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All manuscripts submitted for publication should be addressed to R. E. Buchanan, Room 101 Science Building, Iowa State College, Ames, Iowa.

All remittances should be addressed to P. E. Brown, Bus. Mgr., Room 25 Hall of Agriculture, Iowa State College, Ames, Iowa.

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FIRST SUPPLEMENTARY LIST OF PARASITIC FUNGI FROM IOWA

JOSEPH C. GILMAN

From the Department of Botany, Iowa State College

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Since the publication of the earlier list (29) of the parasitic fungi from the state, in 1929, further collections and publications have increased our list to such an extent as to make advisable a supplementary report. Thirty-five fungi unrecorded in the state have been collected and twenty-six hosts have been added as subject to parasitic invasion. With these are listed the additional hosts for parasites already reported and additional parasites on hosts previously found. These additions bring the total number of new associations of the host and parasite in the state to seventy-seven.

Few additions to the parasitic flora of Iowa have been recorded in the literature during the period under consideration. Wilson (51) has added the most names to the list with three parasites and three hosts. The other contributions have been made chiefly from collections by the staff of the Botany Department at Iowa State College, whose assistance, together with that of Dr. H. S. Conard and of Dr. G. W. Martin of the Lakeside Laboratory at Lake Okoboji, is gratefully acknowledged.

The hosts in this list all belong to families of plants already recorded (29). Of the fungi, members of the genera Amerosporium, Aspergillus, Cladotrichum, Glomerularia, Phlyctaena, Rhinotrichum, Rhynchosporum have not been previously reported for Iowa. To sum up, the total number of hosts in the state now comprises 1035 species, while the fungi number 995. The species not hitherto listed are marked with an asterisk. The figures in parenthesis after the fungus name refer to a citation in which the fungus is described, after the host name to a record of the fungus from Iowa.

1. **Albugo candida** (Pers.) Rouss. (51)
   On *Brassica juncea* L. *Carroll:* Pammel, 1928.
2. ***Amerosporium oeconomium** Ell. & Tracy (24)
   On *Vigna sinensis* Endl. *Muscatine:* Layton, 1928.
3. **Armillaria mellea** (Fr.) Quelet (33)
   On *Quercus rubra* L. *Des Moines:* R. H. Porter, 1929.
4. ***Aspergillus niger.** Van Tiegh. (48)
   On *Allium cepa* L. (cult.) *Pleasant Valley:* Henderson, 1929.
5. **Botrytis cinerea** Pers. (45)
   On *Ranunculus abortivus* L. *Ames:* Buchholtz, 1930.
6. ***Botrytis tulipae** (Lib.) Hopkins (32)
   On *Tulipa sp.* (cult.). *Wapello:* R. H. Porter, 1932.
7. **Cercospora granuliformis** Ell. & Holw. (26)
   On *Viola tricolor* var. *hortensis* D. C. *Webster City:* R. H. Porter, 1929.
8. ***Cercospora mirabilis** Tharp. (47)
   On *Mirabilis jalapa* L. *Muscatine Co.:* Layton, 1928.

9. **Cicinnobolus cesati** De B. (2)
On *Erysiphe polygoni* D. C. on *Lycium halimifolium* Mill. *Ames:* Gilman, 1931.
10. **Cintractia caricis** (Pers.) Magn. (11, p. 33)
On *Carex grisea* Wah. *Lee County:* Fults, 1931.
11. ***Cladotrichum leersiae** Atk. (6)
On *Leersia oryzoides* (L.) Sw. *Arnold's Park:* Gilman, 1931.
12. ***Claviceps nigricans** Tul. (5)
On *Eleocharis palustris* (L.) R. & S. *Clarion:* Melhus, 1929.
cf. Krieger, Fungi Saxonici No. 865.
13. **Colletotrichum graminicolum** (Ces.) Wils. (52)
On *Poa pratensis* L. *Ontario:* Gilman, 1931.
14. ***Colletotrichum violarum** J. J. Davis (16)
On *Viola sororia* Willd. *Estherville:* Gilman, 1931.
15. **Cordyceps sp.**
On *Luperina stipata* Morr. *Ames:* Decker, 1930. (19)
16 ***Cordyceps calvulata** Schw. (38)
On *Lecanium sp. Ames:* Burroughs, 1930.
17. **Cronartium ribicola** Fisch. de Waldh. (4, pp. 122 and 692)
On *Ribes sp.* (cult. black currant). *Centerpoint:* Ness, 1929.
18. **Cylindrosporium apocyni** Ell. & Ev. (13)
Syn. *Stagnospora apocyni* (Pk.) Davis.
On *Apocynum cannabinum L. Osage:* McDonald, 1929.
19. **Cylindrosporium salicinum** (Pk.) Dearn. (17)
On *Salix interior* Rowlee (*Salix longifolia* Auct.). *Ames:* Simonds. 1931.
20. **Discosia artocreas** (Fr.) Tode (20)
On *Vitis vulpina* L. *Ames:* Sylvester, 1931.
21. **Dothichiza populea** Sacc. (31)
On *Populus deltoides* Marshall (Carolina poplar). *Ames:* Gilman, 1930.
22. **Erysiphe cichoracearum** DC. (42)
On *Citrullus vulgaris* L. (greenhouse). *Ames:* Weetman, 1932.
On *Cucumis melo* L. *Muscatine Co.:* Layton, 1928.
On *Zinnia elegans* Jacq. *Blairstown:* Pammel, 1929.
23. **Erysiphe graminis** DC. (42)
On *Agropyron repens* (L.) Beauv. *Ames:* Hershey, 1930.
24. **Erysiphe polygoni** DC. (42)
On *Lycium halimifolium* Mill. *Ames:* Gilman, 1931.
25. ***Glomerularia corni** PK. (37) L.
On *Lonicera tatarica* L. var. *alba* Hort. *Ames:* Melhus, 1929.
cf. Ellis N. Amer. Fungi 1230, Barth. Fungi Columb. 2325.
26. **Gymnosporangium globosum** Farl. (4, p. 204)
On *Juniperus scopulorum* Sarg. *Ames:* Bliss, 1930.
On *Pyrus communis* L. (Cult. pear). *Kent:* Bliss, 1930.
27. **Gymnosporangium juniperi-virginianae** Schw. (4, p. 209)
On *Juniperus scopulorum* Sarg. *Ames:* Bliss, 1930.
28. ***Helminthosporium bromi** Diedicke (22)
On *Bromus inermis* Leyss. *Ames:* Gilman, 1931.
29. **Helminthosporium stenacrum** Drechsler (22)
On *Agrostis maritima* Lam. *Shenandoah:* R. H. Porter, 1931.

30. **Helminthosporium** sp.
On *Bouteloua oligostachya* (Nutt.) Torr. *Lake Okoboji:* Gilman, 1931.
31. **Macrosporium cucumerinum** Ell. & Ev. (8)
On *Cucumis melo* L. (cult. muskmelon). *Janesville:* Layton, 1931.
32. ***Macrosporium porri** Ell. (3)
On *Allium cepa* L. *Homestead:* Henderson, 1929.
33. ***Marssonia sonchi** Dearn. & Bisby (18)
On *Sonchus arvensis* L. *Mason City:* Vestal, 1930.
34. ***Melanconis juglandis** (Ell. & Ev.) Graves (30)
On *Juglans nigra* L. *Ames:* Gilman, 1931.
35. ***Metarrhizium anisopliae** (Metsch.) Sorokin (38)
On *Arcanellus sp. Ames:* Decker, 1930.
36. **Mosaic** (21)
On *Nicotiana affinis* T. Moore. 1927
37. ***Peronospora claytoniae** Farl (27)
On *Claytonia virginica* L. (51).
38. ***Peronospora dicentra** Sydow (28)
On *Dicentra cucullaria* L. *Ames:* Layton, 1930. *Winterset:* Gilman, 1932.
39. ***Peronospora plantaginis** Underw. (49)
On *Plantago aristata* Michx. (51)
40. **Phragmidium uredinis** Link (4, p. 186)
Syn. *Kuehneola uredinis* (L. K.) Arth.
On *Rubrus procumbens* Muhl. (51)
41. ***Phlyctaena linicola** Speg. (7)
On *Linum usitatissimum* L. (cult. var. Bison). *Ames:* Reddy, 1931.
42. ***Phyllosticta catalpae** Ell. & Mart. (43)
On *Catalpa bignonioides* Walt. *Conesville:* Layton, 1929.
43. ***Phyllosticta persicae** Sacc. (43)
On *Prunus persica* L. *Conesville:* Layton, 1929.
44. **Phyllosticta phaseolina** Sacc. (43) (Benth) Wats.
On *Strophostyles pauciflora. Conesville:* Melhus, 1930.
45. ***Phyllosticta podophylli** (M. A. Curt.) Wint.
On *Podophyllum peltatum* L. *Ames:* Weetman, 1932.
cf. Ellis N. Am. Fung. 1156.
46. ***Phyllosticta quercus** Sacc. and Speg. (43)
On *Quercus prinoides* Willd. *Winterset:* Gilman, 1930.
47. **Podosphaera oxyacanthae** (DC.) DeBy. (42)
On *Prunus virginiana* L. *Ames:* Ellis, 1930.
48. **Pseudoperonospora cubensis** (B. & C.) Rostow. (40)
On *Cucurbita pepo* L. *Conesville:* Layton, 1928.
49. **Puccinia asterum** Kern (4, p. 362)
On *Dulichium arundinaceum* (L.) Britton. *Winnebago Co.:* Pammel, 1908.
50. ***Puccinia emiliae** P. Henn. (4, p. 584)
On *Calendula officinalis* L. *Ames:* Berberian, 1931.
On *Dimorphotheca cuneata* (cult.). *Ames:* Berberian, 1931.
51. **Puccinia graminis** Pers. (4, p. 295)
On *Cinna arundinacea* L. *Grinnell:* Conard, 1927.

52. **Puccinia menthae** Pers. (4, p. 405)
On *Mentha gentilis* L. *Grinnell:* Conard, 1923.
This was reported previously as "Mentha sp."
On *Pycnanthemum flexuosum* (Walt.) BSP. (51)
52a. **Puccinia seymouriana** Arth. (4,p. 318)
On *Cephalanthus occidentalis* L. *McGregor:* Hendershott, 1932.
53. **Puccinia sorghi** Schw. (4, p. 277)
On *Euchlaena mexicana* Schrad. *Ames:* Gilman, 1931.
53a.***Puccinia tubulosa** (Pat. and Gaill.) Arth. (4, p. 288)
On *Solanum carolinense* L. *Conesville:* Layton, 1932.
54. **Pucciniastrum americanum** (Farl.) Arth. (4, p. 677)
On *Rubus sp.* (cult. var. Latham red raspberry).
55. ***Rhinotrichum doliolum** Pound and Clements (10)
On *Diderma floriforme* Pers. *Boone, Ledges:* Weetman, 1931.
56 ***Rhynochosporium alismatis** (Aud.) J. J. Davis (15)
On *Alisma plantago-aquatica* L. *Lee Co.:* Fults, 1931.
57. **Sclerotinia bifrons** (Ell. & Ev.) Seaver and Shope (44)
On *Populus tremuloides* Michx. *Allamakee Co.:* Miller, 1929.
58. **Sclerotinia libertiana** Fckl. (55)
On *Aquilegia sp.* (cult.). *Ames:* Bode, 1930.
59. ***Sclerotinia smilacinae** Durand (23)
On *Smilacina racemosa* (L.) Desf. *Ames:* Davis, 1930.
60. ***Septoria atriplicis** Desm. (16)
On *Chenopodium album* L. *Milford:* Gilman, 1931.
61. **Septoria bataticola** Taub. (46)
On *Ipomoea hederacea* Jacq. *Muscatine Co.:* Layton, 1928.
62. ***Septoria citrulli** Ell. & Ev. (24)
On *Citrullus vulgaris* L. *Conesville:* Layton, 1928.
63. ***Septoria coreopsidis** J. J. Davis (14)
On *Coreopsis tripteris* L. (50). *Oskaloosa:* G. W. Wilson, 1929.
63. ***Septoria dracocephali** Thüm. (41, v. 3, p. 540)
On *Dracocephalum parviflorum* Nutt. *Spirit Lake:* R. H. Porter, 1931.
65. **Septoria helianthi** Ell. & Kell. (36)
On *Helianthus annuus* L. *Conesville:* Layton, 1930.
66. ***Septoria mitellae** Ell. & Ev. (35)
On *Mitella diphylla* L. *Iowa City:* Austin, 1930.
67. ***Septoria septentrionalis** H. W. Anderson (1)
On *Ranunculus sp. Ames:* Kopf, 1932.
68. **Septoria virgaureae** Desm. (36)
On *Solidago latifolia* L. *Milford:* Gilman, 1931.
69. **Sporodinia grandis** Link (35)
On *Secotium agaricoides* (Czern.) Hollos. *Ames:* Gilman, 1929.
70. ***Sporotrichum globuliferum** Speg. (38)
On *Arcanellus sp. Ames:* Doecker, 1930.
71. ***Stagonospora caricinella** Brun (12)
On *Carex pennsylvanica* Lam. *Ames:* Gaskill, 1932.
72. ***Stagonospora paludosa** (Sacc. & Speg.) Sacc. (41, v. 3, p. 453)
On *Carex Jamesii* Schw. *Ames:* Hershey, 1930.
73. **Taphrina coerulescens** (Mont. & Desm.) Tul. (50)
On *Quercus macrocarpa* W. *Okoboji:* G. W. Martin, 1929.
On *Quercus velutina* Lam. *Cresco:* Pammel, 1929.

74. **Tuberculina persicina** (Ditm.) Sacc. (41)
On *Aecidium onobrychidis* Burrell on *Apios tuberosa* Moench. (51).
75. **Uncinula salicis** (DC.) Wint. (42)
On *Salix nigra* Marsh. *Ames:* Nagel, 1931.
76. **Urocystis anemones** (Pers.) Schroet. (11, p. 55)
On *Anemonella thalictroides* (L.) Spach. *Ames:* Hershey, 1930.
77. **Uromyces lespedezae-procumbentis** (Schw.) Curt. (4, p. 247)
On *Lespedeza violacea* (L.) Pers. *Eagle Rock:* Pammel, 1920.
On *Lespedeza virginica* (L.) Britt. *Lacey-Keosauqua Park:* Pammel, 1929.
78. ***Uromyces punctatus** Schroet. (4, p. 253)
On *Astragalus canadensis* L. *Ames:* Gilman, 1929.
79. ***Ustilago lorentziana** Thuem (11, p. 9)
On *Hordeum jubatum* L. (51). *Swea City:* Robt. Brown, 1930.
80. **Yellows** (38)
On *Fragaria sp.* (cult. strawberry). *Ames:* R. H. Porter, 1929..

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A STUDY OF CARAMEL COLOR[1]

JOHN B. SHUMAKER WITH J. H. BUCHANAN

From the Department of Chemistry, Iowa State College

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Caramel has been used as a coloring material in foods and beverages for many years. The quality of caramels, however, is quite variable, depending upon the material from which they are made, the method of manufacture, and the nature of the products in which they are to be used as a color.

The coloring powers of caramels vary considerably, even though they may be prepared from the same carbohydrate. A given caramel may show stability at one H-ion concentration, and separate from solution at another H-ion concentration. These facts indicate that caramels may be colloidal in nature and that there is a definite isoelectric point for the different varieties of caramel.

As early as 1838, Peligot (7) examined the products of the dry distillation of sugars. Gélis (4) in 1858, and Stolle (11) in 1899, also did much to establish the nature of the distillates. Sangiori (10) reported the presence in caramel of furfural, acetone, formaldehyde, formic acid, and acetic acid, all in small quantities. By far the greater portion of the distillate is water.

This leads one to believe that the principal reaction promoted by heating sugars is that of dehydration and that other products are formed to a small extent at a temperature slightly above the melting point of sugar.

Gélis (4) also showed that sucrose, when heated at temperatures around 200°C., was converted progressively into a number of dehydration products by successive dehydration. These products he named in the order of their formation:

caramelan—$C_{24}H_{36}O_{18}$
caramelen—$C_{36}H_{50}O_{25}$
caramelin—

Caramelin was a more highly dehydrated product with a rather uncertain formula but decidedly colloidal.

Graham (5) showed that caramelan and caramelen dialyzed readily and that caramelin did not. Cunningham and Dorée (3) prepared caramelan in nearly pure form by heating sucrose at 170°-180°C. until the loss in weight was 12 per cent. After further purification from alcoholic ammonia they were able to verify the formula of Gélis for caramelan by freezing point methods.

Pictet and Andrianoff (8) working at 10-15 mm. pressure at 185°-190°C., were able to produce the entire series including isosaccharosan, $C_{12}H_{20}O_{10}$. This compound is obtained by simple loss of a molecule of water and is nearly colorless. Further dehydration takes place between molecules, forming larger molecules in multiples of C_{12}.

[1]From a thesis submitted to the Graduate Faculty of Iowa State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Ripp (9) prepared caramelan from levulose, proving that sucrose is not the only carbohydrate which will yield caramelan.

Beal and Bowey (1) prepared caramel from glucose with the aid of catalysts such as ammonia, ammonium chloride, sodium carbonate, hydrochloric acid and ammonium sulfate. They showed that with higher temperature and longer heating, caramels of higher coloring power and of less stability were obtained. Beal and Applegate (2) showed that sucrose caramels were equal to or better than glucose caramels.

In order to study caramel more closely and to correlate the colloidal properties with the chemical compounds obtained by previous workers, three series of caramels were prepared from sucrose at temperatures of 190°, 200°, and 210°C. Crtain representative caramels from each series were subjected to various H-ion concentrations, to dialysis, and to electrophoresis. The colloidal properties of the compounds of caramel are clearly shown to be related to the temperature and time of heating of the carbohydrate.

PREPARATION OF CARAMELS

A number of caramels were prepared at different temperatures and with varying intervals of time of heating, without the aid of a catalyst. In order to make a systematic comparison of caramels prepared at a given temperature, the loss in weight during a definite interval of heating was taken as a criterion. The small amounts of substances other than water evolved were considered as negligible, and the loss in weight of the sucrose upon heating was considered as water.

Dry sucrose of a high grade was the carbohydrate employed in the experiments. Charges of two hundred grams of sucrose were used. These were weighed upon a trip balance with an accuracy of 0.1 gram.

It was noted in all previous work that little attention was given to accurate temperature control. Since a given charge of sucrose was to be heated at temperatures of 190° to 200°C. over periods of time ranging from 30 minutes to 130 minutes, a wide variation in weights would result in the final products. Obviously it would be nearly impossible to obtain results which could be accurately duplicated. After some preliminary experiments with various methods of heating, this was found to be the case.

An electrically heated device provided with mechanical stirrers was constructed which gave satisfactory results as is indicated in table 1. These runs are typical of the routine runs which are recorded in tables 2, 3 and 4. The duplicate results (b) check the original runs (a) closely, giving rise to an experimental error of about 0.2-0.3 per cent.

TABLE 1. *Influence of time and temperature on loss in weight*

Series No.	Time min.	Temp.	Loss percentage		
			a	b	average
A-1	40	190	0.5	0.4	0.45
A-3	60	190	5.9	5.8	5.85
B-1	30	200	2.3	2.3	2.30
B-2	35	200	4.45	4.45	4.45
B-3	40	200	5.85	6.05	5.95
B-4	45	200	7.85	7.25	7.75
B-5	50	200	8.40	8.25	8.30
C-1	20	210	2.95	2.85	2.90

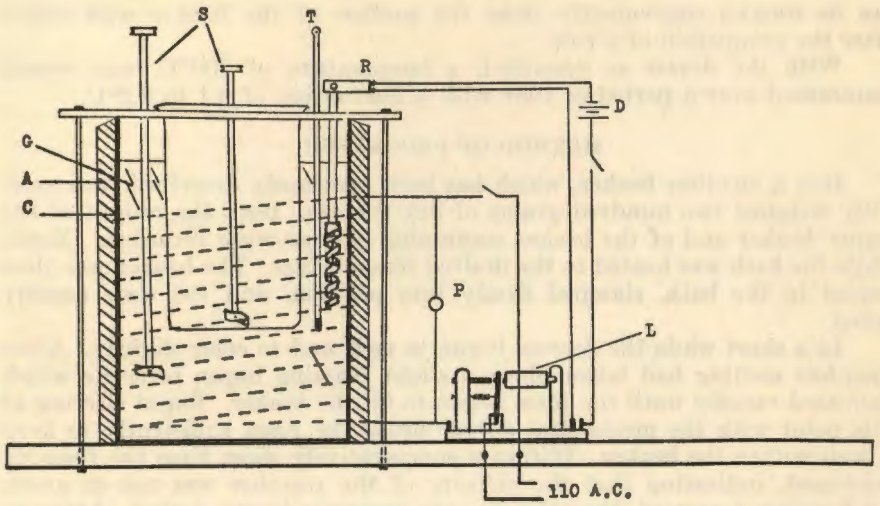

Fig. 1. Design of electric heater for constant temperature bath

DESCRIPTION OF APPARTUS

Figure 1 is a design of the construction of the electric heater. The bath itself is an aluminum cylindrical vessel around which an insulating layer of asbestos paper is closely wrapped. Around the asbestos layer there is placed a coil of twenty turns of No. 18 electric resistance wire. The ends are connected to an electric current. The wire is held in place firmly by a thick layer of fire cement.

A square plate of asbestos board covers the top. Four bolts pass through the corners and into the base upon which the apparatus rests. These bolts serve to hold the bath in a rigid position upon the base. A large hole is cut in the center of the plate of such size that a low-form Griffin Pyrex beaker may be suspended in it and supported only by its rim.

Distributed about the large hole are three small holes. Into one of these is inserted the bath stirrer (S) which keeps the entire bath at uniform temperature by forced circulation. Into another is inserted a thermometer (T) which has been accurately calibrated at the temperature at which the bath is operated. Through the third hole is inserted a regulating device (R) which, by expansion of the metal coil at its lower end, causes the heating current to break when the proper temperature has been reached.

At the top of the regulator are two contact points which, when they touch, close a circuit through the two dry cells (D). This current passes through the magnet on the relay (L). The magnet draws the small vertical arm toward it and causes a break in the 110 volt heating circuit, and the bath ceases to heat. When the temperature of the bath drops sufficiently, the regulator releases the vertical bar, which is pulled over by a small spring and contact is again made in the heating circuit. A pilot light (P), placed across the terminals of the heating unit, indicates when the bath is heating.

The bath medium chosen was glycerin. It becomes very fluid at high temperatures, circulates readily, and has the advantage over oil in that it

can be washed conveniently from the surface of the beaker with water after the completion of a run.

With the device as described, a temperature of 200°C. was evenly maintained over a period of time with a fluctuation of 0.1 to 0.2°C.

METHOD OF PROCEDURE

Into a one-liter beaker, which has been previously described, was carefully weighed two hundred grams of dry sucrose. Both the weight of the empty beaker and of the beaker containing sucrose were recorded. Meanwhile the bath was heated to the desired temperature. The beaker was then placed in the bath, clamped firmly into position, and the time quickly noted.

In a short while the sucrose began to melt and to color slightly. After complete melting had taken place, a slight foaming began to occur which increased rapidly until the foam began to fill the beaker. Rapid stirring at this point with the mechanical stirrer broke the foam sufficiently to keep it well within the beaker. Within a comparatively short time the foaming decreased, indicating that the velocity of the reaction was not so great. As foaming decreased, the color became correspondingly darker. After an interval of time, if the run was carried on for a sufficient period, the foaming ceased, and the mass became quite viscous.

At this point a second stage of foaming began. The mass being very viscous by this time, it was increasingly difficult to stir successfully with the mechanical stirrer. From this point on, stirring by hand became necessary, in order to prevent the very dark viscous mass from rising over the top of the beaker. The velocity of this second reaction slowly decreased and the mass gradually thickened until it was evident that it would be useless to continue the run at the stated temperature.

When the time allotted for the run had expired, the beaker was quickly removed from the bath, the glycerin washed from the outside, and when it had cooled sufficiently, the beaker was weighed. The loss in weight was recorded, as was also the percentage loss in weight.

As soon as one run was completed, a second was immediately started. In this way a series of runs was made at a given temperature, each run being heated a definite number of minutes longer than the previous one.

In this manner, three series of caramels were prepared at temperatures of 190°, 200°, and 210°C. By plotting the time of heating against the percentage loss in weight a number of interesting observations were made and conclusions drawn. Tables 2, 3 and 4 represent the essential data collected for the production of the curves in figure 2.

TABLE 2. *Series A. Caramels prepared at bath temperature 190°C.*

Run no.	Time min.	Loss p'c't'g.	Run no.	Time min.	Loss p'c't'g.
1	40	00.45	7	100	09.4
2	50	3.05	8	110	10.45
3	60	5.85	9	120	10.6
4	70	7.2	10	130	11.3
5	80	8.45	11	140	12.05
6	90	8.7	12	150	11.95

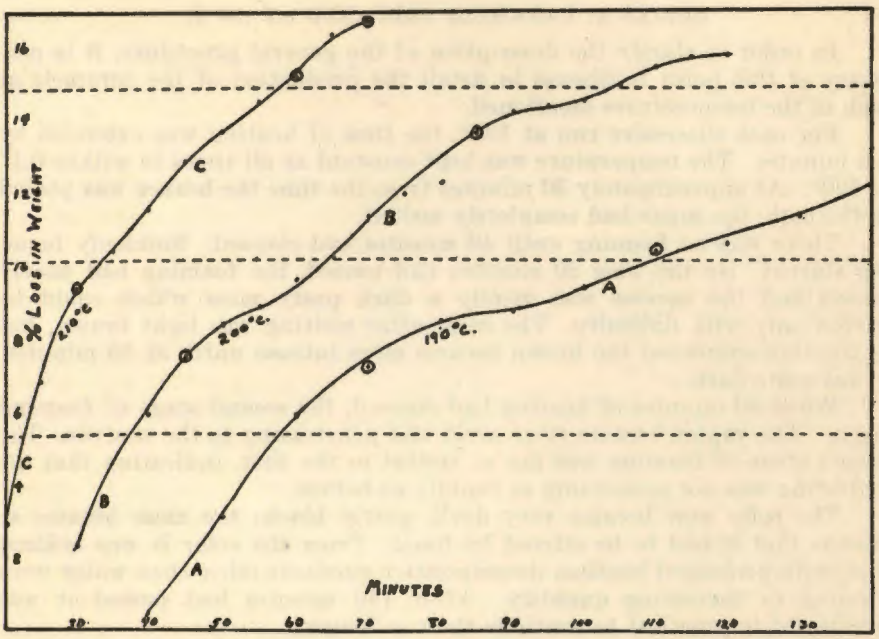

Fig. 2. Curves showing loss in weight during lapse of time of heating

TABLE 3. *Series B. Caramels prepared at bath temperature 200°C.*

Run no.	Time min.	Loss p'c't'g.	Run no.	Time min.	Loss p'c't'g.
1	30	2.3	11	80	12.1
2	35	4.45	12	85	13.65
3	40	5.9	13	90	14.15
4	45	7.55	14	95	14.25
5	50	8.3	15	100	14.50
6	55	8.9	16	105	14.9
7	60	9.6	17	110	15.4
8	65	10.2	18	115	15.65
9	70	11.3	19	120	15.75
10	75	11.9			

TABLE 4. *Series C. Caramels prepared at bath temperature 210°C.*

Run no.	Time min.	Loss p'c't'g.	Run no.	Time min.	Loss p'c't'g.
1	20	2.9	7	50	13.65
2	25	6.85	8	55	14.4
3	30	9.4	9	60	15.15
4	35	10.3	10	65	16.3
5	40	11.5	11	70	16.65
6	45	12.85			

SERIES A. CARAMELS PREPARED AT 190°C.

In order to clarify the description of the general procedure, it is necessary at this point to discuss in detail the production of the caramels at each of the temperatures mentioned.

For each successive run at 190°, the time of heating was extended by ten minutes. The temperature was kept constant at all times to within 0.1° of 190°. At approximately 30 minutes from the time the beaker was placed in the bath, the sugar had completely melted.

There was no foaming until 40 minutes had elapsed. Suddenly foaming started. By the time 80 minutes had passed, the foaming had nearly ceased and the sucrose was merely a dark pasty mass which could be stirred only with difficulty. The color after melting was light brown, but as foaming continued the brown became more intense until, at 80 minutes, it was quite dark.

When 80 minutes of heating had elapsed, the second stage of foaming began. The vapors became more acrid and penetrating to the nostrils. The second stage of foaming was not so violent as the first, indicating that dehydrating was not proceeding so rapidly as before.

The color now became very dark, nearly black; the mass became so viscous that it had to be stirred by hand. From the color it was evident that, with prolonged heating, decomposition products other than water were forming in increasing quantity. After 140 minutes had passed it was considered impractical to continue the run longer.

The above description is that of the longest run made at 190°. All of the shorter runs behaved in an eactly similar manner up to the time allotted to them, when they were removed from the bath.

SERIES B. CARAMELS PREPARED AT 200°C.

At this higher temperature it was to be expected that the sucrose would melt more rapidly, and that the time of heating would be materially shortened in order to form the same caramels which had been produced in series A.

This was found to be the case. The sucrose became completely melted in about 20 minutes and foaming began immediately. The second stage of foaming began in 35 minutes. The third occurred at 55 minutes. A caramel of series B produces much darker solutions than one of series A which had been prepared with the same time of heating.

SEIES C. CARAMELS PREPARED AT 210°C.

The same general observations were made as in series A and B. However, as was to be expected, complete melting had occurred within 15 minutes, and foaming began at once. The second foaming stage began in 19 minutes and the third in 30 minutes.

The data for these three series are recorded in tables 2, 3 and 4. These data were plotted on coordinate paper (fig. 2). With the aid of the curves important deductions were made which are not obvious from the data. In the graph (fig. 1) the loss in weight of the members of each series is plotted against the time of heating in minutes. Three dotted horizontal lines located respectively at 5.23 per cent, 10.52 per cent and 14.03 per cent are included to indicate the points at which isosaccharosan, caramelan, and caramelen should be formed.

In order to prepare isosaccharose ($C_{12}H_{20}O_{10}$), one molecule of sucrose must lose one molecule of water. The weight of water lost would be 5.23 per cent of the molecular weight of sucrose.

The formation of caramelan from sucrose requires the loss of four molecules of water from two molecules of sucrose. The loss in weight required is 10.52 per cent.

Caramelan is formed from three molecules of sucrose by the loss of eight molecules of water. In this case the loss is 14.03 per cent.

These values are theoretical and cannot be attained practically for the reason that other products are formed at the same time to a small extent. Therefore, in order to prepare the purest caramelan that is possible, it would be necessary to heat sucrose until the loss in weight was somewhat over 10.52 per cent. Cunningham and Dorée (3) obtained almost pure caramelan by heating sucrose until the loss was 12 per cent.

Foaming of the caramel as it is heated is indicative of the chemical reaction whereby water is lost. Excessive foaming indicates rapid loss of water, wheeras, little foaming indicates little loss. The greatest foaming occurs at the beginning of a definite chemical reaction and at the end there is practically no foaming.

Therefore, in the study of the curves, the horizontal portions indicate the end of one definite dehydration reaction. The rising portion immediately following indicates the beginning of a new reaction.

Curve A. The running temperature of 190° was selected as being the lowest practical temperature at which caramel could be prepared within a reasonable length of time. The melting point of sucrose is approximately 10° below this.

The first stage of foaming started within 40 minutes and had become essentially complete at the 80 minute interval. No decided break can be seen in this portion of the curve. A break should appear in the region of 5 per cent loss, but the relatively low temperature causes the merging of the two reactions into practically one. No foaming stage was noted again until the 80 minute interval was reached. Here a decided rise in the curve is again noticed, showing the completion of the third reaction and the beginning of the fourth.

The first stage should indicate the formation of isosaccharosan, but because of the low temperature, the reaction is not rapid enough to show a distinct break. Consequently, the beginning of the second stage, or the formation of caramelan, is not discernable. The second stage as shown is then really the third stage, and caramelan begins to form within the 80 minute interval.

The loss should be 10.52 per cent, but the curve indicates that caramelan has been formed when the loss is only about 8.5-9 per cent. In order to account for this apparent discrepancy the temperature and voscosity of the caramel must be taken into account. The viscosity is continually increasing while the temperature remains at 190°.

As viscosity increases it becomes more and more difficult to remove the water as rapidly as it is formed, by stirring. Consequently, although the water may be completely liberated, some of it is retained mechanically for a short time before it can be vaporized. According to the curve, when caramelan has been completely formed, there is still mechanically retained as much as 1-1.5 per cent of unvaporized water. The result is that caramelan has been apparently formed with a loss of only about 9 per cent water instead of 10.52 per cent.

At a higher temperature, water would be eliminated more rapidly as it is formed. On curves B and C this is found to be true.

A slight rise in the curve is again noticeable at the 140 minute interval, although no foaming was observed. It was considered impractical to continue this curve further because of the fact that the mass became unstirrable. At higher temperatures the mass remains liquid until the loss is greater.

At the temperature of 190°, the formation of isosaccharosan cannot be detected. The formation of caramelan is readily observed, however, with a loss of weight about one per cent below the theoretical, which has been explained. Caramelan cannot be prepared satisfactorily at this temperature.

Curve B. Foaming begins in about 20 minutes. At the 35 minute interval a slight break occurs which may be construed as the completion of the reaction, sucrose = isosaccharose. The break occurs at the proper point for the formatio nof isosaccharose, but is not definite, probably because the temperature is high, causing the rapid elimination of water. The second break indicates quite clearly the complete formation of caramelan, and the beginning of formation of caramelen. Here, as in curve A, caramelan is formed with a loss of water about one per cent below the theoretical.

A small break is again observed at the 95 minute interval, although no unusual foaming was observed. The break occurs very close to the 10.52 per cent line, which is theoretical for caramelen. Assuming the mechanical retention of water to be roughly the same, the close agreement between the theoretical loss and the experimental loss can be accounted for by the increase in other decompositio nproducts which vaporize with the water. The higher temperature would favor the formtaion of substances other than water.

This break shows quite clearly the formation of three dehydration products. Isosaccharosan is formed at the proper place. Caramelan formation is indicated quite distinctly with a loss about one per cent below the theoretical. Caramelen appears to be formed at the proper place also. There is probably mechanical retention of some water, but this is offset by the formation of more decomposition products at the higher temperature.

Curve C. In this case three distinct stages of foaming were observed. The high temperature of 210° caused foaming to occur at 15 minutes and again at 19 minutes. However, water was eliminated so rapidyl that it was impossible to record the weights in such a way as to show a break on the curve.

At 30 minutes the third stage beganfl A distinct break occurs here at the 10.52 per cent line. It is probable that at this high temperature water is driven off almost as rapidly as it is formed and the result is that the loss is almost theoretical for that required to form caramelan.

Another depression is to be noted at the 55 minute interval, but no specific foaming was noted at this stage. This depression is indicative of the complete formation of caramelen.

A consideration of all three curves brings out the following observations:

1. Curve B only, indicates the formation of isosaccharosan.
2. All three curves show clearly the formation of caramelan at or near the theoretical point.

. Curves B and C show evidence of the formation of caramelen. Curve A cannot be carried far enough to show a similar point.

4. Variations from the theoretical losses can be explained on a basis of mechanical retention of water due to relative temperature and viscosity.

STUDY OF COLLOIDAL PROPERTIES OF CARAMELS

In order to study the colloidal properties, certain caramels were selected from each series of runs which most closely represented the compounds isosaccharosan, caramelan, and caramelen. The caramels selected were as follows:

1. Caramels on curve A which had been formed by heating 70 and 110 minutes, respectively. These will be designated hereafter as A-70 and A-110.

2. Caramels on curve B—B-45 and B-85.

3. Caramels on curve C—C-30, C-60, and C-70. C-70 was selected because it had undergone the greatest loss at the hightest temperature employed, and therefore represented the most highly caramelized product obtained.

Dialysis. No attempt was made to establish any quantitative relationships in the experiments on dialysis. Collodion membranes were prepared by allowing a film to dry upon the inner surface of a large test tube. These were easily removed from the tube by soaking in water.

Solutions of the selected caramels were made by dissolving a small quantity of the caramel in distilled water until the color was an intense brown. The solutions were still dilute with respect to the quantity of caramel present.

Each of the seven selected caramels were placed in separate collodion bags. Each bag was filled and then suspended in a two-liter beaker containing distilled water. Diffusion began immediately as indicated by the color of the distilled water in the beaker. The water was changed every two hours.

Wtihin 6 hours solutions of A-110, B-85, and C-60 had ceased to dialyze. The solutions inside the bags were almost as dark in color as they originally had been. Apparentl ymost of the caramel in each of these bags did not diffuse through the membrane.

After 24 hours, A-70, B-45, and C-30 had practically ceased diffusing. By the color of the contents of the bags, it was concluded that there was some colloidal material present. However, most of the contents were crystalloid because so much time was required to remove it by dialysis.

In the higher portions of the curves the caramels are shown to be quite colloidal in nature, while the lower caramels are shown to be mostly crystalloidal.

Electrophoresis. According to Holmes (6) the charge of electricity on a colloidal particle in suspension is caused by the preferential absorption of positive or of negative ions from the solution on the surface of the particle. A particle thus charged, on electrolysis, will move toward the electrode of opposite sign. Caramels are no exception to this rule.

To demonstrate the mobility of caramel on electrolysis, an apparatus similar to that described by Holmes (6) was constructed. A layer of clear distilled water was carefully superimposed on the caramel solution, the platinum electrodes were immersed in the water layer, and a direct current was passed through at 110 volts for 60 minutes.

The distance which the colored layer at the negative electrode moved downward was carefully measured and noted in table 5.

TABLE 5. *The downward movement of the layer at the negative electrode*

Caramel sol.	mm. lowering	Caramel sol.	mm. lowering
A-70	1.70	A-110	3.55
B-45	0.85	B-85	1.40
C-30	0.40	C-60	5.10
		C-70	1.00

Caramels on the lower portions of the curves (A-70, B-45, and C-30) undergo electrophoresis to a less extent than the upper members. This fact would indicate that the charge upon the lower members is not so great as that on the higher members. The lower caramels would be considered either as particles which are near their isoelectric point, or as particles which border on true solution particles in size. The results of dialysis favor the idea that the particles are near in size to particles in true solution.

Effect of H-ion Concentration. From the results of dialysis and cataphoresis it is concluded that the caramels which have been prepared are electro-negatively charged colloids. Whatever the source of the charge upon the particles, whether it is merely a difference of potential set up between the particles and the medium, or whether it is a case of preferential absorption of hydroxyl ions, the addition of ions bearing opposite charges should have a neutralizing effect upon the charges already present on the particles.

Assuming that hydroxyl ions are absorbed to a greater extent than hydrogen ions, the colloid becomes negatively charged. Addition of acids, or in other words, addition of hydrogen ions, would tend to neutralize the negative charges and eventually cause the particle to become electrically neutral. At the neutral zone coagulation and precipitation of the particles usually occur and the isoelectric point is said to have been reached.

Acids which are highly ionized would be expected to have a greater precipitating effect upon caramel than slightly ionized acids of equivalent strengths. In order to avoid incorrect conclusions, it would be best to choose acid solutions which contained equal molar concentrations of hydrogen ions from different acids.

For the purposes of the experiment, the acids citric, phosphoric and sulfuric were selected. One of these acids represents the weak organic acids which are used in acid beverages, and one a weak inorganic acid (phosphoric), and one the moderately strong inorganic acid (sulfuric).

In order to secure comparative results as far as H-ion concentrations were concerned, solutions of each acid were prepared having the same approximate pH, namely, 3, 2, and 1.5. These solutions were accurately standardized by the electrometric method, using the hydrogen electrode. Table 6 is designed to give the accurate pH of the solutions mentioned in

the foregoing. For the purposes of discussion, the approximate values will be used.

TABLE 6. *The pH of acids*

Approx. pH	Citric acid	Phosphoric acid	Sulfuric acid
3			2.90
2	1.97	2.00	2.17
1.5	1.46	1.49	1.56

Since a rise in temperature causes a change in the pH, increasing the degree of ionization of the acid, the effect of both the hot and cold acids on the caramels was determined. The procedure is as follows:

To 50cc. of citric acid, pH-1.5, was added a concentrated solution of caramel until a very deep but still transparent color was obtained. Only a few drops of the caramel solution were required to produce the proper color, and the change in pH by the addition of so small a quantity was considered negligible. The flask was then corked carefully to exclude dust and to prevent evaporation, and set aside in diffused daylight for observation.

The limit of time set for observation was two weeks. If precipitation did not occur within that time the caramel was considered stable toward citric acid of pH 1-5. This procedure was carried out in the same way for the other acid solutions previously described—seven in all. This test constitutes the "cold test" of the different acids on the seven caramels which had been selected for examination of their colloidal behavior.

The "hot test" was conducted in a somewhat similar way. The caramel was added to the acid solution in a small flask. The solution was then boiled gently for a period of twenty minutes, tightly stoppered while still boiling hot, and set aside for observation.

In this way the effect of each acid upon each caramel was determined. The results of these tests are recorded as follows:

TABLE 7. *Effect of cold acids on caramels*

Series no.	Approx. comp.	Time required for caramel to ppt.						
		Citric 2	pH. 1.5	Phosphoric 2	1.5	pH. 3	Sulfuric 2	pH. 1.5
A-70	Isosaccharosan	—	—	—	—	—	—	—
B-45	"	—	—	—	—	—	—	—
C-30	"	—	—	—	—	—	—	—
A-110	Caramelan	—	—	—	—	—	—	11d
B-85	"	—	—	—	—	—	—	11d
C-60	"	—	—	—	6d	—	—	75m
C-70	Caramelen	—	—	6d	1m	—	6d	1m

d = days, h = hours, m = minutes.
— indicates no apparent precipitate found within two weeks.

TABLE 8. *Effect of hot acids on caramels*

Series no.	Approx. comp.	Time required for caramel to ppt.						
		Citric 2	pH. 1.5	Phosphoric 2	Phosphoric 1.5	pH. 3	Sulfuric 2	pH. 1.5
A-70	Isosaccharosan	—	—	—	—	—	—	6d
B-45	"	—	—	—	—	—	—	6d
C-30	"	—	—	—	—	—	—	3h
A-110	Caramelan	—	—	—	6d	—	—	60m
B-85	"	—	—	—	?	—	—	75m
C-60	"	—	—	—	6d	—	—	60m
C-70	"	—	—	—	1m	—	—	30m

d = days, h = hours, m = minutes.
— indicates no apparent precipitate found within two weeks.

Considering the tables given it is found that cold citric acid, phosphoric and sulfuric acids have no precipitating effect upon the caramels A-70, B-45 and C-30 which represent isosaccharosan on the curves (fig. 1), in two weeks time. From the results of dialysis and electrophoresis, caramels in this region have been shown to be only partly colloidal.

These caramels were found to dialyze much more than the higher caramels and also migrated a shorter distance when subjected to the action of the electric current. When subjected to acid solutions ranging in pH from 1.5 to 2 and 3, there was no precipitation in the cold.

Sulfuric acid with pH 1.5 when hot caused precipitation after an interval of six days had elapsed. In the case of C-30, precipitation occurred much sooner, because C-30 represents a caramel formed by a slightly greater loss of weight, and therefore, is somewhat more colloidal in nature than A-70 or B-45.

The caramels A-110, B-85 and C-60, which represent caramelan on the curves, are not affected by citric either hot or cold. However, cold phosphoric acid (pH 1.5) affects C-60 while hot phosphoric (pH 1-5) affects all the caramels. Sulfuric acids with pH2 and 9 do not affect these caramels when either hot or cold.

C-70, representing caramelen, is not affected by citric acid, but phosphoric (pH 1.5) and sulfuric (pH 1.5) both precipitate it quickly.

The two mineral acids, phosphoric and sulfuric, are more effective precipitating agents than the organic citric acid. When the mineral acids are hot they are more effective than when cold.

SUMMARY

1. Three series of caramels have been prepared, all of which, when loss of weight is plotted against time of heating, show the same general regions in which definite compounds form. These regions correspond in general to the requirements for formation of isosaccharosan, caramelan and caramelen.

2. Caramels of low molecular weight are shown to possess low color value, and to dialyze largely. The higher caramels are shown to have opposite properties and are clearly colloidal. All caramels behave as electronegative colloids under electrophoresis.

3. The effect of H-ion concentrations may be summarized as follows:

   a. The caramels do not precipitate in citric acid solutions having pH 2 and 1.5 within two weeks.

   b. The lower caramels are stable to cold sulfuric acid at pH of 1.5, but are precipitated by the same acid when hot.

   c. Phosphoric acid, pH 2 and sulfuric acid, pH 3 and 2 have no effect either hot or cold.

   d. The caramels are most stable in the presence of citrate ions and less stable in the presence of sulfate ions. Phosphate ions have intermediate precipitating action.

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CONJUGATED SYSTEMS IN FURAN TYPES

HENRY GILMAN AND JOSEPH B. DICKEY

From the Chemical Laboratory of Iowa State College

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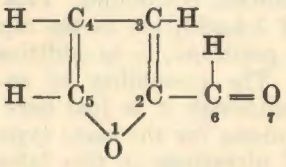
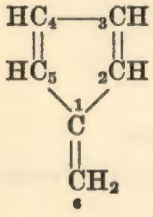
INTRODUCTION

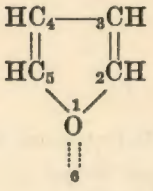
The pronounced and almost extraordinary tendency of many furan types to undergo nuclear substitution reactions indicates that these heterocycles have super-aromatic characteristics. Current explanations of nuclear substitutions, in general, turn on the preliminary formation of addition compounds. Because of the marked tendency of conjugated systems to undergo addition, it was advisable to determine whether furan types have more active conjugated systems than other related cycles like benzene, and also which conjugated systems are involved in the addition reactions.

The compounds selected were those having lateral unsaturation which might be considered as forming conjugated systems with units of nuclear unsaturation. Furfural, for example, may be considered to have a multiple series of conjugated systems.



Such systems are (2, 3, 4, 5) and (3, 2, 6, 7); and a 1,6-addition system is (5, 4, 3, 2, 6, 7). Also, if we assume that the oxygen is capable of functioning, latently or otherwise, in an oxonium form, then we have a hypothetical double bond emerging from this oxygen which would give rise to two other conjugated systems: namely, (1, 5, 4) and (1, 2, 3). This latter consideration is highly attractive because it would give a system of crossed conjugated linkages which generally are distinctly more active than a simple conjugated system just as a simple conjugated system is more active than a non-conjugated system or a simple unsaturated unit like an olefinic linkage or a carbonyl group. An illustration would be the comparison of a highly active crossed conjugated system like fulvene with the related furan,





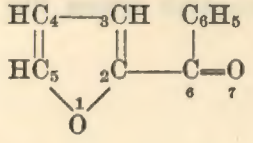
the partial valences emerging from the furan oxygen might then give rise to the crossed conjugated systems (6, 1, 2, 3) and (6, 1, 5, 4). The related

crossed conjugated systems are patent in the molecule of fulvene as written. Such a system would serve to emphasize a tendency to add to the oxygen in furan. Actually, such addition was postulated by Moureu, Dufraisse and Johnson[1] in the bromination of furylacrylic acid.

If addition does occur initially at the oxygen, then we are of the opinion that rearrangement sets in so that the addenda find themslves on the 2- and 5-carbons, with a resulting shift of unsaturation to the position (3, 4). In short, this is a 1,4-addition to the system (2, 3, 4, 5) and has already been suggested as an explanation of some nuclear substitution reactions[2]. Such a mode of addition finds support in the interesting and valuable application by Diels and co-workers of maleic anhydride as a reagent for the characterization of conjugated systems. It is worth mentioning, that maleic anhydride appears not to be generally applicable with furan types, particularly those having negative substituents.

It is necessary at this point to emphasize that probably all nuclear substitution reactions of furan do not necessarily proceed by the same mechanism. Even a given type of substitution like nitration may take place by different mechanisms depending on the kind of furan compound. It does appear altogether reasonable that what first happens is addition to the oxygen, particularly where substitution involves interaction with compounds like the halogens, nitric acid, sulfuric acid, etc. Second, there is a rearrangement to an addition compound. This addition compound may result as a consequence of 1,4-addition to the *alpha*-carbon atoms; or 1,2-addition to the 3,4- or 4,5- positions; or as addition to a single *alpha*-carbon as previously suggested. The possibility of so-called addition by ring opening is not excluded, although it is just here that emphasis should be placed on different mechanisms for the same type of substitution. For example, in a miscellany of nitrations in this laboratory we have observed in some cases so-called intermediate compounds; in other cases no intermediates are formed; and, finally, in some reactions *part* of the product appears to be an intermediate and *part* of the product is the ultimate compound. For purposes of generalization and as a working hypothesis until more adequate experimental data are accumulated it seems probable that 1,4-addition to the *alpha*-carbon atoms is the most useful and general mechanism of preliminary addition.

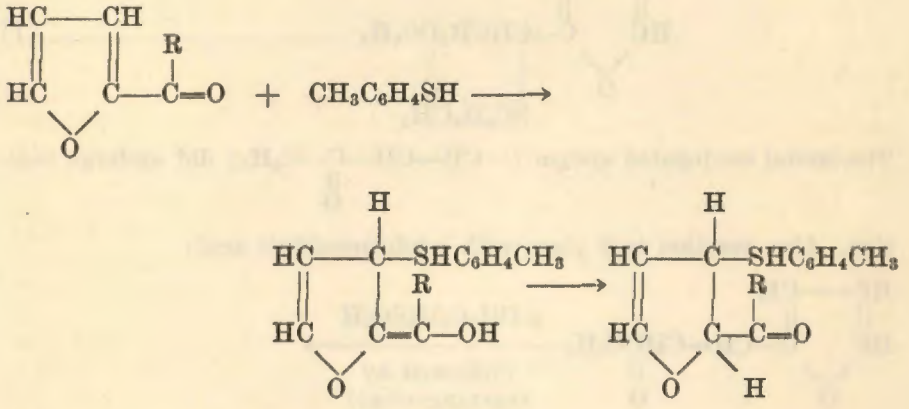
In order to establish the possible presence of other active conjugated systems, like (3, 2, 6, 7) in furfural (and the same system in benzoyl furan or furyl phenyl ketone)



[1]Moureu, Dufraisse and Johnson, *Ann. chim.*, 7, 8 (1927).

[2]Gilman and Wright, *J. Am. Chem. Soc.*, 52, 3349 (1930); Freure and Johonson, *ibid.*, 53, 1142 (1931).

these types were treated with *p*-toluenesulfinic acid (p-$CH_3C_6H_4SO_2H$), *p*-thiocresol (p-$CH_3C_6H_4SH$) and *p*-thiocresylmagnesium iodide (p-$CH_3C_6H_4SMgI$). These reagents are indicated for such a purpose, having been used effectively for related conjugated systems. If *p*-thiocresol added to such a system one might expect the following transformations, where R is (H) or (C_6H_5).



The net result would be, as with such systems, addition to the (2,3)-linkage. However, the very high recovery of reactants proves that either no such addition occurred or if it did take place it was followed by 1,4-elimination to give the original compounds. The latter possibility is somewhat remote in view of earlier studies with related conjugated systems. Accordingly, it is reasonable to conclude that the addition reactions which precede nuclear substitution of such furan types do not proceed through the conjugated system (3, 2, 6, 7) and are very probably confined to the (2, 3, 4, 5) conjugated system. Likewise, one can conclude that there is no 1,6-addition to the system (5, 4, 3, 2, 6, 7). This type of 1,6-addition would be necessary, rearrangements excluded, to account for the fact that nuclear substituents are almost invariably found on the *alpha* or 2- or 5-carbon atoms if either *alpha*-position is available, and that direct *beta* or 3- or 4-carbon atom substitution practically never occurs. It should be emphasized here that the related phenyl compounds also do not undergo such addition reactions to a nuclear-lateral conjugated system. However, addition of this kind has been noted in the reaction between highly phenylated compounds and the Grignard reagent[3].

In order to establish with some certainty that the reagents employed for the above-mentioned purpose are reliable for furan compounds having a conjugated system which is entirely lateral and not a mixed nuclear-lateral conjugated system (as with furfural), the same reactions were carried out with compounds like furfuralacetophenone:

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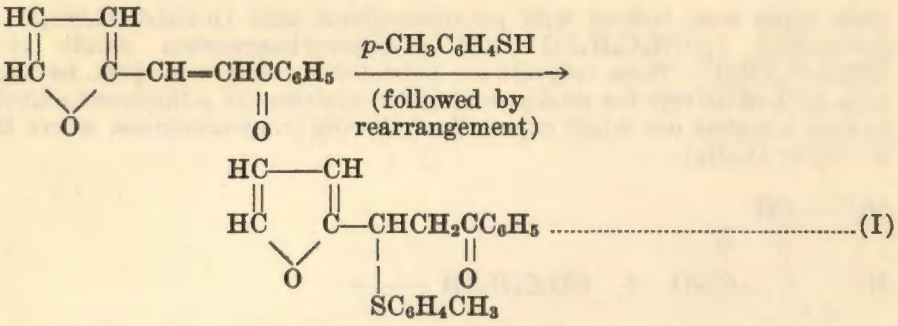
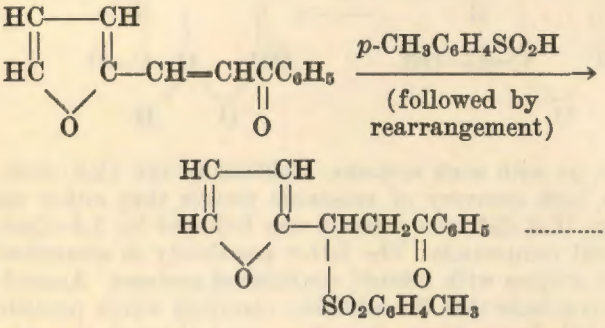
[3]Gilman, Kirby and Kinney, *J. Am. Chem. Soc.*, 51, 2252 (1929); Kohler and Nygaard, *ibid.*, 52, 4128 (1930).



(I)

The lateral conjugated system (—CH=CH—C—C_6H_5) did undergo reaction. Also, reaction took place with *p*-toluenesulfinic acid:



(II)

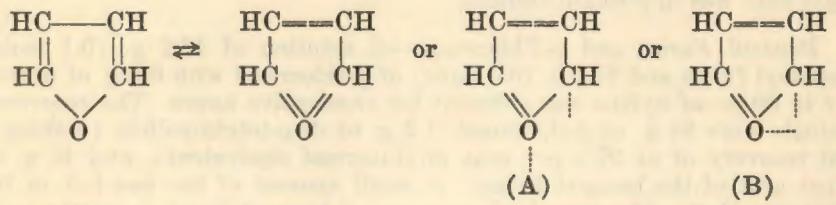
Similar reactions have been studied by numerous investigators[4] using benzene but not furan types.

These studies on lateral conjugated furan types revealed some interesting differences from the related phenyl types, and so make it necessary to proceed with caution in drawing too broad generalizations between such aryl types. First, in contrast with the ready addition of *p*-thiocresylmagnesium iodide to benzalacetophenone[4d], negative results were obtained with furfuralacetophenone. Only extensive refluxing with furfurlacetophenone gave a small quantity of the addition compound, and there is always the possibility that this very limited reaction took place with *p*-thiocresol formed by the hydrolysis of a small quantity of *p*-thiocresylmagnesium iodide, despite precautions observed to exclude moisture. Second, no condensation was effected with *p*-thiocresol and furylacrylic acid, although such condensation has been noted with the related cinnamic acid[4c]. Third, *p*-toluenesulfinic acid did not react with furylacrylic acid, although reaction does take place with cinnamic acid[4a]. In this connection it is interesting to note that when the reaction was carried out in ethyl alcohol, esterification took place and gave a satisfactory yield of ethyl furylacrylate. Finally, the accepted methods for oxidizing the sulfide probably obtained

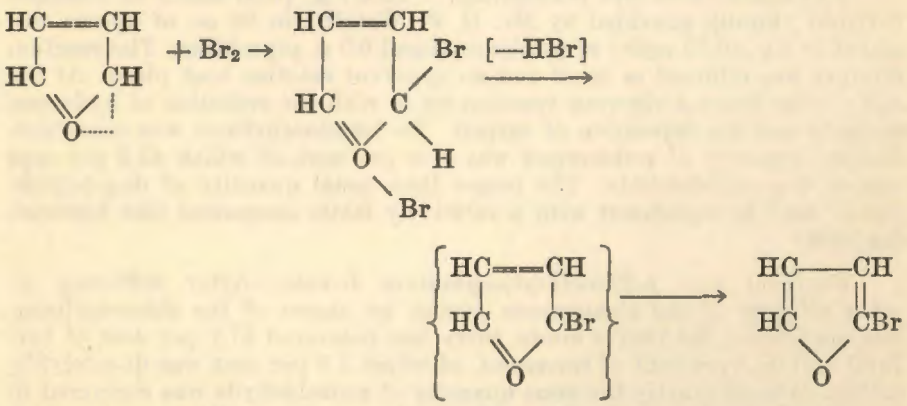
[4](a) Kohler and Reimer, *Am. Chem. J.*, **31**, 163 (1904); (b) Ruhemann, *J. Chem. Soc.*, **87**, 17, 461 (1905); (c) Arndt, Flemming, Scholz and Löwensohn, *Ber.*, **56**, 1269 (1923); (d) Gilman and King, *J. Am. Chem. Soc.*, **47**, 1136 (1925).

in Reaction (I) to the sulfone obtained in Reaction (II) were not successful. This difficulty is not due entirely to any instability of the furan nucleus to oxidizing agents as is attested by the high recovery of sulfide in some oxidations. In this connection, attention should be directed to the ready polymerization of furfuryl mercaptan[5].

In connection with preliminary addition to the nuclear oxygen and the ease of *alpha*-substitution, there is a possibility that the analogies between furan and other aromatic types like benzene can be extended to include the phenomenon of oscillating linkages in the related nuclei. We might, for example, have the following in a system like that proposed by Kekulé in his revised formula for benzene:



With Formulas (A) and (B) the active parts would be the oxygen and an *alpha*-carbon atom. In such a representation, with a dynamic system, bromination (as a type) may take place in the following manner:



The bracketed hypothetical bromofuran need not have trivalent oxygen and trivalent carbon, but may have a structure like that represented in Formula (B) which then undergoes a re-allocation of linkages to give the ultimate product. It may be objected that an interpretation of this kind might warrant the expectation of addition to other unsaturated parts of the nucleus, like the ethylenic linkage between carbon atoms 3 and 4. The same objection is patent in the interpretation of preliminary addition in substitution reactions of benzene. Possibly it is just such a labilized system which is necessary to account for the uncommon tendency of furan types to form tetrahalogen *addition* compounds.

[5]Gilman and Hewlett, *Iowa State Coll. J. of Sci.*, 5, 19 (1930). See, also, Bost, Turner and Norton, *J. Am. Chem. Soc.*, 54, 1985 (1932), who could not successfully oxidize a furyl sulfide.

EXPERIMENTAL PART

All reactions were carried out in a three-necked flask of suitable size, equipped with a mercury sealed stirrer, separatory funnel and condenser. A trap[6] was used to reduce atmospheric contamination.

Furfural and p-Thiocresol.—A number of experiments were carried out in both benzene and toluene and for varying periods of refluxing up to twenty-four hours. The ratio of *p*-thiocresol to furfural was varied up to 2:1. With 0.1 mole of furfural, about 0.4 g. of piperidine was used as a catalyst. The furfural recovered was as high as 67 per cent. The *p*-thiocresol recovered was as high as 95.6 per cent (in which experiment about 10 per cent was di-*p*-tolyldisulfide).

Benzoyl Furan and p-Thiocresol.—A solution of 17.2 g. (0.1 mole) of benzoyl furan and 37.2 g. (0.3 mole) of *p*-thiocresol with 0.5 g. of piperidine in 60 cc. of xylene was refluxed for twenty-five hours. The recovered products were 35 g. of *p*-thiocresol, 1.2 g. of di-*p*-tolyldisulfide (making a total recovery of of 97.3 per cent of thiocresol equivalent), and 16 g. or 93 per cent of the benzoyl furan. A small amount of tar was left in the distillation flask. The very high recovery of benzoyl furan is particularly significant from the point of view of the thermal stability of some furan compounds.

5-Bromofurfural and p-Thiocresol.—To 8.7 g. (0.05 mole) of 5-bromofurfural (kindly provided by Mr. G. F. Wright) in 50 cc. of xylene was added 18.5 g. (0.15 mole) of *p*-thiocresol and 0.5 g. piperidine. The reaction mixture was refluxed as usual and no apparent reaction took place. At the end of four hours a vigorous reaction set in with the evolution of hydrogen bromide and the deposition of carbon. No 5-bromofurfural was recovered. But the recovery of *p*-thiocresol was 91.8 per cent, of which 43.2 per cent was as di-*p*-tolyldisulfide. The larger than usual quantity of di-*p*-tolyldisulfide may be significant with a relatively labile compound like 5-bromofurfural.

Furfural and p-Thiocresylmagnesium Iodide.—After refluxing an ether solution of the components (using an excess of the thiocresylmagmesium iodide) for twelve hours, there was recovered 67.7 per cent of furfural and 95.3 per cent of thiocresol, of which 1.3 per cent was di-*p*-tolyldisulfide. Almost exactly the same quantity of benzaldehyde was recovered in an earlier related study[4d] with *p*-thiocresylmagnesium iodide.

When refluxing was carried out in an ether-xylene solution at 66-71° for twenty hours the tarry mixture yielded no furfural, and 90 per cent of *p*-thiocresol, of which 13.3 per cent was di-*p*-tolyldisulfide.

Furfural and Benzoyl Furan with p-Toluenesulfinic Acid.—The furfural in benzene at 60-65° was completely decomposed and di-*p*-tolyldisulfoxide, $CH_3C_6H_4SO_2SC_6H_4CH_3$, was recovered. This disulfoxide undoubtedly arises from the sulfinic acid. The sulfinic acid under milder conditions probably adds to furfural as it does to benzaldehyde[4a], but this addition compound was not isolated and analyzed.

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[6]Gilman and Hewlett, *Rec. trav. chim.*, **48**, 1124 (1929).

Benzoyl furan under corresponding conditions is much more resistant to the action of *p*-toluenesulfinic acid. After refluxing an ether-xylene solution of 15 g. (0.087 mole) of benzoyl furan and 27.1 g. (0.174 mole) of *p*-toluenesulfinic acid at 80° for twenty-four hours, 90 per cent of the benzoyl furan was recovered in addition to di-*p*-tolyldisulfoxide.

Furfurlacetophenone and p-Thiocresol (see Reaction (I)).—A vigorous reaction took place between 19.8 g. 0.1 mole) of furfuralacetophenone, 12.4 g. (0.1 mole) of *p*-thiocresol and 0.5 g. of piperidine in 50 g. of benzene. After standing for twenty-four hours the mixture was worked up to give a 90 per cent yield of 1-benzoyl-2-furyl-2-*p*-thiocresylethane (see reaction (I), which when crystallized from a mixture of 90 per cent petroleum ether (b.p. 40-60°) and 10 per cent benzene melted at 78.5°. This stable, yellow crystalline compound is soluble in chloroform, carbon tetrachloride and hot alcohol.

Anal. Calcd. for $C_{20}H_{18}O_2S$: S, 9.96. Found: S, 10.6, 10.11.

Several unsuccessful attempts were made to oxidize this sulfide to the corresponding sulfone. With 30 per cent hydrogen peroxide in acetic acid, tars were formed which so far have resisted crystallization. With chromic oxide in acetic acid at 40° about 70 per cent of the sulfide was recovered; and at 100°, decomposition apparently set in. The same phenomena were observed with sodium dichromate in sulfuric acid. With potassium permanganate and acetic acid at moderate temperatures about 50 per cent of the sulfide was recovered.

Furfuralacetophenone and p-Toluenesulfinic Acid (see Reaction (II)).—The reaction mixture of 19.8 g. (0.1 mole) of furfuralacetophenone and 15.6 g. (0.1 mole) of *p*-toluenesulfinic acid in 75 cc. of alcohol, filled with needle crystals after twenty-four hours. After standing for four days it was worked up to yield 13.4 g. or 35 per cent of the sulfone: namely, 1-benzoyl-2-furyl-2-*p*-tolylsulfonylethane (see Reaction (II)). On crystallization from a mixture of 80 per cent benzene and 20 per cent petroleum ether it melted at 141°.

Anal. Calcd. for $C_{20}H_{18}O_4S$: S, 9.06. Found: S, 9.26, 9.20.

Furfuralacetophenone and p-Thiocresylmagnesium Iodide.—The general conditions were those used in the related study with benzalacetophenone[4d]. Despite the fact that some sort of reaction occurred, as evidenced by refluxing of the ether and the separation of a red layer, 90 per cent of the thiocresol was recovered. In other experiments using two equivalents of *p*-thiocresylmagnesium iodide and heating on a water bath for one or two days, 92 per cent *p*-thiocresol was recovered. The tar and red gum, probably condensation products of the furfuralacetophenone, have as yet resisted crystallization.

From one experiment with 4 equivalents of *p*-thiocresylmagnesium iodide and refluxing on a water bath for four days, there was recovered 90 per cent of *p*-thiocresol. The oily residue, after several fractional precipitations with petroleum ether (b.p., 40-60°) and benzene yielded a small quantity of the 1-benzoyl-2-furyl-2-*p*-thiocresylethane (see Reaction (I)). It was identified by a mixed melting point determination.

Furylacrylic Acid and p-Thiocresol.—The general procedure was that of Arndt[4c]. From the reaction mixture there has been isolated so far some unaltered furylacrylic acid, *p*-thiocresol and an oil which is probably *p*-thiocresyl acetate, $CH_3C_6H_4SCOCH_3$, for it yields *p*-thiocresol on hydrolysis. It owes its formation to acetylation of *p*-thiocresol by the acetic acid and acetic anhydride used as the medium.

Ethyl Furylacrylate and p-Thiocresol.—Equimolecular portions of the reactants in benzene, with piperidine as a catalyst, were allowed to stand for a week. A practically quantitative yield of initial materials was obtained. Similar results were noted in a check experiment carried out at 50-60° The authors are grateful to Mr. G. F. Wright for the ester.

Furylacrylic Acid and p-Toluenesulfinic Acid.—The general procedure followed was that of Kohler and Reimer[4a]. From a first experiment carried out in water, there was a practically quantitative recovery of furylacrylic acid.

From a second experiment with 0.025 mole of reactants in 40 cc. of alcohol, refluxed for fifteen hours, there was obtained 48 per cent of ethyl furylacrylate.

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The authors are grateful to Mr. W. F. Schulz for a helpful suggestion.

SUMMARY

In connection with mechanisms concerned with nuclear substitution reactions of furan types, a study has been made of conjugated systems which are undoubtedly involved in the addition reactions which precede substitution. The precursory reaction of 1,4-addition to a conjugated system is probably the formation of an oxonium compound by addition to the oxygen which because of a crossed conjugated system or because of oscillating double bonds has an exalted activity. The conjugated system involved in preliminary addition is probably that comprising the two *alpha*-carbon atoms and not a nuclear-lateral system. Completely lateral conjugated systems participate in some characteristic 1,4-additions. However, the exceptions from the related benzene compounds are sufficiently marked to emphasize caution in drawing broad generalizations between the phenyl and furyl series.

THE STABILIZING EFFECT OF NUCLEAR NITRO GROUPS IN FURAN TYPES

5-NITRO-2-FURFURYL CHLORIDE AND 5-NITRO-2-FURFURYL METHYL ETHER

HENRY GILMAN AND ROBERT R. BURTNER

From the Chemical Laboratory of Iowa State College

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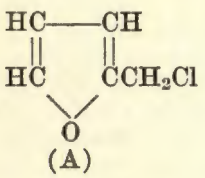
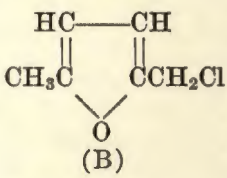
INTRODUCTION

Current studies on nuclear substitution reactions of furan demonstrate in a striking manner the effect of various substituents on the stability of the furan nucleus. In general, some of these effects might have been predicted from other aromatic types. However, the effect appears to be exaggerated with furan types. For example, no simple aminofuran has as yet been prepared, and the simple *alpha-* or 2-monohalides like chloro-, bromo- and iodo-furan are decidedly less stable than the corresponding analogues in the benzene series. The stability of such types is increased by the introduction of a negative group like carboxyl so that the corresponding amino- (or acetamino) and halogen-furoic acids are of a relatively high order of stability. Such stability is further increased by the introduction of a nitro group as in 4-nitro-5-amino-2-furoate[1].

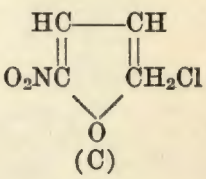
Furfuryl chloride (A) is unstable[2]. 5-Methyl-2-furfuryl chloride (B) has recently[3] been shown to be much less stable than furfuryl chloride. However, 5-nitro-2-furfuryl chloride (C) is relatively highly stable.



(A)



(B)



(C)

The 5-nitro-2-furfuryl chloride has been synthesized by two different methods. The first of these methods, nitration of furfuryl chloride, is somewhat unusual because of the apparent instability of furfuryl chloride to acids. However, a distinction must be made between halogen acids and other so-called mineral acids; also, it is possible to prepare furfuryl chloride from furfuryl alcohol and hydrogen chloride if due precautions are taken to exclude moisture[2].

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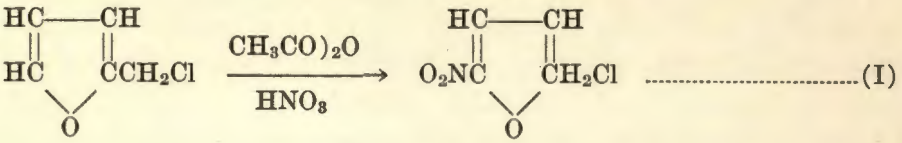
[1]Studies by G. F. Wright indicate that the effect of the negative groups on the amino group in this molecule is such as to endow the amino group with very slightly basic or even acidic properties. Solubility in alkali is very probably due to a transformation like that observed with *o-* and *p*-nitroanilines and derivatives.

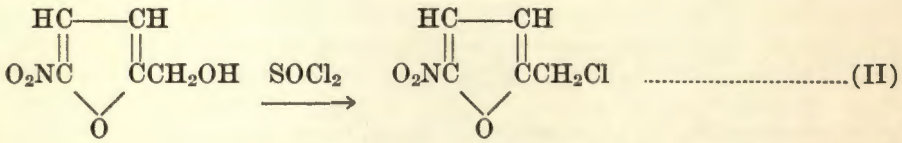
[2]v. Braun and Köhler, *Ber.*, **51**, 87 (1918); Gilman and Vernon, *J. Am. Chem. Soc.*, **46**, 2576 (1924); Kirner, *ibid.*, **50**, 1955 (1928).

[3]Reichstein and Zschokke, *Helv. Chim. Acta.*, **15**, 249 (1932).



(I)

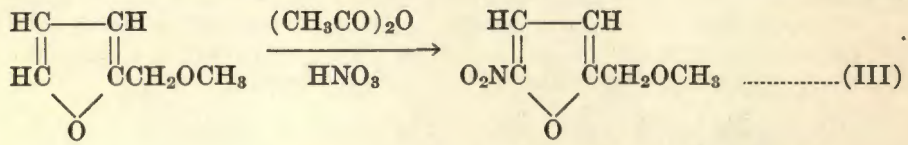
The second method is the action of thionyl chloride on the recently accessible 5-nitro-2-furfuryl alcohol[4].



(II)

The 5-nitro-2-furfuryl chloride was characterized by conversion, by means of silver acetate, to 5-nitro-2-furfuryl acetate[4]. Physiological tests on 5-nitro-2-furfuryl chloride show that the lethal concentration for mice on ten minutes exposure is greater than 7 mg. per liter. Particular care should be exercised in working with 5-nitro-2-furfuryl chloride because of its vesicant action, which is about 1/150 that of mustard gas.

Another illustration of the stabilizing effect of the nitro group is found in 5-nitro-2-furfuryl methyl ether which was synthesized by the nitration of furfuryl methyl ether.



(III)

Although furfuryl methyl ether is relatively unstable unless special precautions are observed, the 5-nitro-2-furfuryl methyl ether not only is highly stable without the use of solvents or diluents, but is also resistant to the action of acids. This is strikingly illustrated by experiments with hydriodic acid under conditions for the splitting of ethers. Not only is the heterocylic ether unit rendered stable to the action of hydriodic acid, but the methoxyl group also appears to be relatively unaffected by this reagent, which is commonly employed in the Zeisel analysis for the splitting of methyl ethers. This unusual stability of a lateral function, induced in large part by the nuclear nitro group, has been observed in another study by Mr. R. V. Young on 5-nitro-2-furfuralmalonic ester wherein the ester groups are uncommonly resistant to hydrolysis.

The nitration of furfuryl chloride and furfurl methyl ether to give an *alpha-* or 5-nitro substituent is additional evidence that with furan types the tendency to substitute in an *alpha*-position is essentially independent of the group already present. That is, substituents which in the benzene series orient either *ortho* and *para* or *meta,* orient in the furan series to an *alpha* position, if such be available.

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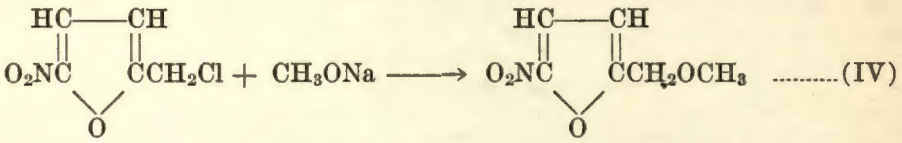
[4]Gilman and Wright, *J. Am. Chem. Soc.*, 53, 1923 (1931).

The position of the nitro group in nitrofurfuryl methyl ether was established by converting 5-nitrofurfuryl chloride to the nitro-furfuryl methyl ether obtained by nitration of furfuryl methyl ether:



This reaction (IV), carried out under anhydrous conditions, is of more than passing interest because of the great sensitivity of *alpha*-nitro compounds to alkali. The stability of 5-nitrofurfuryl methyl ether to hydrogen iodide was ascertained incidental to some attempts to hydrolyze this ether to the known 5-nitrofurfuryl alcohol.

EXPERIMENTAL PART

5Nitro-2-Furfuryl Chloride.—This compound was prepared by two different methods.

First, 50.0 g. (0.43 mole) of furfuryl chloride dissolved in 120 cc. of acetic anhydride was added dropwise with stirring at —25° to the nitrating mixture prepared by the dropwise addition of 130.0 g. (2.0 moles) of fuming nitric acid to 360 cc. of acetic anhydride at —5°. Following the addition, the mixture was allowed to stir for an additional hour at the indicated temperature and then poured into 1.0 kg. of cracked ice. Neutralization was effected by adding 50 per cent sodium hydroxide until the mixture was faintly acid to methyl orange; then neutralization was completed with a suspension of sodium bicarbonate in water. The mixture was then extracted with ether three times and 100 g. of pyridine added to the ethereal extract. After standing at room temperature for twenty-four hours the ether was distilled off, and approximately three-quarters of the pyridine was removed by distillation under reduced pressure. The residual material was then acidified with dilute hydrochloric acid and extracted with ether. The ethereal extract was dried over anhydrous sodium sulfate and the solvent removed by distillation, fractionating the residue under reduced pressure to obtain 15.0 g. or a 25 per cent yield of a light yellow oil boiling at 122-124°/6 mm.; sp. g. $_{20}^{20}$ 1.429; n_D^{20} 1.5688.

Anal. Calcd. for $C_5H_4O_3NCl$: Cl, 21.93. Found: Cl, 21.59.

Second, 59.0 (0.5 mole) of thionyl chloride was added dropwise with stirring at 0° to 71.5 g. (0.5 mole) of 5-nitro-2-furfuryl alcohol[4]. Following the addition of the thionyl chloride the mixture was allowed to stir for two hours longer at 0°, at the end of which period the temperature was slowly raised to 60° and maintained at this point for four hours. The resulting solution was then washed several times with water, taken up with 75 cc. of ether and dried over anhydrous sodium sulfate. The solvent was distilled off and the residue fractionated under reduced pressure, collecting the fraction boiling at 125-129°/7 mm. The yield was 34.0 g. or 41.6 per cent. A small amount of the nitro alcohol was recovered unchanged, showing that the reaction mixture should have been refluxed longer with a slight excess of thionyl chloride. Without additional refluxing, but with a 50 per cent excess of thionyl chloride the yield was 50.5 per cent.

Despite the superior yields of 5-nitro-2-furfuryl chloride by the action of thionyl chloride on 5-nitrofurfuryl alcohol, the method involving direct nitration of furfuryl chloride has its merits. First, it appears to be less time consuming. Second, the over-all yield starting with furfuryl alcohol is about the same in both processes. Third, the distillation of 5-nitrofurfuryl alcohol may, at times, be troublesome due to decompositions in varying degrees of violence. Over and against such advantages, it should also be observed that occasionally the purification of furfuryl chloride is attended with deep-seated decompositions or condensations, as is sometimes observed with benzyl chloride.

5-Nitro-2-Furfuryl Acetate.—5.0 g. (0.03 mole) of nitrofurfuryl chloride and 7.6 g. (0.045 mole) of silver acetate were placed in a small three-necked flask with 75 cc. of absolute ether and refluxed with stirring for a period of four hours. The solution was then filtered and the ether removed under reduced pressure, leaving a small crop of yellow crystals. This material was pressed dry on a porous plate and was found to melt at 45°. A mixed melting point with an authentic sample of 5-nitro-2-furfuryl acetate showed no depression.

5-Nitro-2-Furfuryl Methyl Ether.—Fifty-six g. (0.05 mole) of furfuryl methyl ether[5] dissolved in 100 cc. of acetic anhydride was added dropwise with stirring at —15° to the nitrating mixture prepared by dropwise addition of 85.0 g. (1.3 moles) of fuming nitric acid to 287 cc. of acetic anhydride at —5°. Following the addition, the reactants were allowed to stir for an additional hour in the freezing mixture and then poured into 2 kg. of cracked ice. The mixture was treated with 50 per cent sodium hydroxide until the reaction is faintly acid to methyl orange and the neutralization then completed with a suspension of sodium bicarbonate in water. The resulting solution was extracted three times with ether and 125 cc. of pyridine was added to the ethereal extract. After standing for twenty-four hours at room temperature the ether was distilled off and approximately three-quarters of the pyridine removed by fractionation under reduced pressure. The remainder of the pyridine was then removed by acidification with dilute hydrochloric acid and the resulting solution extracted with ether. The ethereal extract was dried over anhydrous sodium sulfate and the ether distilled off. The residual material was then fractionated under reduced pressure collecting the light yellow oil boiling at 114-117°/4 mm.; sp. g. $_{20}^{20}$ 1.283; n_D^{20} 1.5343. The yield was 42.6 g. or 54.2 per cent.

Anal. Calcd. for $C_6H_7O_4N$: N, 8.91. Found: N, 9.23.

Attempted Splitting of 5-Nitro-2-Furfuryl Methyl Ether.—15.7 g. (0.1 mole) of the nitro furfuryl methyl ether was heated at 60° under a reflux condenser with 33.6 g. hydriodic acid (sp. g. 1.68) containing a small amount of red phosphorus, for a period of one and one-half hours. The mixture was then poured into 400 cc. of ice water and neutralized with

[5]Pummerer and Gump, *Ber.*, **56**, 999 (1923).

sodium bicarbonate solution. The reaction mixture was extracted with ether, washed with a solution of sodium thiosulfate, and dried over anhydrous sodium sulfate. The ether was then distilled off and the residual material fractionated under reduced pressure, collecting 11.5 g. of the nitro furfuryl methyl ether unchanged.

A second attempt at splitting of the ether was made using the same amounts of materials but refluxing the mixture at 135° for two hours. Ten g. of the ether was recovered. Part of the remainder of the ether was probably lost by decomposition during the final distillation.

5-Nitrofurfuryl Chloride and Sodium Methylate.—A cooled solution of sodium methylate prepared from 2.3 g. (0.1 atom) of sodium and 32 g. of absolute methyl alcohol was added dropwise and with vigorous stirring to a solution of 16.1 g. (0.1 mole) of 5-nitrofurfuryl chloride in 16 cc. of absolute methyl alcohol contained in a three-necked flask immersed in an ice bath. The rate of addition was such that there was practically no rise in temperature.

Subsequent to the addition of the 5-nitrofurfuryl chloride, the dark brown reaction mixture was stirred for ten minutes at room temperature. It was then poured upon about 200 g. of cracked ice and immediately acidified with 10 per cent sulfuric acid. The mixture was extracted with ether; the extract dried over sodium sulfate; the ether removed by distillation; and the residue fractionated under reduced pressure. The small quantity of distillate was shown to be 5-nitrofurfuryl methyl ether by its boiling point and refractive index.

Oxidation of 5-Nitrofurfuryl Alcohol.—In establishing the constitution of 5-nitrofurfuryl alcohol[4] it was oxidized to the known 5-nitrofurfural. Subsequent to that study, Mr. G. F. Wright showed that the nitro alcohol could be oxidized, as might have been expected, to 5-nitro-2-furoic acid. Fourteen and three-tenths g. (0.1 mole) of nitrofurfuryl alcohol, 20 g. of manganese dioxide and 50 cc. of 50 per cent sulfuric acid were mixed in a 125 cc. flask and allowed to stand for forty-eight hours at 40-45°. At the end of this time 5 g. more of manganese dioxide was added and the reaction continued for twelve hours longer. It was then cooled, extracted three times with ether and the ether extract washed three times with saturated sodium bicarbonate solution. This bicarbonate solution was then acidified with hydrochloric acid and the solution was extracted with ether. Evaporation of the ether left 1.1 g. of 5-nitrofuroic acid melting at 168°. Crystallization of this acid from water raised the melting point to 182°. A mixed melting point with authentic 5-nitrofuroic acid showed no depression. The yield of crude acid was 7 per cent.

The original ether extract after washing with sodium bicarbonate was dried with sodium sulfate and distilled under reduced pressure. The fraction boiling at 125-130°/4-5 mm. was found to be 2.9 g. of 5-nitrofurfural as proved by conversion to the oxime and a mixed melting point with 5-nitrofurfural. The yield was 21 per cent.

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The authors are grateful to Mr. G. F. Wright and Mr. W. H. Zugschwerdt for assistance.

SUMMARY

The nitration of furfuryl chloride and of furfuryl methyl ether introduces a nitro group in the 5-position. The resulting compounds are unusually stable. For example, wheras furfuryl chloride is unstable and 5-methyl-2-furfuryl chloride is much more unstable, 5-nitro-2-furfuryl chloride is of a relatively high order of stability. The high stability of 5-nitro-2-furfuryl methyl ether is strikingly illustrated by its resistance to the action of hydrogen iodide, both the nuclear and the lateral ether linkages remaining essentially unaltered.

5-Nitro-2-furfuryl chloride was also prepared by the action of thionyl chloride on 5-nitro-2-furfuryl alcohol. Oxidation of 5-nitro-2-furfuryl alcohol gives a mixture of the corresponding nitro-aldehyde and nitro-acid.

5-Nitro-2-furfuryl chloride has a vesicant action.

THE SULFUR AND NITROGEN OF WOOL

ELEANOR WINTON AND RACHEL EDGAR[1]

From the Department of Chemistry, Iowa State College

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Although Chevreul (51) in 1806 found less sulfur in black hair than in red or white hair, no work was reported comparing the total sulfur of different kinds of wool until 1867, when Reich and Ulbricht (35) analyzed eight fleeces for sulfur and raised the question of the dependence of the sulfur content upon such factors as the age, breed, sex and portion of the fleece of the sheep from which the wool was obtained.

Trotman and Bell (46, 48) analyzed three wools, found the variation in sulfur slight, and attributed the lack of agreement among the analyses of other investigators to faulty methods of analysis, the probability that the labile sulfur is not entirely a constituent of the protein molecule, and the presence in wool of two or more proteins of different sulfur contents. Marston's work (31) with Australian wools indicated very slight variation in the proportions of sulfur and of nitrogen. Others reported variation in the proportions of sulfur and of nitrogen in wool from different sheep and related these differences to the color, diameter and portion of the fiber, and to the diet of the sheep.

Colored wools have been shown of lower nitrogen content (17, 3). According to Barritt and King (4, 7) and others (2, 3, 1, 44, 24, 8) fine wools contain a higher percentage of sulfur than coarser medullated wools. Yet Bonsma's work (11) with South African wools, the only investigation reported with sheep in a controlled environment, indicates no such relation; Bonsma concluded that the sulfur content of wool was chiefly an individual characteristic, but that nutrition was an important factor in the incorporation of sulfur into the wool fiber. The analysis of the tips and roots of wool fibers has shown that the distribution of sulfur along the length of the fibre is not always uniform. Barritt and King (5) found the tips contained more sulfur than the portions nearer the root and attributed this lack of uniformity to the increased incorporation of cystine after shearing. Such an increase would need to be great enough to overcome the loss of sulfur reported in the oxidation of the tips in light (52, 33, 25). Bonsma found no significant variation in the sulfur of different portions of wool fibers from sheep fed a controlled diet.

Less extensive investigation has been made of the nitrogen than of the sulfur of wools. Total sulfur has been suggested and the determination of nitrogen has been used (39) as a basis for the proximate analysis of the wool of mixtures containing no other source of these elements. The quantitative determinations of sulfur and nitrogen reported in this article were made to obtain more data as to the accuracy of these proximate analyses

[1]The authors wish to thank those who gave the wools and Mrs. Vera Berg who developed the method for cleaning the wool.

for wool. Table 1 is a résumé of values reported in the literature for the total sulfur and nitrogen of wools.

TABLE 1. *Values reported for the total sulfur and nitrogen of wools*

Year	Investigator	Sulfur Percentage	Nitrogen Percentage	Description of Wool
1840	Chevreul (13)	1.78, 2.20		
1841	Scherer (40)	2.66	17.71	
1856	Von Bibra (53)	0.81, 0.92		mixed
1863	Grothe (18)	1.9, 2.0, 2.1		carded
		2.4, 2.5, 2.7		carded
		2.0, 2.3, 2.4		English
		2.4, 2.5, 2.5		English
		3.0, 3.2, 3.4		Hardschnucke
		1.6, 1.8, 1.8		worsted
1867	Reich and Ulbricht (35)	3.37		Lincoln
		2.85		Lincoln Merino yearling
		3.41		Rambouillet ewe
		3.55		Rambouillet Elektoral
				Negretti yearling
1869	Märcker and Schulze (30)	3.41	16.01	Landschafen
		3.57	16.08	Landschafen
		3.66	15.86	Landschafen
		3.73	15.55	Landschafen
		3.43	15.73	Rambouillet
		3.69	15.54	Rambouillet
1870	Henneberg (20)	3.50, 3.62	16.01	wethers
1878	Schützenberger (41)	3.1	17.7	
1882	Bleunard (10)	3.01	12.63	
			12.90	Australian wool
1885	Bowman (12)	3.0	19.1	Irish
		2.5	18.1	Lincoln
		2.3	18.5	Northumberland
		3.8	17.8	Southdown
	Mulder (12)	5.4	16.8	
	Hummel (22)	3.66	15.86	German
1889	Knecht and Appleyard (26)	3.34, 3.35		
1895	Mohr (34)	3.68		
1901	Washburn (55)	3.42		
1909	Ruszkowski and Schmidt (39)		14.23	washed fabric
			13.96	washed yarn
1911	Strunk and Priess (43)	3.56	15.14	
1913	———— (18)		14.00	flannel
	Gortner (17)		15.11	black
			16.27	white
1920	Waentig (54)		16.37	
1922	Trotman (47)	3.74	16.01	yarn
1923	Meunier and Latreille (32)	3.0		

TABLE I—Continued

Year	Investigator	Sulfur Percentage	Nitrogen Percentage	Description of Wool
1924	Herzog and Krahn (21)	3.46	16.60	
	Trotman (45)		15.70	
			15.96	
			16.94	
1926	Barritt and King (4)	3.76		Australian Merino 100's
		3.33		Blackface (coarse)
		3.82		Blackface (fine)
		3.24		Blackface (kempy)
		4.00		Cape Merino (Kaffrarian)
		3.94		Cape Merino (LeGrange)
		3.10		Lincoln (white)
		3.26		Lincoln (yellow)
		3.73		Merino lamb
		3.75		Peruvian (1924)
		3.82		Peruvian (1925)
		3.34		Ripon fleece (fine)
		3.76		Romney (Monte Video)
		3.97		Welsh mountain P64
		3.79		Welsh mountain S71
	Farrar and King (15)	3.92		
	Trotman and Bell (46)	2.9, 3.2, 3.3		Blackface
		3.0, 3.2, 3.3		Lester hog
		3.0, 3.3, 3.4		Lester wether
1927	Barritt (2)	3.35		crossbred
		3.64		Merino
	Küster, Kumpf and Köppel (28)		15.94	
1928	Barritt (3)		16.57	Australian Merino 60's C22
			16.63	Australian Merino 64's A6
			16.63	Australian Merino 58's A3
			16.74	Australian Merino 60's E67
			16.76	Australian Merino 64's F87
			16.78	Australian Merino 60's A4
			16.79	Australian Merino 60'c F86
			16.82	Australian Merino 64's F85
			16.86	Australian Merino 60's E64
			17.01	Australian Merino 64's E76
			16.69	Australian Merino lamb No. 2
			16.73	Australian Merino lamb No. 1
			16.71	Devon lamb
			16.16	Jacob black
			16.52	Jacob white
			16.61	La Concordia Lincoln
			16.80	Lincoln (white)
			16.62	Lincoln (yellow)
			16.71	Mazemet
			17.07	Monte Video
			16.62	New Zealand matching 50's
			16.71	New Zealand matching 50's
			16.50	Peruvian merino
			16.72	Ripon fleece (fine)
			16.63	Scotch blackface (fine)
			16.74	Scooth blackface (medium)
			16.54	Welsh mountain S10
			16.60	Welsh mountain P5
			16.68	Welsh mountain S71

TABLE I—Continued

Year	Investigator	Sulfur Percentage	Nitrogen Percentage	Description of Wool
	Marston (31)	3.523	17.9	Leicester
		3.521	17.6	Lincoln
		3.546	17.7	Merino
		3.560	17.8	Merino
		3.562	17.9	Merino
		3.570	17.9	Merino
		3.581	17.7	Merino
		3.585	17.8	Merino
		3.585	18.0	Merino
		3.580	17.9	Merino lamb
		3.535	17.8	Polwarth
		3.541	17.8	Polwarth
		3.530	17.8	Shropshire
	Trotman, Trotman and Brown (49,50)		15.78	crossbred
			15.88	
			15.90	
			16.01	
			16.16	Botany
1929	Barritt and King (5)	3.66		Albury tip 56's
		3.40		Albury root 56's
		3.72		Albury II
		3.08		Albury hogs, tip 58's
		3.00		Albury hogs, root 58's
		3.84		Australian Barratta, New South Wales 60's
		3.73		Australian Barratta, New South Wales 60's
		3.74		Australian Barratta, New South Wales 64's
		3.56		Australian Dalkeith stud ram, New Souht Wales 64's
		3.48		Australian Dalkeith stud ram, New South Wales 60's
		3.53		Australian Dalkeith 6-tooth ewe 64's
		3.97		Australian Meribee Co. Ltd. stud ram, New South Wales 60's
		3.74		Australian Meribee Co. Ltd. stud ram, New South Wales 60's
		3.69		Australian Millbrae stud 6-tooth ewe, S. Australian 58's
		3.83		Australian Millbrae stud 6-tooth ewe S. Australian 60's
		3.90		Australian Millbrae aged ewe 56's
		3.88		Australian, Mt .Crawford estate, 3 year ram, 58's
		3.91		Australian, Mt. Crawfrod estate, 3 year ewe, 60's
		3.46		Australian, Mt. Crawford estate, 2½ year ewe, 64's
		3.82		Australian, Triangle Exp. Farm stud ewe, 4 years, 64's
		3.99		Australian, Trangie Exp. Farm wether, 4 years, 70's

TABLE I—Continued

Year	Investigator	Sulfur Percentage	Nitrogen Percentage	Description of Wool
1929	Barritt and King (5)	3.93		Australian, Trangie Exp. Farm stud ram, 2 years, 60's
		3.46		Australian, Trangie Exp. Farm stud ram, 5 years, 64's
		4.11		Australian, Trangie Exp. Farm stud ram, 5 years, 60's
		3.77		Australian, Trangie Exp. Farm stud ram, 4 years, 60's
		3.41		Australian Merino tip, E66, 64's
		3.27		Australian Merino root
		3.62		Australian Merino tip E68, 58's
		3.39		Australian Merino root
		3.68		Badger face, white
		3.93		Badger face, white
		3.47		Badger face, brownish black
		3.67		Cape Merino
		3.60		Jacob's flock A, white
		4.05		Jacob's flock B, white
		3.47		Jacob's flock A, black
		3.74		Jacob's flock B, black
		4.01		La Concordia Lincoln
		3.65		Lincoln tip
		3.58		Lincoln II
		3.53		Lincoln III
		3.59		Lincoln root
		3.86		Mazemet
		3.22		New Zealand Romney Corriedale
		3.24		Ripon fleece (fine) tip
		3.33		Ripon fleece (fine) II
		3.40		Ripon fleece (fine) III
		3.27		Ripon fleece (fine) root
		3.22		Romney Corriedale 50's
		3.94		South African Merino
		4.00		South African Merino
		3.67		South African Merino
		4.00		Welsh mountain
		3.98		Welsh mountain
		4.13		Welsh mountain
		4.08		Welsh mountain
		3.75		Welsh mountain
		3.78		Welsh mountain
		4.01		Welsh mountain, all first clip
1929	Barritt and King (6)	3.66		Australian crossbred
		3.48		Australian Merino
		3.34		Devon lamb
		3.27		fabric
		3.47		New Zealand 50's
	Küster and Irion (27)	3.3	16.1	
	Rimington (36)	3.67		Cape Merino
		3.46		Crossbred 50's I
		3.47		Crossbred 50's II
		3.54		Crossbred 50's III
		3.34		Devon lamb
		4.08		Welsh mountain
	Rimington (37)	3.03		Crossbred 40's

TABLE I—Continued

Year	Investigator	Sulfur Percentage	Nitrogen Percentage	Description of Wool
1931	Bonsma (11)	3.30		half-bred wethers
		3.27		Irish
		3.14		Kent
		3.32		Lincoln
		3.13		Shropshire
		3.55		Shropshire wethers
		3.57		Southdown ewes
		3.43		Southdown tegs
		3.62		Veldt fleece A tip
		3.44		Veldt fleece A middle
		3.71		Veldt fleece A root
		3.60		Veldt fleece A average
		3.54		Veldt fleece B tip
		3.38		Veldt fleece B middle
		3.71		Veldt fleece B root
		3.56		Veldt fleece B average
		3.66		Veldt fleece C tip
		3.63		Veldt fleece C middle
		3.81		Veldt fleece C root
		3.70		Veldt fleece C average
		3.52		Veldt fleece D tip
		3.48		Veldt fleece D middle
		3.48		Veldt fleece D root
		3.48		Veldt fleece D average
		3.31		Veldt fleece E tip
		3.30		Veldt fleece E middle
		3.35		Veldt fleece E root
		3.32		Veldt fleece E average
	Rimington (38)	2.72, 3.06		Welsh mountain birth-coat
	Sidney (42)	3.02		Corriedale
		3.21		Corriedale
		3.16		Romney
		3.17		Romney

EXPERIMENTAL

The wools analyzed were from registered sheep and were obtained in the grease, the Leicester wool from Mr. J. H. Bohendrier of Elk River, Minnesota, the Cotswood III from Mr. C. H. Hartman of Mt. Emons, Utah, the Karakul wools from the Karakul Fur Sheep Farm at Fayetteville, New York, the Lincoln wool from Dr. D. T. Knight of Marlette, Michigan, the Blacktop Delaine Merino, Cotswold I, Dorset, Hampshire I, Merino B type, Oxford, Rambouillet, Shropshire I, and Southdown I from Michigan State College, the Tunis wool from Mr. R. E. Owen of Fulton, New York, the Cotswold II from Rita M. Smalley of Staatsburg, New York, the Romney from Mr. Eugene Tribble of Lodi, California, and the Columbian, Corriedale, Hampshire II, Shropshire II, and Southdown II from the United States Department of Agriculture.

The raw wool was boiled for 15 minutes in eighty volumes of 0.5 per cent solution of olive oil soap, rinsed thoroughly, immersed in a fresh volume of 0.5 per cent solution of soap for 15 minutes at 50°C., rinsed, again treated for 15 minutes with the soap solution, rinsed, and dried. The wool fibers were then separated by hand in order to remove stained portions and foreign substances. After this sorting the wool was treated

for 15 minutes with 0.5 per cent solution of soap at 50°C., rinsed thoroughly, and, after a repetition of this treatment, immersed for 15 minutes in eighty volumes of water, rinsed, dried, and extracted continuously with ether for 18 hours in a modified Soxhlet extraction apparatus.

The Benedict-Denis method (9, 14) was used for the estimation of sulfur in samples of from two to five grams of wool dried to constant weight at 105°C. The precipitates of barium sulfate were collected on Gooch crucibles (16) and ignited to constant weight at dull red heat in an electric muffle furnace. Blank determinations were made on the reagents with each set of sulfur determinations.

The Kjeldahl-Gunning-Arnold method (29) was used for the estimation of nitrogen in samples of from one and one-half to three grams of wool dried to constant weight at 105°C. Blank determinations were made on the reagents.

Table 2 shows the percentage of sulfur and nitrogen obtained for the wools.

TABLE 2. *Amount of sulfur and nitrogen obtained for the wools*

Wool	Sulfur percentage	Nitrogen percentage
Blacktop Delaine Merino	3.74, 3.75, 3.77, 3.78	15.92, 15.94
Columbia	3.31, 3.32, 3.35	
Corriedale	3.34, 3.36	
Cotswold I	3.33, 3.35, 3.37	16.21, 16.23
Cotswold II	2.99, 3.00, 3.00, 3.01, 3.02	16.25, 16.27
Cotswold III	2.98, 2.99, 3.00	15.81, 15.83
Dorset	3.51, 3.51, 3.54, 3.55	15.92, 15.97
Hampshire I	3.18, 3.19, 3.20	16.34, 16.35
Hampshire II	3.47, 3.50	
Karakul (black)	3.03, 3.04, 3.07, 3.08	15.84, 15.85
Karakul (mixed black and yellow)	2.82, 2.90	15.89, 15.96
Karakul (root)	2.83, 2.86, 2.88	
Karakul (tip)	2.62, 2.64, 2.74	
Leicester	3.44, 3.45	16.30, 16.31
Lincoln	2.91, 2.92, 2.93	15.92, 16.01
Merino B type	3.61, 3.61, 3.63, 3.65	16.18, 16.21
Oxford	3.31, 3.33, 3.34, 3.36	16.20, 16.25
Rambouillet	3.56, 3.59, 3.60, 3.60	15.99, 16.02, 16.03
Romney	2.97, 3.00, 3.02, 3.04, 3.05	16.17, 16.24
Romney (root)	3.21, 3.22	
Romney (tip)	3.60, 3.64	
Shropshire I	3.49, 3.49, 3.50	16.16, 16.17, 16.20
Shropshire II	3.31, 3.32, 3.37	
Southdown I	3.30, 3.33, 3.34, 3.34	16.33, 16.34
Southdown II	3.52, 3.54, 3.57, 3.57	
Tunis (root)	3.46, 3.48	
Tunis (tip)	3.35, 3.35	

SUMMARY

1. Sulfur was determined in sixteen wools by the Benedict-Denis method and nitrogen was determined in thirteen wools by the Kjeldahl-Gunning-Arnold method.

2. Analyses of the tips and roots of three wools showed a non-uniform distribution of sulfur along the length of the fiber.

3. The sulfur and nitrogen contents do not furnish bases for the proximate analysis of the wool of mixtures unless blank determinations are made on the wool.

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CONTENTS

Abstracts of Doctoral Theses

Note:—Complete copies of these theses can be consulted at the Library, Iowa State College, Ames, Iowa.

SOME OF THE RELATIONSHIPS AMONG THE ORGANISMS IN BUTTER CULTURES[1]

MERLE PORTER BAKER

From the Department of Dairy Industry, Iowa State College

Accepted for publication June 15, 1932

Studies on the bacteriology of butter cultures were made from the standpoint of relationships among the types of organisms present. The work reported is divided into two parts. Part I is a study of the numbers of the two types of organisms present in butter cultures under various conditions. It involves (a), attempts to find a method for determining the numbers of each of the two types of organisms present and (b), studies on the variations occurring in the growth relationships of the organisms in butter cultures under various conditions, including those existing when new cultures are being developed. Part II involves studies on the effect of the addition of calcium carbonate to milk on the keeping qualities of butter cultures made from it.

PART I. STUDIES ON THE NUMBERS OF EACH OF THE TWO TYPES OF ORGANISMS PRESENT IN BUTTER CULTURES UNDER VARIOUS CONDITIONS

In studying the numbers of each of the two types of organisms present in butter cultures under various conditions several methods for making differential counts were tried. Attempts to distinguish between the two types by the appearance of colonies on agar to which indicator had been added were not successful. The small amounts of acid produced by the colonies diffused out through the medium so that on plates where the number of colonies was large the color change extended over the entire area. On plates where the number of colonies was small they usually all appeared to have produced acid and were assumed to be *Streptococcus lactis*. *S. lactis* is normally present in butter cultures in larger numbers than are the associated organisms and in amounts of butter culture small enough to produce only a few colonies the associated organisms were probably diluted out.

Attempts to determine the numbers of each of the two types of organisms in butter culture by inoculating varying small amounts of butter cultures into litmus milk and using the aroma produced as an index to the presence of the associated organisms and coagulation of the casein or reduction of the litmus as an index to the presence of *S. lactis* were also unsuccessful. The detection of the aroma was difficult in many of the trials and the results secured were not conclusive. The difficulty, in part, was due to the small amount of aroma materials involved and also to the heated odor caused by sterilizing the milk.

The method finally used to determine the approximate numbers of each of the two types of organisms present in butter cultures was to inocu-

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[1]Original Thesis submitted June, 1931.

late varying small amounts of butter culture into series of flasks containing sterile skimmed milk and, after incubation at 21°C., determine the amount of volatile acidity produced as an index to the presence of the associated organisms and observe coagulation as an index to the presence of *S. lactis*. A comparison of the numbers of each of the two types of organisms in butter cultures of different qualities showed, that in general there were higher numbers of the associated organisms in satisfactory than in unsatisfactory butter cultures, but also, that there were wide variations in the numbers as well as the ratios of the two types of organisms in satisfactory and also in unsatisfactory butter cultures.

Studies were made in which the numbers of each of the two types of organisms added to combinations were varied in order to determine whether adding the associated organisms in the larger numbers and thus giving them a chance to grow more rapidly was a factor in developing satisfactory butter cultures. The results secured showed that adding the associated organisms in large numbers as compared to the numbers of *S. lactis* added tended to increase the amount and also the rate of volatile acid production. However, results secured with this procedure were not constant and showed that under some conditions the associated organisms grow well in combinations even when added in small numbers at the time of preparing the mixtures.

Attempts made to develop butter cultures from combinations which in the original mixtures produced volatile acidities comparable to those produced by satisfactory butter cultures resulted in failures to secure a desirable flavor and aroma.

This indicates that the ability of combinations of associated organisms and *S. lactis* to produce high volatile acidities is not a satisfactory basis on which to select them for use in butter cultures.

In order to determine whether or not some of the difficulty encountered in securing satisfactory butter cultures from certain combinations of associated organisms and *S. lactis* might be avoided by using freshly isolated cultures, experiments comparing freshly isolated and old cultures of *S. lactis* were carried out. The results secured indicated that freshly isolated cultures were no better than old cultures for developing butter cultures. Freshly isolated cultures of associated organisms were not tried because of the time required for their isolation and identification.

PART II. THE EFFECT OF THE ADDITION OF CALCIUM CARBONATE TO MILK ON THE KEEPING QUALITIES OF BUTTER CULTURES MADE FROM IT

The studies on the effect of the addition of calcium carbonate to milk on the butter cultures made from it included, (1) the effect on the keeping qualities of butter cultures held at room temperature, (2) the relationship between the temperature of holding and the effect of the addition of calcium carbonate on the keeping qualities of butter cultures and (3) the keeping qualities of butter cultures held for long periods of time at room temperature.

There was a small increase in the keeping qualities of butter cultures held at room temperature (25°-32°C.) in milk to which calcium carbonate had been added. The advantage due to the presence of calcium carbonate was greater in the butter cultures held for the longer periods than in the butter cultures held for the shorter periods. In the short holding periods

(5 days or less) the advantage due to the presence of calcium carbonate was only slight. There was no correlation between the effect of the addition of calcium carbonate and the individual butter cultures used.

When comparisons were made of 21°C. and 37°C. as holding temperatures the results showed a considerable increase in the keeping qualities of the butter cultures held at 21°C. in milk with added calcium carbonate but no increase at all in the keeping qualities of the butter cultures held at 37°C. The disadvantages incident to the higher holding temperature were greater than the advantages because of the presence of calcium carbonate. Variations in the keeping qualities secured in the different runs, however, indicated that factors other than the temperature of holding were also important.

Only one of 28 trials in which butter cultures were held in milk with added calcium carbonate at room temperature for long periods of time (154 to 272 days) yielded a satisfactory butter culture when transferred back to pasteurized milk. Microscopic examinations of the material held in the trials usually showed gram positive rods which presumably had survived the pasteurization incident to the preparation of the milk for inoculation. Examinations for the numbers of each the associated organisms and *S. lactis* were made on the material held in four of the trials. These showed wide variations in the proportions of the numbers of the two types of organisms and indicated that some factor or factors other than the loss of one of the types were probably responsible in part at least for the failure of the material to again produce satisfactory butter cultures.

I. AN X-RAY INVESTIGATION OF THE IRON-COPPER SYSTEM[1]

JAMES HAL CARTER

From the Department of Chemistry, Iowa State College

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It is quite generally accepted that an immiscibility gap exists in the liquid state of the iron-copper system. Ruer (4) and Benedicks (1) propose that the liquid immiscibility gap intersects the liquidus curve of the phase diagram. On the other hand, Muller (3) claims that there is a lower critical point for the immiscibility gap at about 1500°C. Ruer clams that Muller's results are due to impurities.

The above observations were made by means of thermal and microscopic examinations. The literature contains no report of an x-ray examination of this system.

EXPERIMENTAL

A series of pure iron-copper alloys, covering the entire range of compositions, were prepared by melting Armco iron and pure copper in a magnesia crucible in connection with an Ajax induction furnace. Nitrogen was passed over the charge during melting to prevent oxidation. The alloys were cooled in air and the condition of the iron and copper in the solidified alloys represents the condition which existed at the time of solidification.

The alloys were examined by application of the Hull-Debye-Sherrer method of x-ray analysis. The alloys were heated at 1700°F. for twelve hours and x-ray photograms made of the annealed samples as well as the original samples. Photomicrographs were made of the samples before and after annealing.

RESULTS AND CONCLUSIONS

Only iron lines appear in the x-ray photogram of alloys containing up to 13.02 per cent copper. The photograms of alloys containing from 13.02 per cent copper up to and including 82.31 per cent copper show the superimposed x-ray spectra of iron and copper. The alloys containing 83.39 per cent copper at 100 per cent copper show only copper lines.

Heat treatment at 1700°F. for twelve hours produced no change in the photograms, but the photomicrographs of the samples indicate that a change in structure has started. Evidently the time of annealing was insufficient.

The results obtained are in fair agreement with those of Ruer, 23.8 per cent and 85 per cent copper, respectively, for the high copper side of the diagram, but vary for the low copper side. The value obtained for the low copper side agrees well with the result of Benedicks (1).

Future work will be carried out to determine the effect of annealing on the miscibility limits as obtained above. By quenching from high temperatures, it is hoped that further information may be obtained concerning the miscibility gap at temperatures above the melting points of the alloys.

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[1]Original Thesis submitted December, 1931.

II. A STUDY OF THE CORROSION OF GALVANIZED SHEET IRON

Localized, or point, corrosion occurs quite generally on galvanized sheet iron. This type of corrosion is more serious than general, or uniform, corrosion because the attack is concentrated on a small area and penetration of the metal results in a very short time. Evans (2) has explained this type of corrosion by his "differential aeration theory."

The present study was undertaken with the view of isolating a certain range of gas compositions most favorable to the progress of localized corrosion.

EXPERIMENTAL

Samples of 18 gauge galvanized Armco sheet iron, submerged in conductivity water, were subjected to the corrosive action of various mixtures of oxygen, carbon dioxide and nitrogen gases. The compositions of the different gaseous mixtures used are given in table 1.

TABLE 1

Mixture number	P'c't'g. O_2	P'c't'g. O_2	Mixture number	P'c't'g. O_2	P'c't'g. CO_2
1	5	0.00	11	5	0.06
2	10	0.00	12	10	0.06
3	15	0.00	13	15	0.06
4	20	0.00	14	20	0.06
5	25	0.00	15	25	0.06
6	5	0.03	16	5	0.10
7	10	0.03	17	10	0.10
8	15	0.03	18	15	0.10
9	20	0.03	19	20	0.10
10	25	0.03	20	25	0.10

The samples were perforated at five points on each side by means of a small power drill. The perforations just penetrated the zinc coating.

Three sample cans, each containing sixteen corrosion samples, were fed by each gaseous mixture. The sample cans were also made of the 18 gauge material. The samples were arranged circularly within the can, eight samples at the top and eight at the bottom. The gaseous mixture was introduced at a level between the two layers of samples. The plan of the set-up was that the water in the upper half of the can should be saturated with the gas and a concentration gradient exist in the lower half of the can. As will be pointed out later, this condition did not exist.

The samples were kept at constant temperature, 26°C. $\pm$ 2°, by placing the sample cans in a thermostat tank.

The rate at which the gas bubbled through the sample can was about one bubble per second, as measured by a small glass tip through which the gas entered.

The experiment extended from March 1, 1931, to July 24, 1931.

OBSERVATIONS AND CORRELATIONS

The total number of points on each sample which were attacked and the degree of corrosion were noted. The pH values of solution from the top and bottom portions of forty cans were determined colorimetrically. The dissolved solids of samples of water from twenty cans were determined. The results of these observations are given in tabular form.

Photographs of representative samples corresponding to each gaseous mixture are given in the complete thesis.

The total number of points attacked were summed up for all cans having the same gas composition. The results are given in table 2.

TABLE 2

Pc't'g. CO_2	5 per cent O_2	10 per cent O_2	15 per cent O_2	20 per cent O_2	25 per cent O_2
0.00	282	224	151	136	240
0.03	161	268	111	38	91
0.06	71	82	95	64	227
0.10	100	157	117	227	189

For any constant carbon dioxide content it is found that the corrosion passes through a minimum as the oxygen content increases. Likewise, when the oxygen content is constant and the carbon dioxide is increased the corrosion passes through a minimum. The minimum corrosion occurred at 20 per cent oxygen and 0.03 per cent carbon dioxide.

It is the opinion of the writer that the data obtained on pH values and dissolved solids are insufficient to warrant any conclusions. No correlation could be detected for the data at hand.

The top samples were decidedly more corroded than the bottom samples. Evidently the gases diffused to the top and there was not enough stirring action, nor enough gas, to maintain as high concentration in the lower part of the can as in the upper part. Thus the plan for a concentration gradient in the lower part of the can failed.

Wherever corrosion had proceeded to the extent of formation of a tubercle, it was found that the greatest amount of corrosion had occurred at a point immediately below the original perforation. On the basis of the differential aeration theory, this phenomenon may be explained as follows: corrosion products form about the perforated point on the corrosion sample and stream downward. In the presence of relatively high oxygen concentration, the $Fe(OH)_2$ formed is oxidized to $Fe(OH_3$. The less soluble $Fe(OH)_3$ forms a film over the metal surface which is highly resistant to the diffusion of oxygen and thereby causes a region of low oxygen concentration beneath the film| This unaerated portion is anodic to the surrounding aerated portion and electrochemical action results. Iron goes into solution at the anode as $Fe(OH)_2$ and is deposited as $Fe(OH)_3$ at the cathodic area. This deposition of $Fe(OH)_3$ results in the formation of a membranous mantle around the anode. When once the walls of the mantle have formed around the anodic point they will protect it from oxygen, and thus the anodic attack will persist indefinitely.

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STUDIES ON THE STERILIZATION OF SOLUTIONS OF GLUCOSE AND SUCROSE[1]

WENDELL BURNHAM COOK

From the Department of Chemistry, Iowa State College

Accepted for publication June 15, 1932

Glucose, also known as dextrose, corn sugar and cerelose, is being economically produced at the present time by the hydrolysis of corn starch. Upon the modification of the food law regulations in January, 1931, this sugar may now be used in food products without declaring the fact on the label. A promising field thus opened to the producers of glucose is that of the carbonated beverage industry. One of the chief problems of this industry is the production of a product that will not spoil after a period of several months storage (3, 2). Since 85 per cent of the spoilage in carbonated beverage is caused by yeast (5), it is important to study the comparative ease of sterilizing solutions of glucose and sucrose.

Seven strains of spore forming yeasts, typical of those causing spoilage in carbontaed beverages, were selected for the experimental work. In order to obtain a sufficient amount of spore material to carry out all of the experiments, spores were prepared by growing them on carrot juice calcium sulfate agar in Kolle flasks. After scraping off the spores, they were dried and diluted by grinding with sterile powdered sugar in an agate mortar.

The concentration of sugars studied were in the range that would be practicable for the manufacturer. The highest concentration of glucose that may be obtained at 25°C. is one of 27° Baumé strength (49.5 per cent). Two other concentrations of syrups were studied—24° Baumé (44 per cent sugar) and 20° Baumé (36 per cent sugar).

The effects of the two sugars on yeast spores were studied in syrups and in syrups containing small amounts of citric acid, both at room temperature (28°C.) and at 60°C. One experiment was conducted using vegetative cells.

For the experiments carried out at room temperature, the following procedure was employed: 100 cc. of a sterile syrup was inoculated with 5 cc. of a solution containing 2 per cent glucose and from one to four million yeasts. At definite intervals, 5 cc. portions of syrup were taken out of the flask and placed in 45 cc. of sterile tap water. The number of organisms present were determined by plating out dilutions on Wort agar and incubating at 28°C. for five days.

The apparatus and techniques used in experiments conducted at 60°C. were similar to those employed by Levine, Buchanan and Lease (4) and Hall (1).

In the experiments conducted at room temperature the results showed that at given concentrations (20°, 24°, 27° Baumé), sucrose syrups favor growth considerably better than glucose. An interesting point brought out was the decrease in the number of organisms in glucose solutions during the first few days of the experiment. In 20° Baumé glucose syrups, the count dropped to 62 per cent of the original number inoculated before

[1]Original Thesis submitted August, 1931.

growth occurred; 24° Baumé showed the same decrease, and 27° Baumé showed a decrease of 50 per cent of the initial count. The extent of the lag period was longer in glucose than in sucrose. A 27° Baumé glucose solution showed a lag period of five to seven days, while the same density sucrose solution showed a lag period of one day.

Upon the addition of small amounts of citric acid (.04M) to 27° Baumé syrups, the yeasts in both sugar solutions slowly decreased in number. There was no significant difference on the killing spores between the two sugars.

However, when the same experiment was conducted on yeasts from a 48 hour broth culture, glucose containing .02M and .04M citric acid killed the cells more rapidly than the corresponding sucrose solution.

In the experiments conducted at 60°C., the results showed that it required less time to kill 99 per cent of the yeasts in a sucrose solution than in the corresponding glucose solution.

TABLE 1. *Killing time for 99 per cent of yeast in sugar solutions at 60°C.*

Density Baumé	Citric acid concentration	Glucose	Sucrose
		Time in minutes	
24°	0	72	52
	.02M	34	23
	.04M	27	17
27°	0	106	90
	.02M	43	35
	.04M	38	33

An explanation given for the behavior of the sugar solutions was that the glucose syrup, containing almost twice as many molecules as the sucrose syrup, had a considerably higher osmotic pressure than the sucrose syrups. At room temperature this condition prevented growth of the yeasts for a period until the cells became acclimated to the high osmotic pressure. At higher tempertaures, the solutions with the higher osmotic pressure exert a protective action on the cells, tending to hold water in the sugar solution, thus preventing the passage of hot water into the cell.

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THE PREPARATION OF PER ACIDS AND THEIR SALTS[1]

ROBERT ROY COONS

From the Department of Chemistry, Iowa State College

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There are a number of acids to which the prefix "per" has been applied, for example, perchloric, periodic and permanganic. In these cases the prefix "per" denotes only that they contain relatively more oxygen than the chloric, the iodic and the manganic acids. Price[2] defines true per acids "as those which are either formed by the action of hydrogen peroxide on ordinary acids, or else give rise to hydrogen peroxide on treatment with dilute sulphuric acid; with concentrated sulphuric acid many of them evolve ozonized oxygen, thus behaving similarly to the metallic peroxides and to hydrogen peroxide itself."

Price[2] groups the elements forming per acids. Leaving out of consideration the elements in the first two short series of the periodic table, it will be observed that the formation of per acids is confined to groups IV, V and VI, and more particularly, with the exception of tin and selenium to the A family of these groups.

Respecting the elements of family B, Tanatar[3] obtained a perstannic acid by the action of 30 per cent hydrogen peroxide on stannic acid which had been precipitated from a solution of stannic chloride by sodium carbonate. Dennis and Brown[4] reported an impure perselenate, analagous to potassium persulphate, prepared by the electrolysis of a saturated solution of potassium selenate containing a little free selenic acid. Neither Dennis and Koller[5] nor Bauer and Wilkinson[6] were able to confirm this result. Alvarez[7] claims to have prepared sodium perarsenate by dissolving disodium arsenate in water and alcohol, cooling to 0°, and then adding sodium peroxide. Aschkenasi[8] reported perarsenates which were prepared by treating arsenic acid with barium peroxide and hydrogen peroxide, the solid being obtained by the evaporation of the solutions with gentle heating and under reduced pressure.

PURPOSE OF THE INVESTIGATION

Since no element of a B family has been definitely shown to form per acids or salts, the purpose of this work was to apply to arsenic, a typical element of a B family, all of the known methods for the preparation of per acids.

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[1] Original Thesis submitted September, 1931.

[2] Price, Per-acids and their salts, pp. 1-8 (1912).

[3] Tanatar, Ber., **38**, 1184 (1905).

[4] Dennis and Brown, Jour. Am. Chem. Soc., **23**, 358 (1901).

[5] Dennis and Kohler, *ibid*, **41**, 949 (1919).

[6] Bauer and Wilkinson, Unpublished Thesis, Library, Iowa State College, Ames, Iowa (1927).

[7] Alvarez, Chem. News, **94**, 269 (1906).

[8] Aschkenasi, German Patent, 296796 (1914).

METHODS

1. HYDROGEN PEROXIDE METHOD

Disodium arsenate was dissolved in a 5 per cent solution of hydrogen peroxide, at room temperature, and the resulting solution was evaporated over concentrated sulphuric acid under reduced pressure.

A second preparation was made by treating a solution of disodium arsenate, saturated at 0°, with 30 cc. of 30 per cent hydrogen peroxide. Before being mixed, the two solutions were cooled to 5°C. No precipitate formed. The mixture was evaporated over concentrated sulphuric acid under reduced pressure and a white residue was left which was very hygroscopic.

The residues were analyzed by the Mohr[9] method for the per cent of arsenic, and by the Bunsen[10] method for the oxidizing equivalent. The second residue was analyzed for the percentage of hydrogen peroxide.

No active oxygen was found in the first residue. From the analytical data of the second residue, which contained 8.45 per cent active oxygen, the calculations show that the product approaches the formula $25Na_2HAsO_4 \cdot 32H_2O_2 \cdot 16H_2O$, which is probably a mixture.

(2) BARIUM PEROXIDE METHOD

Small quantities of barium peroxide were added, at ten minute intervals, to a concentrated solution of arsenic acid kept cold with salt and ice. The filtrate from this preparation was evaporated over concentrated sulphuric acid under reduced pressure.

A second concentrated solution of arsenic acid at 8° was treated with barium peroxide. Sodium hydroxide was added slowly to this mixture, and after filtering, the filtrate was evaporated as indicated in the preceding paragraph.

To a cold solution of disodium arsenate barium peroxide was added in excess. A white solid was obtained by evaporating the filtrate over concentrated sulphuric acid under reduced pressure.

The three residues were analyzed by the Bunsen method for the oxidizing equivalent. No active oxygen was found in the first two residues; the third showed 0.47 per cent active oxygen.

3. ELECTROLYTIC METHOD

(A) *Disodium Arsenate*

A solution of disodium arsenate, saturated at 0°, which was made the anolyte inside a porous cup, and a solution of arsenic acid, which was made the catholyte around a porous cup inside a liter beaker, were electrolyzed, at a temperature of 0° to 5°, in the Elb's[11] cell for sixteen hours with an anodic current density of one ampere per square centimeter.

With an anodic current density of 0.7 amperes per square centimeter, a second solution of the same salt, saturated at 0°, was electrolyzed for eight hours at a temperature of 0° to 10°. Two platinum wires were used as electrodes[12].

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[9]Scott, Standard methods of chemical analysis, 1, p. 36 (1927).

[10]Foulk, Notes on quantitative analysis, p. 181 (1930).

[11]Elbs, Electrolytic preparations, p. 35 (1903).

[12]Partington, A textbook of inorganic chemistry, p. 518 (1927).

A third solution of the same salt and of the same concentration was electrolyzed for twelve hours, the temperature ranging from 0° to 8°, with an anodic current density of 1.5 amperes per square centimeter.

Since no precipitate was obtained in either case, the three solutions were analyzed by the Bunsen method for the oxidizing equivalent. No active oxygen was found in the first two preparations, while the third contained 0.0137 g .of active oxygen per 100 cc. of solution.

(B) *Disodium Arsenate and Sodium Fluoride*

Approximately 125 cc. of a solution of disodium arsenate, saturated at 0° and containing 1 g. of sodium fluoride, was electrolyzed for ten hours, at 0-12°, using an anodic current density of 0.7 to 1.8 amperes per square centimeter.

An equal volume of the same disodium arsenate solution containing 2.5 g. of sodium fluoride was electrolyzed for sixteen hours with an anodic current density of two to three amperes per square centimeter, the temperature ranging from 0° to 10°. (See reference 12 for the apparatus used.)

Both of these preparations were analyzed for the oxidizing equivalent and neither showed any active oxygen.

4. SODIUM PEROXIDE METHOD

To 25 g. of disodium arsenate, dissolved in 1500 g. of distilled water in a four-liter round bottom flask, 1700 cc. of 95 per cent ethyl alcohol were added. Fifty grams of sodium peroxide were added to this mixture in small quantities and at intervals of five to ten minutes to prevent an appreciable increase in the temperature. The contents of the flask were kept at —10° throughout the procedure. About 500 cc.. of absolute ethyl alcohol were added and the copious white precipitate, after being filtered out, was dried over phosphorus pentoxide under reduced pressure.

The precipitate was analyzed and the results are shown in table 1:

TABLE 1. *Analysis of Na_2O_2 precipitate*

Bunsen method percentage Na_2O_2	$KMnO_4$ method percentage Na_2O_2	Mohr method percentage arsenic	Total sodium percentage	Titration with HCl percentage sodium
23.26	22.74	6.26	21.25	17.48
23.36	22.87 22.84	6.23 6.24	21.34	17.16 17.38
23.10	22.90	6.22		17.53
23.23				
23.24				
21.30				

From the data of this table the calculations show that the product approaches the compound $2Na_3AsO_4.7Na_2O_2.2NaOH.75H_2O$, but is probably a mixture. No perarsenate was present.

5. FLUORINE METHOD

The apparatus used in generating free fluorine was similar to that employed by Jones[13]. The gas was obtained by the electrolysis of fused potassium bifluoride.

[13]Jones, Jour. Phys. Chem., 33, 801 (1929).

(A) Acid Solution. For three hours gaseous fluorine was passed into a solution of disodium arsenate, saturated at 0°, the temperature remaining constant. A small amount of a blue precipitate was formed. The filtrate was placed in an automatic refrigerator until analyzed. The precipitate was dried over concentrated sulphuric acid under reduced pressure, and then over phosphorus pentoxide for three days at room temperature.

The filtrate and the precipitate were analyzed by the Bunsen method for the oxidizing equivalent, and contained neither perarsenate nor active oxygen.

(B) Basic Solution. A solution of normal sodium arsenate, with a slight excess of sodium hydroxide, was saturated at 0°. A portion of this filtrate, maintained at zero degrees $\pm$ 2°, was treated with free fluorine for three and one-half hours. A bluish-gray precipitate was obtained.

Analyses of the filtrate by the Mohr method for the oxidizing equivalent showed 0.0142 g. of active oxygen per 100 cc. of solution, and neither ozone nor peroxide were found. The precipitate contained no active oxygen.

CONCLUSIONS

(1) Five per cent hydrogen peroxide gives no evidence of the formation of a per salt but the 30 per cent hydrogen peroxide shows the formation of a compound with an active oxygen content of 8.45 per cent. The formula indicated is $25Na_2HAsO_4.32H_2O_2.16H_2O$, which is apparently a mixture.

(2) Barium peroxide forms no perarsenate with either disodium arsenate or arsenic acid.

(3) There is no evidence of a perarsenate being formed by the electrolysis of a solution of disodium arsenate either in the presence or absence of fluorides.

(4) Sodium peroxide precipitates a compound from an alcoholic solution of disodium arsenate whose analysis indicates the compound $2Na_3Aso_4.7Na_2O_2.2NaOH.75H_2O$, but this is undoubtedly a mixture since different preparations vary in analyses.

(5) Fluorine gas passed into a solution of trisodium arsenate gives but little evidence of the formation of a per salt.

STUDY ON THE UTILIZATION OF XYLOSE[1]

HSI CH'OU FANG

From the Department of Chemistry, Iowa State College

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Xylose occurs widely distributed as a condensation product in all cellulose plant material. Studies on the utilization of plant residues which constitute our greatest agricultural waste must necessarily include the study of this constituent. The investigations reported in this work for the utilization of xylose are limited to the three following phases of the general problem:

I. Oxidation of xylose to xylonic acid;

II. Preparation of pyrrole from ammonium xylonate;

III. Fermentation of xylose to butyl alcohol. The present investigation is limited to the development of an analytical method for the determination of a mixture of butanol, acetone and ethanol.

EXPERIMENTAL

I. OXIDATION OF XYLOSE TO XYLONIC ACID

Studies were made on the oxidation of xylose solutions by means of gaseous chlorine using ammonium hydroxide as a neutralizing agent. For every volume of gas, room temperature and barometer reading were recorded and the gas volume corrected to standard conditions. At the end of the reaction, that is, after an equivalent amount of chlorine had been absorbed, the solution was concentrated under reduced pressure until ammonium chloride crystallized. After filtering out ammonium chloride, the process of concentrating was repeated several times until no ammonium chloride crystals were shown when the solution was syrupy.

The thin syrup was stored in the ice box for about three weeks, with frequent stirring. About 30 per cent of ammonium xylonate was obtained. M.P. 120-122°. $(\alpha)^{27}_{D}° = +6.2$

Anal. Calcd., 7.617 per cent N. Found, 7.618 per cent N
7.618 per cent N

Experiments showed that the yield depended on the pH, but no practical method of controlling this factor was found.

II. PREPARATION OF PYRROLE FROM AMMONIUM XYLONATE

Dry distilation of ammonium xylonate yielded pyrrole in quantities of about 25 per cent of the theoretical. It seems possible that, with larger quantities which would permit more detailed studies, these yields would be increased. This salt would thus be as practical a source of pyrrole as mucic acid if it could be made available.

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[1]Original Thesis submitted June, 1931.

When other salts such as ammonium arabonate, ammonium gluconate and ammonium galactonate were dry distilled, they also yielded pyrrole; but the yield was very low.

III. ANALYSIS OF AQUEOUS SOLUTIONS OF BUTANOL, ETHANOL AND ACETONE

A rapid and accurate method of analysis of the products formed is necessary for the study of any fermentation process. Bogin[2] reports that turbidity determinations can be used for the analysis of ternary mixtures of butanol, ethanol and acetone where one constituent is less soluble than the others. The method used by Reilly and his co-worker[3] is limited to the binary system and requires the chemical determination of one constituent for the analysis of the mixture.

The method as developed in this laboratory depends on (a) the determination of acetone by the standard iodine titration method, (b) the measurement of specific gravity of the ternary solution, (c) the measurement of refraction of the ternary solution, and (d) the determination of butanol and ethanol by means of simultaneous equations (as shown below) involving the additive properties of specific gravity and refractivity measured above. Since the method of analysis was designed for our own particular fermentation mixtures, special attention has been devoted to the ratio of solvents which are about 6 parts of butanol, 3 parts of acetone and 1 part of ethanol.

The following equations are for the calculation:

$$S_{B+A+E} = -0.0014C_B - 0.0015C_A - 0.0017C_E + 1 \qquad \text{Equ. 1}$$

$$R_{B+A+E} = 2.80C_B + 1.98C_A + 1.73C_E + 13.26 \qquad \text{Equ. 2}$$

where A = Acetone,

B = Butanol,

E = Ethanol,

S = Specific gravity,

C = Concentration of solute in grams per 100 cc. of solution, and

R = Reading of immersion refractometer on original solution.

The accuracy of the method is shown in table 1.

TABLE 1. *Results*

Soln.	Specific gravity	Reading of immersion refracto-meter	Gms. per 100 cc. of solution				
			Acetone by iodine titration	Butanol calc.	Butanol actual	Ethanol calc.	Ethanol actual
1	0.9943	23.22	1.2	2.6	2.4	0.19	0.4
2	0.9914	28.12	1.8	3.83	3.6	0.31	0.6
3	0.9884	32.96	2.4	5.05	4.8	0.50	0.8
4	0.9825	42.78	3.6	7.27	7.2	1.15	1.2

[2]Bogin, Ind. Eng. Chem., 16:380-385 (1924).

[3]Reilly & Ralph, Sci. Proc. Royal Dublin Soc., 15:597-60 (1919).

ABNORMAL REACTIONS OF ORGANOMETALLIC COMPOUNDS[1]

A. THE ABNORMAL REACTION OF CINNAMYL CHLORIDE WITH MAGNESIUM

STANTON A. HARRIS

From the Department of Chemistry, Iowa State College

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The object of this work was to find an explanation for the formation of the two hydrocarbons from cinnamyl chloride and magnesium. Rupe and Burgin[2] obtained dicinnamyl ($C_6H_5CH{=}CHCH_2CH_2CH{=}CHC_6H_5$ (1)) and a liquid hydrocarbon whose formation they explained by the following reactions:

$$C_6H_5CH{=}CHCH_2MgCl + C_6H_5CH{=}CHCH_2Cl \longrightarrow$$

$$\begin{array}{lcl} C_6H_5CHCH(MgCl)CH_2MgCl & \xrightarrow{HOH} & C_6H_5CHCH_2CH_3 \\ \qquad\quad | & & \qquad\quad | \\ \qquad CH_2CH{=}CHC_6H_5 & & (II)\,CH_2CH{=}CHC_6H_5 \end{array}$$

A new explanation was found in the formation of rearranged products when so-called cinnamylmagnesium chloride was treated with a variety of reagents. The equations illustrating this rearrangement are given on page 426.

Ordinary methods for the preparation of a Grignard reagent gave poor yields with cinnamyl chloride, because of the unusual reactivity of the halogen which causes coupling reactions to take place. Some of the factors which were found to affect the yield were: the purity of the cinnamyl chloride; the amount of surface of magnesium which was available; the rate of addition of the halide; and the amount of ether that was used.

Cinnamyl chloride of high purity, as shown by the absence of tarry residues on distillation, was made in 83 per cent yield by adding a chloroform solution of thionyl chloride. The hydrogen chloride was removed from the reaction mixture by a slight excess of pyridine.

The Grignard reagent was made in 82-87 per cent yield by slowly adding cinnamyl chloride, dissolved in fifteen molecular equivalents of ether, to six atomic equivalents of 30-60 mesh magnesium. The reaction was first started with a small quantity of the cinnamyl chloride solution. Rapid stirring was used throughout the addition of the halide which took 2.25 hours for a two-tenths mole run.

This Grignard reagent acted as if it had the following structure: $C_6H_5\underset{\displaystyle MgCl}{\underset{|}{C}H}CH{=}CH_2$. With carbon dioxide, phenylvinylacetic acid

[1]Original Thesis submitted July, 1931.

[2]Rupe and Bürgin, *Ber.*, 43, 172 (1910).

($C_6H_5CHCH{=}CH_2$) was obtained. It was crystallized from petroleum
|
COOH

ether (b.p. 40-60°) at —10° and was found to melt at 23-24°. Its structure was proved; first, by ozonization which gave formaldehyde; and, second, by reduction to α-phenylbutyric acid. The liquid acid was converted to methylatropic acid ($C_6H_5C{=}CHCH_3$) by the action of dilute acid, dilute
|
COOH

base, or by heat alone.

Phenyl isocyanate, with this Grignard reagent, gave phenylvinylacetanilide ($C_6H_5CHCH{=}CH_2$) which was found to be identical with the
|
$CONHC_6H_5$

anilide obtained from phenylvinylacetic acid, thionyl chloride and aniline. Ethyl chlorocarbonate gave an ester which yielded methylatropic acid on hydrolysis. The product from gaseous formaldehyde was not identified. On oxidation it yielded only benzoic acid. Similar oxidation of residues from several cinnamylmagnesium chloride runs did not yield any *o*-phthalic or terephthalic acids. This proved that no rearrangement to the ring had taken place, after the kind of rearrangement shown by benzylmagnesium chloride types.

The products obtained from cinnamylmagnesium chloride with oxygen and with ethyl sulfate seemed to support the rearrangement reaction. The attempted catalytic reduction of this Grignard reagent resulted in no addition of hydrogen. The reaction with benzophenone yielded no benzopinacol which showed that this RMgX compound did not dissociate appreciably into R— and —MgX.

The liquid hydrocarbon from cinnamyl chloride and magnesium was carefully separated from dicinnamyl, purified and analyzed. The analysis, ozonization, reduction and comparison of the reduced liquid hydrocarbon with synthetic 1,4-diphenylhexane showed it to have structure (III) instead of structure (II). The following equations were given to explain the formation of the derivatives from cinnamylmagnesium chloride:

$$C_6H_5CH{=}CHCH_2Cl + Mg \longrightarrow C_6H_5CH{=}CHCH_2{-} + {-}MgCl$$

$$C_6H_5CH{=}CHCH_2{-} \longrightarrow C_6H_5CHCH{=}CH_2$$

$$C_6H_5CHCH{=}CH_2 + {-}MgCl \longrightarrow C_6H_5CHCH{=}CH_2$$
|
MgCl

$$2C_6H_5CH{=}CHCH_2{-} \longrightarrow (I)\,C_6H_5CH{=}CHCH_2CH_2CH{=}CHC_6H_5$$

$$C_6H_5CHCH{=}CH_2 + C_6H_5CH{=}CHCH_2{-} \longrightarrow C_6H_5CHCH{=}CH_2$$
|
(III) $CH_2CH{=}CHC_6H_5$

$$2C_6H_5CHCH{=}CH_2 \longrightarrow \underset{\text{(IV)}\ C_6H_5CHCH{=}CH_2}{\overset{C_6H_5CHCH{=}CH_2}{|}}$$

Dicinnamyl (II) was obtained in small yields, but 3,4-diphenylhexadiene-1,5 (IV) was not isolated from the products.

1,4-Diphenylhexane was made by the following reactions:

$$C_6H_5CH_2CH_2CH_2OH + SOCl_2 \longrightarrow C_6H_5CH_2CH_2CH_2Cl + SO_2 + HCl$$

$$C_6H_5CH_2CH_2CH_2Cl + Mg \xrightarrow{\text{ether}} C_6H_5CH_2CH_2CH_2MgCl$$

$$C_6H_5CH_2CH_2CH_2MgCl + C_6H_5\overset{\overset{O}{\|}}{C}CH_2CH_3 \xrightarrow{HOH} C_6H_5\overset{\overset{OH}{|}}{\underset{\underset{CH_2CH_2CH_2C_6H_5}{|}}{C}}{-}CH_2CH_3$$

$$C_6H_5\overset{\overset{CH_2CH_2CH_2C_6H_5}{|}}{C}{-}CH_2CH_3 + HI \xrightarrow[\text{pressure}]{140\text{-}150^\circ} C_6H_5\overset{\overset{CH_2CH_2CH_2C_6H_5}{|}}{C}HCH_2CH_3$$

Ozonization of cinnamyl chloride gave formaldehyde. The molecular refraction showed an exaltation which was over two. This showed the presence of conjugation in the molecule which supports the following structure: $C_6H_5CH{=}CHCH_2Cl$. The Grignard reagent acts as if it had the following structure: $C_6H_5\overset{\overset{MgCl}{|}}{C}HCH{=}CH_2$. However, it is possible that the normal product ($C_6H_5CH{=}CHCH_2MgCl$) is first formed and that rearrangement takes place during the formation of a derivative.

B. GRIGNARD REAGENTS FROM HALOGENATED TERTIARY AMINES

Inorganic lead compounds have been used with varying success in cancer therapy. For this reason, it is desirable to obtain water soluble organolead compounds which might have some value for the same purpose. If a Grignard reagent could be made that contained a tertiary amine group in its hydrocarbon radical, it could then be introduced into organolead compounds to give them water solubility.

This work was the continuation of the problem which was initiated by Gilman and Heck[8]. A variety of halogen substituted pyridines were prepared by previously described reactions. 3-Bromopyridine and 3,5-dibromopyridine did not react with activated magnesium-copper alloy in ether solution. They could be made to react at elevated temperatures with or without the presence of ether. No Grignard reagent could be isolated from the tarry mass that was obtained. A color test for the Grignard reagent was obtained if Michler's ketone was added before the reacion went to completion.

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[8]Gilman and Heck, *Ber.*, **62**, 1379 (1929).

2-Iodopyridine reacted readily with magnesium in ether solution to give an intensely green solution and an ether insoluble tar. The solution would give no color test, but the entire contents of the reaction flask would give the color test if Michler's ketone was added immediately. No derivatives with carbon dioxide or phenyl isocyanate were obtained. The polymerization reactions were thought to be due to the addition of the Grignard reagent to the carbon-nitrogen double bond.

o-Iododimethylaniline and *o*-bromodimethylaniline were found to react very readily with magnesium in ether solution with a small amount of iodine as a catalyst. In 0.05 mole runs the yields were approximately 90 per cent. No derivatives have as yet been made. These results were quite different from those which have been reported for *p*-bromodimethylaniline. Its Grignard reagent is made with extreme difficulty and in small yields.

THE DESIGN OF A PLANT FOR THE PRODUCTION OF INSULATION BOARD FROM AGRICULTURAL WASTES AND COST DATA ON THIS PROCESS[1]

CHARLES EARL HARTFORD

From the Department of Chemical Engineering, Iowa State College

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The development of the design of a plant for the production of insulation board from agricultural wastes and the establishment of cost data on this process have resulted from commercial scale work which has been carried on at Dubuque, Iowa, since November, 1929.

The small scale development work which was done prior to and which led up to the commercial scale development which is here reported, was carried on by the United States Bureau of Standards and the Iowa Engineering Experiment Station at Ames, Iowa.

The Dubuque process, from which the following data have been taken, consists of the following steps: the raw material is cut to small pieces, cooked with hot water, washed, put through a series of refining engines, formed into a sheet, pressed, dryed and sawed to various sizes.

Field corn, broom corn, pop corn, sweet corn, cornstalks and straw have been tried as raw materials. Field corn cornstalks proved to be the best all-around material. Sweet corn and broom corn cornstalks require a longer cooking time than the field corn cornstalks. Pop corn cornstalks yield a board of lower strength than field corn. Straw gives satisfactory results in every way except that the board is apt to show more coarse particles on the surface.

Four machines, the Williams swing hammer mill shredder, Smalley cutter, Taylor-Stiles rag cutter, and Fox bale breaker and cutter have been tried for dry-cutting the stalks. The power consumed by these machines ranges from 15 kw-hr. per ton of board for the Smalley to 26 for the Williams. The Fox machine, which consumes 16 kw.-hr., is the most satisfactory cutter when all factors have been considered.

A Williams swing hammer mill has been used successfully as a shredder for the wet pulp following the dry-cutting and cooking steps. Its power consumption is 12 kw.-hr. per ton of board.

The Claflin, Jordan and Bauer machines, which are used to complete the refining of the fibers, consume respectively 57, 165 and 135 kw.-hr. per ton of board.

Close control is required in the cooking proces. Undercooked pulp gives a board which is light in weight and low in strength, while an overcooked pulp results in a board which is too dense. The correct cooking conditions were found to be 5,000 pounds raw material, 2,200 gallons of water, and a cooking period of one hour and thirty minutes after a temperature of 280°F. has been reached. The temperature is brought to 316°F. and maintained at this point by introducing steam into the pulp.

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[1]Original Thesis submitted September, 1931.

The rosin-alum method of sizing the pulp is used at Dubuque. Three per cent rosin size is used based on the weight of finished board. The rosin is introduced at a point following the wet shredder, and alum is added just before the pulp is formed into a sheet. Enough alum (about 6 per cent) is used to adjust the pH to 4.5.

The refined and sized pulp is diluted to a consistency of 2.5 per cent with "white water" from the forming machine and press and is then run onto a specially designed forming machine. In this machine a spiral weave reinforced wire carries the pulp over a draining area, then over a suction box which reduces the moisture content of the mat to about 90 per cent, then through a series of five press rolls which reduce the moisture content to about 70 per cent.

The board emerging from the press is cut into 20-foot lengths and fed automatically to the eight decks of a steam heated Coe continuous drier. The drying time is about 168 minutes, and the steam cost is about $1.00 per thousand square feet of one-half inch thick board.

As the board emerges from the drier it is automatically cut into standard size sheets, and may be stapled or glued to various thicknesses for special purposes.

In locating the factory the most important consideration is nearness of raw material. If the plant is located in a good corn producing area the average haul for the raw material should be about four miles, making the hauling cost $1.54 per ton of board. This cost increases to $6.70 for a haul of 225 to 250 miles. Other important considerations in choosing the location are: nearness to market, cost of electric power, water supply, cost of fuel for heating drier.

Cost figures indicate that board can be manufactured for $18.84 per thousand square feet in a plant designed to produce a board 4 feet wide; for $15.74 in an 8 foot plant; and for $14.89 in a 12 foot plant. The 4 foot plant cost is $215,550; 8 feet, $292,900; and 12 foot, $381,750.

By reducing the cooking to a mere soaking in hot water, and mixing in about 15 per cent newsprint, the cost of producing the board in the 12 foot plant is reduced to about $12 and the cost of the plant to $352,000. The board produced by this process is light grey in color compared to the tan color of the cooked board. Its strength, weight, moisture resistance and insulation value are about the same as for the cooked pulp board.

THE PHYSIOLOGICAL ACTION OF CYSTINYL PEPTIDES AND GUANIDINE DERIVATIVES[1]

H. JAMES HARWOOD

From the Department of Chemistry, Iowa State College

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I. GUANIDINE DERIVATIVES

The observation that the administration of guanidine hydrochloride resulted in a decrease in blood sugar suggested to a number of workers the possibility that a substance might be discovered which could be used as a substitute for insulin. Of the many compounds studied, one, synthalin (decamethylene diguanidine), because of its very marked hypoglucemic action was recommended for clinical use as a substitute for insulin. Further study of this compound, however, showed it to have an action fundamentally different from that of insulin. Synthalin was also unreliable and in many cases quite toxic. As a result of these findings the search for an insulin substitute has been continued. In the present work a variety of guanidine derivatives and related substances have been prepared for testing as to their effects on blood sugar.

The following compounds were prepared by methods described in the literature: phenylguanidine, diacetoneguanidine, acetyldiacetoneguanidine, formylguanidine, dibenzoylguanidine, anhydrodiacetaylguanidine, dicarbethoxyguanidine, β-carboxylpropionylguadinidine, malonylguanidine, thiobarbutic acid, iminimethyluracil, N′-allyl-N″-guanyl-thiourea, dipiperidyl disulfide, dipiperidyl sulfone, dethylaminomethyl ethyl sulfide, benzimino phenylthioether, diformamidine disulfide hydrochloride and propionomidine hydrochloride.

p-Bromophenylguanidine, *p*-carboxylphenylguanidine and benzylguanidine were prepared by the action of a salt of the corresponding aniline derivative on cyanamide. Benzylguanidine, furfurylguanidine sulfate and β-phenylethylguanidine sulfate were prepared by the action of the corresponding amine on methylisothiourea sulfate. Guanidine benzoate was prepared from guanidine -and -benzoic -acid. *1,3*-Diphenyl-*3*-guanidino-propane-*1* was obtained by the action of guanidine on benzalacetophenone. Furylacrylylguanidine was prepared by the action of guanidine on ethylfurylacrylate and, similarly, cinnamylguanidine was prepared by the action of guanidine on ethyl cinnamate. γ-Guanidinopropyl *n*-butyl sulfide was prepared by the action of γ-aminopropyl *n*-butyl sulfide on methylisothiourea sulfate. This amine was obtained by treating γ-bromopropylphthalmide with *n*-butyl mercaptan in sodium ethylate solution; the resulting phthalimido compound was then hydrolyzed to the amine. Similarly, γ-guanidinopropyl β-phenylethyl sulfide and γ-guanidinopropyl cyclohexyl sulfide were obtained starting with β-phenylethyl mercaptan and cyclohexyl mercaptan, respectively. *n*-Butylthiobarbituric acid was prepared from *n*-butylmalonic ester and thiourea.

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[1]Original Thesis submitted September, 1931.

The physiological tests were made by intravenous injections into starved rabbits followed by periodical blood sugar determinations[2].

Compounds which had no effect on the blood sugar were: *p*-carboxyl phenylguanidine, diacetoneguanidine, furylacrylylguanidine hydrochloride, cinnamylguanidine hydrochloride, formylguanidine, dibenzoylguanidine, anhydro-diacetylguanidine, γ-guanidinopropyl *n*-butyl sulfide sulfate,*n*-butylthiobarbituric acid, iminomethyluracil, N′-allyl-N″-guanyl-thiourea, dipiperidyl disulfide, dipiperidyl sulfone and diethylaminomethyl ethyl sulfide.

Compounds which raised the blood sugar were: *1, 3*-diphenyl-*3*-guanidino-propanone-*1* and hydrochloride, β-carboxylpropionylguanidine, γ-guanidinopropyl cyclohexyl sulfide sulfate, malonylguanidine, thiobarbituric acid, benzimino phenylthioether, hydrochloride, diformidine disulfide hydrochloride and propionamidine hydrochloride.

Compounds which lowered the blood sugar were: phenylguanidine, *p*-bromophenylguanidine nitrate, benzylguanidine nitrate, furfurylguanidine sulfate, β-phenylethylguanidine sulfate, guanidine benzoate, acetyldiacetoneguanidine, dicarbethoxyguanidine, and γ-guanidino-propyl β-phenylethyl sulfide sulfate.

While the above compounds showed hypoglucemic action, the action in no case approached that of insulin or synthalin and in most cases was accompanied by toxic effects. None of the compounds studied could be recommended as a substitute for insulin.

II. CYSTINYL PEPTIDES

The preparation of insulin as a crystalline product with definite physical properties was followed by a large amount of work on the chemical behavior of the hormone. These studies showed insulin to possess the properties of a protein or polypeptide containing the amino acids cystine, tyrosine, arginine, histidine, leucine and perhaps lysine. Whether the activity of insulin is due to the molecule as a whole or to some group within the molecule has not been determined. It appears, however, that the activity bears some relation to the sulfur content of the molecule and that it is also in some way dependent upon the presence of a free amino or imino and carboxyl group.

In the present work a number of polypeptides were prepared consisting of cystine in combination with other amino acids found in insulin. The hope was to find some grouping which would display the hypoglucemic action of insulin. Cystine was included in all of these combinations since it contains the very reactive disulfide linkage which, it has been suggested, might be concerned as a catalyst in the metabolic oxidation of sugar.

Dicarbethoxytryrosylcystine diethylester was prepared by the action of dicarbethoxytyrosyl chloride on cystine diethylester. The latter compounds were prepared by methods described in the literature. Dicarbethoxycystinyltyrosine ethylester was prepared by the action of dicarbethoxycystinyl chloride on tyrosine ethylester. Dicarbethoxycystinyl chloride was obtained by the action of ethyl chlorocarbonate on an alkaline solution of cystine followed by treatment of the free acid with phosphorus pentachloride. Dicarbethoxycystinyltyrosine was prepared by hydrolysis of

[2]The compounds were tested in the laboratories of Parke-Davis and Company.

the ester and also by the action of dicarbethoxycystinyl chloride on an alkaline solution of tyrosine. Dicarbethoxycystinylhistidine methylester was prepared by the action of dicarbethoxycystinyl chloride on histidine methylester. Dicarbethoxycystinylarginine was prepared by the action of dicarbethoxycystinyl chloride on an alkaline solution of arginine. The esters of tyrosine and histidine were prepared by methods described in the literature.

Several different physiological tests (including the effect on blood sugar) of the above polypeptides have not yet been completed[3].

[3]The compounds are being tested in the laboratories of Parke-Davis and Company.

THE EFFECTS OF MOLYBDENUM AND CHROMIUM ON THE MALLEABILIZATION OF WHITE CAST IRON[1]

EVERETTE LEE HENDERSON

From the Department of Chemistry, Iowa State College

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The present investigation was undertaken with the object of determining the quantitative effect of molybdenum and chromium, separately and in combination, upon the rates of graphitization of white cast iron.

EXPERIMENTAL

A. PREPARATION AND CHEMICAL COMPOSITION OF THE ALLOYS

Seventeen alloys were prepared from a commercial white cast iron base. Five of these alloys contained from one to five per cent molybdenum by weight. Nine of these alloys contained combinations of molybdenum and chromium from one to five per cent of each element. The remaining three alloys contained one, three and five per cent of chromium and no molybdenum.

Five thousand grams of the commercial cast iron were melted in a plumbago crucible by means of a 35 kv. induction furnace. The calculated quantities of ferro-molybdenum or ferro-chromium were added to the molten metal. Small amounts of powdered graphite and ferro-silicon were added to compensate for the burning out of these elements at the high temperature. The molten alloy was stirred with a pure iron rod just before pouring to assure a homogeneous composition. The alloys were cast into sand moulds at a pouring temperature of approximately 1350°C. and were allowed to cool to room temperature before breaking open the moulds.

The chemical composition of the white cast iron base and the molybdenum alloys is given in table 1.

TABLE 1

	Weight percentage					
Melt No.	Mo	C	Si	Mn	S	P
S-1-1	0.922	2.79	0.781	0.21	0.033	0.148
S-6-2	1.85	2.52	0.806	0.20	0.037	0.151
S-7-3	3.07	2.63	0.644	0.19	0.037	0.150
S-14-4	4.10	2.58	0.650	0.21	0.035	0.149
S-15-5	4.96	2.61	0.615	0.20	0.033	0.151
Com. Cast	0	2.61	0.781	0.20	0.033	0.154

No analysis of the chromium containing alloys was made because it was learned early in the investigation that graphitization was completely stopped in alloys containing as low as one per cent of chromium.

Samples of all compositions were found to be extremely hard as cast and some difficulty was experienced in preparing them for chemical analysis.

[1]Original Thesis submitted July, 1931.

B. HEAT TREATMENT

As a preliminary operation, one inch samples of each composition were placed in cast iron pipe containers. The packing material was twenty mesh gas carbon. The samples and containers were placed in a Hump electric annealing furnace at 932°C. A container, with a sample of each composition, was removed at the following intervals:

½ hour, 10 hours, 48 hours, 100 hours, 74 hours, 257 hours

Upon removal at the stated intervals, the samples were ground down to a depth of about three-sixteenths inch. They were then polished and examined microscopically, both etched and unetched, to determine the progress of the decomposition of the massive cementite. A five per cent solution of concentrated nitric acid in ethyl alcohol was employed as the etching solution.

As soon as the approximate time for the primary stage of graphitization was determined, the above procedure was repeated until the exact time was found for each composition at the elevated temperatur of 932°C. At least four samples of each composition was given the predetermined treatment.

A similar procedure was undertaken to determine the minimum time required for the secondary stage at 704°C. The samples, which had previously been given the required treatment at 932°C., were placed in the furnace at 932°C. and held at this temperature for one-half hour. The furnace was then cooled to 704°C. at a controlled rate of 18.5°C. per hour. This temperature was then maintained and samples were removed at twenty-four hour intervals and examined microscopically to determine the progress of the secondary graphitization stage. After the proper time had been found, all samples were returned to the furnace at proper intervals to insure that all samples of the same composition would have the same time at 704°C. When the proper time had elapsed, the furnace was cooled under control to 450°C. at a rate of 6.8°C. per hour. The samples were then removed from the furnace and air cooled in the carbon pack.

The samples were then ready for final microscopical examination and photographic study.

PRESENTATION OF RESULTS

The following table shows the optimum time required for the completion of the primary and secondary stages of graphitization. Percentages of molybdenum are given in round numbers.

TABLE 2

	Weight percentage		Time for complete graphitization	
Alloy no.	Mo	Cr	At 932°C.	At 104°C.
S-1-1	1.00	0	75 to 80 hrs.	125 hrs.
S-6-2	2.00	0	90 to 100 hrs.	160 to 165 hrs.
S-7-3	3.00	0	150 to 155 hrs.	195 to 200 hrs.
S-13-4	4.00	0	200 to 205 hrs.	245 to 250 hrs.
S-14-5	5.00	0	Incomp. 255 hrs.	Incomp. 250 hrs.
X-9	1.00	1.00	No effect 406 hrs.	No effect 255 hrs.
X-13	0	1.00	No effect 406 hrs.	No effect 255 hrs.
Com. Cast	0	0	60 to 65 hrs.	100 to 105 hrs.

These results were obtained by examintaion of the samples under the microscope and by a study of the micro-photographs. Photographs were made of the samples at intermediate stages of graphitization and of the completely graphitized alloys. These show that the molybdenum serves to refine the grain structure. This partially explains the increased hardness and toughness of those alloys which contain molybdenum.

CONCLUSIONS

Molybdenum, when present up to five per cent in white cast iron, exhibits an inhibiting effect upon the decomposition of the iron carbide, but does not completely stop the graphitization process. Both stages of graphitization show a decreasing rate with increasing quantity of molybdenum as indicated by a smooth curve which results by plotting the time required for completion of the graphitization against the percentage of molybdenum in the alloys.

Chromium has been found to completely stop the decomposition of the iron carbide under a heat treatment of 406 hours at 932°C. and 255 hours at 704°C.

The actual requirements as regards the necessary heat treatment for completion of graphitization are given in table 2.

No attempt has been made to correlate the physical properties with the chemical composition except the observations that were made during the breaking, grinding and polishing of the samples. Such observations have led to the belief that molybdenum imparts increased hardness, toughness and tensile strength to the malleablized iron. The alloys show a pronounced refinement of grain structure.

SUMMARY

A study of the quantitative effects of molybdenum, chromium and a combination of these two elements up to five per cent, on the stability of cementite has been made.

2. Rates of the first and second stages of graphitization have been determined for alloys containing from one to five per cent of molybdenum.

3. It has been shown that the presence of chromium completely stops the graphitization process.

FURFURAL AND SOME OF ITS DERIVATIVES[1]

AMIOT P. HEWLETT

From the Department of Chemistry, Iowa State College

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INTRODUCTION

Furfural may be secured by the acid hydrolysis of practically all vegetable matter. Farm waste products, therefore, are the chief sources of this substance. At the present time, the furfural of commerce is largely prepared from oat hulls. The annual possible production of furfural in this country, from farm waste products, is practically unlimited. The total available quantity from oat hulls and corncobs alone is about 1,104,200,000 pounds per year, while the total production for the year 1929 was only 1,500,000 pounds. Therefore, the production of furfural depends only on the demand for that substance. At the present time, furfural is being used as a substitute for the more expensive formaldehyde in the preparation of resins of the bakelite type, as insecticides and fungicides and as an embalming fluid.

This is a part of the work being done at Iowa State College on utilization of agricultural waste products. In this work the problem of utilization of furfural is being attacked from the point of view of chemistry. This consists of the preparation of furfural derivatives, which are to be used in the synthesis of more valuable and more useful compounds and also the synthesis of antiseptics and dyes.

EXPERIMENTAL

5-Bromofurylacetenylmagnesium Bromide. This compound was prepared by the addition of 5-bromofurylacetylene[2] to a solution of ethylmagnesium bromide in ether. Treatment of the resulting solution with carbon dioxide gave the corresponding 5-bromofurylpropiolic acid in yields of 21.8 per cent, while treatment with α-naphthyl isocyanate gave the corresponding α-naphthalide, melting at 150°, in yields of 35 per cent.

The 5-bromofurylacetylene was prepared by bromination of furylacryloyl chloride[3] to produce 5-bromofurylbromoacryloyl chloride in yields of 96 per cent (crude) boiling at 90°-95°/24 mm. and melting at 69°-70°. The pure compound boils at 182°-183°/21 mm. and melts at 72°. The 5-bromofurylbromoacryloyl chloride was hydrolyzed with dilute sodium hydroxide to yield 69.5 per cent (crude) of 5-bromofurylpropiolic acid melting at 127°. The pure acid melts at 143°. The crude 5-bromofurylpropiolic acid was subjected to steam distillation to produce 29.4 per cent of 5-bromofurylacetylene boiling at 63°-64°/24 mm. This compound was con-

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[1]Original Thesis submitted December, 1931.

[2]Gibson and Kahnweiler, *Am. Chem. J.*, 12, 314 (1890).

verted to the diacetylene, melting at 126°, which agrees with the value reported in the literature[2].

Furyl-Alkyl Halides. 1-Furyl-3-chloropropane and 1-tetrahydrofuryl-3-chloropropane were prepared by the action of thionyl chloride on the corresponding alcohols. The 1-furyl-3-chloropropane was prepared in yield of 20 per cent from furylpropyl alcohol [4] by the method used by Kirner[5] in the preparation of α-furfuryl chloride. This compound boils at 60°/5 mm. and is a colorless liquid, d_{25}^{25} 1.0813; n_D^{25} 1.4730.

Analysis. Calculated for C_7H_9OCl: Cl, 24.57. Found: Cl, 24.49 and 24.73.

The 1-tetrahydrofuryl-3-chloropropane is best prepared by addition of one mole of thionyl chloride to a boiling solution of one mole of the tetrahydrofurylpropyl alcohol[4] in benzene. The yield of the chloride boiling at 75°/4 mm. was 82.5 per cent. This compound is a colorless liquid, d_{25}^{25} 1.0425; n_D^{25} 1.4540.

Analysis. Calculated for $C_7H_{13}OCl$: Cl, 23.90. Found: Cl, 23.74 and 23.76.

The 1-furyl-3-chloropropane did not react with magnesium in some preliminary tests, while the 1-tetrahydrofuryl-3-chloropropane reacted vigorously to form the corresponding Grignard reagent in yields of 91 per cent, by acid titration. This Grignard reagent was treated with carbon dioxide to yield 47.5 per cent of tetrahydrofuryl-*n*-butyric acid boiling at 145°/5 mm. This acid is a colorless liquid with an unpleasant odor, d_{25}^{25} 1.2286; n_D^{25} 1.4572; neutralization equivalent 165, the calculated value being 158.

Analysis. Calculated for $C_8H_{14}O_3$: C, 60.76; H, 8.86. Found: C, 60.89 and 60.72; H, 8.66 and 8.69.

The 1-tetrahydrofuryl-3-chloropropane was converted quantitatively into tetrahydrofurylpropyl thiocyanate by heating a mixture of one mole of the chloride in 500 cc. of alcohol and 1.5 moles of potassium thiocyanate in a sealed tube at 120° for 12 hours. Tetrahydrofurylpropyl thiocyanate is a colorless liquid boiling at 138°/8 mm., d_4^{30} 1.0660; n_{D3}^{0} 1.4890.

Analysis. Calculated for $C_8H_{13}ONS$: S, 18.71. Found: S, 18.93.

The 1-tetrahydrofuryl-3-chloropropane was converted into tetrahydrofurylpropyl mercaptan in yields of 34 per cent by heating a mixture of equimolecular proportions of the chloride and thioureau and treating the resulting product with ammonium hydroxide. The mercaptan is a liquid with an extremely offensive odor, boiling at 85°/10 mm., d_4^{25} 1.0006; n_D^{24} 1.4807.

Anlysis. Calculated for $C_7H_{14}OS$: S, 21.91. Found: S, 21.60.

5-Chlorofuroic Acid. This compound was formerly prepared by Hill and Jackson[6]. 5-Chlorofuroyl chloride was prepared by treatment of furoyl chloride with chlorine at a temperature of 100°. This compound was secured in yields of 66 per cent, boiling at 92°-95°/10 mm. and was converted to the corresponding amide which melted at 154°, which is in agreement with the value reported elsewhere[6]. The chloro-amide was hydrolyzed to yield 54 per cent of 5-chlorofuroic acid melting at 177°[6].

[2]Gilman and Hewlett, *Iowa State College Jour. Science*, **4**, 31 (1930).
[4]Adams and Bray, *J. Am. Chem. Soc.*, **59**, 2101 (1927).
[5]Kirner, *J. Am. Chem. Soc.*, **50**, 1955 (1928).
[6]Hill and Jackson, *Am. Chem. J.*, **12**, 22 (1890).

Lachrymators of the Furan Series. The lachrymatory power of furoyl chloride, 5-chlorofuroyl chloride, tetrahydrofuryl iodoacetate and tetrahydrofuryl chloroacetate has been determined. While these substances are lachrymators, they are less effective than chloroacetophenone. The tetrahydrofuryl chloroacetate was prepared in yields of 99 per cent, boiling at 110°/5 mm. by addition of one mole of choloracetyl chloride to a boiling benzene solution of one mole of tetrahydrofurfuryl alcohol. This compound was previously prepared by Gilman and Dickey[7]. The iodoacetate was prepared by heating a solution of the choloroacetate in alcohol with sodium iodide. The yield of iodoacetate boiling at 130°/5 mm. was 65 per cent.

Analysis. Calculated for $C_7H_{11}O_3I$: I, 47.21. Found: I, 47.14.

Antiseptics. The phenolic esters of furoic acid were prepared by the Schotten-Baumann reaction, using furoyl chloride and an alkaline solution of the phenol, with the exception of catechol and hydroquinone furoates, which were prepared by heating a mixture of the acid chloride and the phenolic compound. The esters of furylacrylic acid were prepared by refluxing a benzene solution of the acid chloride and the phenolic compound. The full ester was always secured in the case of the furoates, while the half esters were secured in the case of the furylacrylates, with the exception of resorcinol furylacrylate. In the case of this compound, either the half or full ester was secured, depending upon the quantity of furylacryloyl chloride used. Tetrahydrofurfuryl oxalate was prepared by heating anhydrous oxalic acid with a 200 per cent excess of the alcohol. Tetrahydrofurylpropyl oxalate was prepared by treating the sodium salt of the alcohol in benzene with equimolecular proportions of oxalyl chloride. Tetrahydrofurfuryl salicylate was prepared by refluxing a solution of salicyloyl chloride[8] and tetrahydrofurfuryl alcohol in benzene. The esters were all hydrolyzed and the acids identified by mixed melting point. In cases where the composition of the ester was in doubt, verification was effected by determination of saponification equivalent and the melting points of mixtures of the ester with the acid and also with the phenol.

A study of the antiseptic property of these compounds is now being made. The results will be reported at a later date.

Insecticides of the Furan Series. The insecticide property of a number of furan compounds has been studied. They were all found to be relatively inactive, with the exception of tetrahydrofurylpropyl thiocyanate, benzoylfuran[8] and the *p*-cresol and phenyl esters.

Furfural Diacetate. Following the method of Gilman and Wright[9] it was found possible to secure furfural diacetate in yields of 60-70 per cent by the use of one gram of stannous chloride per mole of furfural and allowing the reaction mixture to stand in an ice box for 48 hours. Also, yields of 50 per cent were secured by addition of one mole of acetic anhydride to a solution of two grams of stannous chloride in one mole of furfural at a temperature of 20°-30°. The mixture was then stirred for two hours, filtered, crystallized from carbon disulphide and distilled.

[7]Gilman and Dickey, Unpublished results.

[8]Adams and Ulich, *J. Am. Chem. Soc.*, 42, 604 (1920).

[9]Gilman and Wright, *Iowa State College Jour. Sci.*, 4, 35 (1929).

TABLE 1. *Data on esters*

No.		M. p. °C.	B. p. °C.	P. mm.	Yield Pctg.
1	Phenyl furoate	42	145	44	94
2	Phenyl furylacrylate	.....	185	4	84
3	Guaiacol furoate	76	175	5	82
4	Guaiacol furylacrylate	105	210	6	70
5	*p*-Cresol furoate	55	152	5	64
6	*p*-Cresol furylacrylate	75	195	6	71
7	*m*-Cresol furoate	40	155	5	90
8	*m*-Cresol furylacrylate	.....	185	5	61
9	Resorcinol furoate	130	.....	....	37
10	Catechol furoate	116	.....	....	34
11	Hydroquinone furoate	200	.....	....	27
12	Resorcinol furylacrylate	128	.....	....	35
13	Hydroquinone furylacrylate	173	.....	....	30
14	Catechol furylacrylate	132	.....	....	35
15	Resorcinol di-furylacrylate	112	.....	....	95
16	Tetrahydrofurfuryl oxalate	.....	203	4	58
17	Tetrahydrofurylpropyl oxalate	.....	210	3	51
18	Tetrahydrofurfuryl salicylate	.....	166	5	78

Analyses

No.	Carbon, Pctg.		Hydrogen, Pctg.		Saponification equivalent
	Calcd.	Found	Calcd.	Found	
1	70.21	70.16	4.25	4.42	
2	72.88	72.78	4.67	4.87	
3	66.05	65.92	4.69	4.74	225
4	68.85	69.23	4.92	5.12	
5	71.28	71.66	4.95	4.82	
6	73.68	73.56	5.26	5.31	
7	71.28	70.95	4.95	4.90	
8	73.68	73.96	5.26	5.39	
9	64.43	64.10	3.35	3.19	147
10	64.43	64.73	3.35	3.61	145
11	64.43	64.59	3.35	3.81	150
12	67.83	67.95	4.35	4.50	208
13	67.83	67.50	4.35	4.34	208
14	67.83	67.76	4.35	4.72	208
15	68.57	69.02	4.00	4.23	165
16	55.81	55.79	6.98	7.21	127
17	61.15	61.00	8.25	8.14	157
18	63.26	63.25	5.77	5.92	

PREPARATION OF SECONDARY AND TERTIARY FURYL CARBINOLS

Following the work of Hale, McNally and Pater[10] and of Peters and Fischer[11], respectively, diphenyl furyl and phenyl furyl carbinols were prepared. These compounds are unstable, but seem to be more stable than was formerly indicated. Di-*tert.* butyl furyl carbinol was prepared by the action of ethyl furoate and also furoyl chloride on *tert.* butylmagnesium chloride. The di-*tert.* butyl furyl carbinol was not secured in the pure condition, analyses indicating that the substance was a mixture. This material, however, is quite stable. 5-Chlorofuryl and 5-bromofuryl carbinols were prepared by the action of the corresponding aldehyde on phenylmagnesium bromide. These compounds undergo decomposition on standing, with elimination of halogen acid and with the formation of a small amount of a stable, halogen free, compound melting at 86°. The same substance is secured from both of the above mentioned compounds. The 5-bromofurfural was prepared by the method of Gilman and Wright[12]. The 5-chlorofurfural was prepared by the action of sulfuryl chloride on a solution of furfural diacetate in carbon disulphide according to the method of Gilman and Wright[13].

Di-*tert.* butyl furyl carbinol is a yellow liquid boiling at 80°85°/1 mm., d_4^{34} 0.9486; n_D^{34} 1.4749. The yield was 25 per cent.

Analysis. Calculated for $C_{13}H_{22}O_2$: C, 74.29; H, 10.48. Found: C, 73.56 and 73.45; H, 9.87 and 9.73.

5-Chlorofuryl phenyl carbinol is a white solid melting at 113°, yield 24 per cent.

Analysis. Calculated for $C_{11}H_9O_2Cl$: Cl, 17.07. Found: Cl, 17.28 and 17.19.

The 5-bromofuryl phenyl carbinol is a white solid melting at 128°, yield 23.6 per cent.

Analysis. Calculated for $C_{11}H_8O_2Br$: Br, 24.69. Found, Br, 24.38 and 24.11.

Dyes of the Furan Series. *p*-Dimethylaminophenylmagnesium bromide was prepared in yields of 40-45 per cent, based on acid titration, by the action of *p*-bromodimethylaniline on magnesium in the presence of one equivalent of "magnesious iodide." This Grignard reagent was treated with ethyl furoate to prepare the furyl analog of malachite green, formerly described by Renshaw and Naylor[14]. The yields were very unsatisfactory and no stable derivatives of the carbinol base were secured. The furyl analog was also prepared by the method of Renshaw and Naylor[14] and the only comparison made between the compounds from the two sources was that of the color produced on fabric and the analyses of the platinum salts.

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[10]Hale, McNally and Pater, *Am. Chem. J.*, **35**, 68 (1906).

[11]Peters and Fischer, *J. Am. Chem. Soc.*, **52**, 2079 (1930).

[12]Gilman and Wright, *J. Am. Chem. Soc.*, **52**, 1170 (1930).

[13]Unpublished results.

[14]Renshaw and Naylor, *J. Am. Chem. Soc.*, **44**, 862 (1922).

The 5-bromofuryl, 5-chlorofuryl and nitrofuryl analogs were prepared by the condensation of the corresponding aldehyde with dimethylaniline in the presence of zinc chloride. In the case of the nitrofuryl analog, no condensation agent was necessary, the mixture merely being allowed to stand for one week. The nitrofurfural was prepared by the method of Gilman and Wright[15]. The leuco bases were oxidized according to the usual method for malachite green[14] and the zinc chloride double salts were prepared and found to be stable dyes, producing blue colors on silk, artificial silk, wool and mordanted cotton. The platinum salts of the carbinol bases were prepared and analyses for platinum found to agree with the theoretic values. The furyl analog, leuco base, was secured in yields of 6.9 per cent melting at 87°; the 5-bromofuryl analog in yields of 12.9 per cent melting at 132°; the 5-chlorofuryl analog in yields of 14.1 per cent melting at 117°; and the nitrofuryl analog in yields of 23.5 per cent melting at 135°.

Analyses. Calculated for $C_{21}H_{23}BrN_2O$ (bromofuryl analog): Br, 20.05. Found: Br, 19.88. Calculated for $C_{21}H_{23}ClN_2O$ (chlorofuryl analog): Cl, 10.01. Found: Cl, 10.11 and 10.09. Calculated for $C_{21}H_{23}N_3O_3$ (nitrofuryl analog): C, 69.04; H, 6.30. Found: C, 68.66 and 68.93; H, 6.27 and 6.42.

5-Bromofurfural, 5-chlorofurfural and nitrofurfural were treated with a mixture of aniline and aniline hydrobromide in an attempt at preparation of the analogous hydroxyglutaconaldehydedianilides and 3-hydroxy-1-phenylpyridinium salts of Stenhouse and others [16]. It was found impossible to secure the nitro analog, complete decomposition resulting. However, the same identical substance was secured from both 5-bromofurfural and 5-chlorofurfural when treated with a mixture of aniline and aniline hydrobromide. Analyses show that the halogen substituent of the ring is lost during the condensation. This compound melts at 145° and was secured from both sources in yields of 63 per cent. This compound is yellow in color, slightly soluble in water and dyes silk, artificial silk, wool and cotton in a golden yellow color which is very fast. This compound is not converted into the pyridinium salt by the methods used for the conversion of the compound of Stenhouse and others [16]. For this reason, the structure suggested for this substance is $PhN{=}C{=}CH{-}CH{=}C(OH){-}CH{=}NPh{\cdot}HBr{\cdot}H_2O$.

Analysis. Calculated for $C_{17}H_{15}BrN_2O{\cdot}H_2O$: Br, 22.16. Found: Br, 22.16 and 21.94.

[15]Gilman and Wright, *J. Am. Chem. Soc.*, 52, 2550 (1930).

[16]Stenhouse, *Ann.*, 156, 199 (1870); Zincke and Mulhausen, *Ber.*, 38, 3824 (1905); Konig, *J. prakt. Chem.*, 69, 105 (1904); *ibid*, 72, 555 (1905); Dieckmann and Beck, *Ber.*, 38, 4122 (1905).

SUMMARY

5-Bromofurylacetenylmagnesium bromide has been prepared and characterized by conversion to the corresponding acid and α-naphthalide.

1-Furyl-3-chloropropane and 1-tetrahydrofuryl-3-chloropropane have been prepared and described. The latter reacts with magnesium to form the Grignard reagent, and was also converted to the corresponding thiocyanate and mercaptan.

5-Chlorofuroyl chloride has been prepared and described. This compound was converted to the corresponding acid and amide.

The lachrymatory properties of a group of furan compounds have been studied.

The furyl and furylacrylic esters of phenol, guaiacol, *p*-cresol, *m*-cresol, resorcinol, catechol and hydroquinone and the tetrahydrofurfuryl and tetrahydrofurylpropyl esters of oxalic acid and the tetrahydrofurfuryl esters of salicylic acid have been prepared and described.

Di-*tert.* butyl furyl, 5-bromofuryl phenyl and 5-chlorofuryl phenyl carbinols have been prepared and described.

The 5-bromofuryl, 5-chlorofuryl and nitrofuryl analogs of malachite green have been prepared and described. A condensation product of 5-chloro and 5-bromofurfural with aniline and aniline hydrobromide has been prepared and described.

A STUDY OF SOME OF THE LACTOBACILLI[1]

LINCOLN SPENCER HYDE

From the Department of Dairy Industry, Iowa State College

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Certain members of the genus Lactobacillus (Beijerinck) are of particular importance to the dairy industry; namely, *L. acidophilus* (Moro), *L. bulgaricus* (Grigoroff), and *L. casei* (v. Freudenreich). The first two have in recent years occasioned increasing interest because of their suggested therapeutic value in the treatment of intestinal disorders and because they are so alike culturally that some doubt still exists as to definite differentiating characters. The third, although recognized several years prior to the others, has received comparatively little attention except in its relationship to the ripening of certain types of cheese.

This investigation of various organisms usually considered as belonging to these three species was made with a view to correlating some of the more outstnding characters, which were considered valuable in poper identification and classification.

RESULTS OBTAINED

The 86 cultures studied were secured from various sources: 33 were from research and commercial laboratories and included 9 cultures of *L. acidophilus,* 7 *L. bulgaricus,* and 17 *L. casei;* 53 were isolated, 16 from the fecal matter of humans and animals were considered as *L. acidophilus,* while 37 from raw milk and cheddar cheese were considered as *L. casei.*

Since fewer failures resulted, the Heymann acetic acid bouillon method of isolating *L. acidophilus* strains from fecal material proved more certain than a dilution method or direct plating, which were apparently successful only under particularly favorable conditions.

Milk proved very satisfactory as an enrichment medium when isolating lactobacilli from milk and cheese. The frequency with which *L. casei* cultures were secured from milk and cheese showed their prevalence in these products; numerous investigators have reported similar results.

For convenience in discussion the organisms were classified into two groups according to the size of the cells; one designated as large, the other as small.

The ability of the cultures to grow at various temperatures seemed to show some correlation between this character and the size of the organisms. For the most part the cultures classed as large among the *L. bulgaricus* and *L. acidophilus* groups were able to grow at 45°C., but were unable to grow or grew poorly at room temperature and at 15-20°C. Conversely, the small cultures in these groups were unable to grow or grew poorly at 45°C., but were for the most part able to grow at the lower temperatures. Twelve large *L. casei* cultures failed to grow at any of these temperatures, while at 37°C. these cultures grew very slowly. Eleven *L. casei* cultures, all except one classed as small, grew at 45°C., but rather poorly, while prac-

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[1]Original Thesis submitted June, 1927.

tically all except the twelve noted grew well at room temperature and at 15-20°C. It appears from these results that in general the large organisms grew better than the small at 45°C., while the small grew better than the large at the lower temperatures. Since *L. bulgaricus* has long been recognized as having a high optimum growth temperature these relationships seemed to be significant. Studies of other characters emphasized this relationship.

Thirteen trials were made using several different media and depressants to determine the effect of lowered surface tension of the medium on the growth of the organisms. Sodium ricinoleate was found to be superior as a depressant to sodium taurocholate, sodium glycocholate or sodium oleate so in most of the trials sodium ricinoleate was used. When the surface tension of the medium was depressed much under 40 dynes the first cultures failing to grow were apparently in the group classed as large. In medium X (Albus and Holm) all of the 60 cultures tested grew at 40 dynes; at 37.4 dynes 23 of the 82 cultures failed to grow—16 of these were large and 7 small. In whey peptone broth at 39 dynes, 9 of 87 cultures failed to grow and all were classed as large; at 37.3 dynes, 58 of 87 failed to grow and it was noticeable that the small *L. acidophilus* and *L. bulgaricus* strains were among the few which grew. In beef infusion bouillon 17 of 60 cultures failed to grow and 16 of these were classed as large. Among 72 cultures tried in medium M (medium X with maltose substituted for lactose), 4 large strains of *L. bulgaricus* and *L. acidophilus* failed to grow without depressant; at 39 dynes, 9 failed to grow and all were classed as large. From these results it is evident that a close relationship exists between the large *L. acidophilus* and *L. bulgaricus* organisms in respect to their ability to grow in media with reduced surface tensions. The results also indicate that 40 dynes is a critical surface tension for the *L. bulgaricus* types, and that the small *L. acidophilus* and *L. casei* types are able to grow at nearly 37 dynes. The suitability of the medium without depressant as well as the depressant used undoubtedly influence the ability of the organisms to grow at reduced surface tensions.

A study of the type of lactic acid produced in milk by 12 cultures considered as *L. acidophilus* showed variations from pure active acid of the dextro modification to practically pure inactive acid. Four of six large cultures produced largely or entirely inactive acid. In other characters, such as growth at 45°C., but not at 15-20°C., failure to grow in medium X at 37.4 dynes, and the failure of one of them to ferment lactose, these cultures closely resembled the *L. bulgaricus* cultures classed as large. These four were among five from other laboratories studied for the type of lactic acid produced.

Determinations of the total and volatile acids produced in milk by 66 cultures did not show any particularly striking relationships. Five of six cultures, two *L. acidophilus* from laboratory sources and three *L. bulgaricus*, producing over 2.0 per cent acid were classed as large, grew at 45°C., but not at 15-20°C., and failed to grow at the lower surface tensions. The volatile acidities varied widely, the values ranging from 4.7 to 41.7. The *L. acidophilus* cultures seemed to produce slightly higher volatile acidities since the values obtained with several were over 30, while none over 30 were obtained with the *L. bulgaricus* cultures and with only an occasional *L. casei* culture.

Sixteen cultures were studied as to their proteolytic activity in milk both with and without $CaCO_3$ during an incubation of 30 days. All of the cultures caused some increase in soluble nitrogen; the values varied from 1.0 to 6.7 per cent in the milk without $CaCO_3$, and from 1.3 to 19.2 per cent in the milk with $CaCO_3$. In nearly all instances a culture caused higher increases in milk with than without the carbonate. When a culture caused the highest increase with $CaCO_3$ it also caused the highest without $CaCO_3$. No particularly significant relationships seemed to exist between the amount of proteolysis and other characters.

These studies of the various characters of the organisms seemed to bring out correlations which would justify certain conclusions as to their inter-relationships. Most of the large cultures in the *L. acidophilus* groups exhibited characteristics which indicated close relationship to the large *L. bulgaricus* cultures. On the other hand, the small *L. acidophilus* types showed close relationship to the *L. casei* cultures since their reactions to the conditions mentioned have been pointed out as being opposite to those shown by the large types. It seems that many of the large types previously considered as *L. acidophilus* should have been considered as *L. bulgaricus* and the small types as *L. casei* or else that two types of *L. acidophilus* exist, one of which is closely related to *L. bulgaricus* and the other to *L. casei*. If the latter view is to be accepted, *L. casei* seems to bear closer relationships to both *L. acidophilus* and *L. bulgaricus* than these two species do to one another. If any one species is to be considered a central type, it appears from this study that *L. casei* rather than *L. acidophilus* should be accepted as the central type. In any case it must be recognized that certain forms are found which apparently are borderline strains lying between two species so that no absolutely definite line of demarcation can be drawn between them. The close relationship between the *L. casei* and *L. acidophilus* types suggested the probability that *L. casei* could be implanted in the intestinal tract since indications are that *L. acidophilus* can be.

Feeding experiments were conducted with four young men and with rats, the effects being determined by bacteriological examination of the feces.

The men consumed daily, with the regular diet, one quart of milk fermented with several cultures of *L. casei* isolated from milk and cheese. The rats were fed suspensions of pure cultures of single strains of *L. casei* or *L. acidophilus* with a basal diet of fresh white bread and fresh beef, with lactose added except in the case of the control rats during the latter part of the trials. Results obtained seemed to justify the conclusion that transformaion of the intestinal flora took place with the men. The change in flora with the rats was less definite although sufficient for an unbiased observer to detect microscopically which fecal smears were from rats receiving cultures and which were from the controls. No such proportions of Gram-positive organisms were found in any fecal smears as have been reported by several investigators. The results were more in agreement with those reported by Kopeloff (1926).

The ability to become implanted in the intestinal tract further emphasized the close relationship between *L. casei* and *L. acidophilus*, particularly between the small strains. It also indicated the probability that many reports of favorable changes in the intestinal flora from milk feeding have been due in part at least to the presence of *L. casei* types

in the milk. The importance to the dairy industry of the prevalence of these types in milk and cheese is further brought out by the results of these trials.

A satisfactory white acidophilus milk was produced by two exposures of a good quality milk at 195-200°F. for one hour each, with an incubation between of three hours at 100°F., although large Gram-positive rods very evidently not *L. acidophilus* were present in every instance. This method was not as consistently successful as when the milk was sterilized.

Storage of acidophilus milk at refrigerator temperature was found to be more satisfactory than at room temperature both from the standpoint of palatability and the number of organisms remaining viable after seven days. The organisms were also found to be able to survive freezing in the form of lacto; the average of six trials showed that 56.7 per cent of the original number were viable after seven days. This was considered a new and satisfactory medium for supplying viable *L. acidophilus* to persons who would not take acidophilus milk in the usual form.

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STUDIES IN VITAMIN A TECHNIC[1]

MARGARET HOUSE IRWIN

From the Department of Foods and Nutrition, Iowa State College

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An application of statistical method to the data of 469 vitamin A feeding tests was made with a view to determining the factors influencing the weight-gains of the test-animals. The data of this study indicated a possible difference in the reactions of rats fed vitamin A-free diets containing different amounts of fats. These data showed also that the quantity of basal diet ingested was the measured variable having the greatest percentage effect upon the weight-gains of the animals.

An experiment was conducted to test the difference in the reactions during the depletion period of vitamin A test animals fed diets containing and not containing fat. One hundred and twenty-three animals were fed the basal diet containing fat and 60 animals the fat free basal diet. The mean initial weight, mean gain, mean days to depletion, and mean basal diet eaten daily for each of these two groups of animals are recorded in table 1.

The difference in the mean gains in weight was not found to be significant, but the difference in the number of days to depletion was significant. These data show that a vitamin A-free basal diet containing fat is preferable to a fat-free basal diet as it shortens the depletion period.

An analysis of the reactions of vitamin A test animals from three different stock colonies was made. One of these colonies came from the Chemistry Department of Iowa State College and was maintained in this laboratory without an organized scheme of mating. The second colony was obtained from the Wistar Institute. These animals were from a strain that had been inbred for fifty generations and were maintained in this laboratory by strictly brother and sister matings. The data for the third colony were very kindly furnished by Dr. Hazel E. Munsell of the Bureau of Home Economics, U. S. D. A., Washington, D. C. The difference in the mean in-

TABLE 1

	Osborne & Mendel vitamin A-free diet containing fat	Sherman & Munsell vitamin A-free diet containing no fat
Mean initial weight, gms.	51.9 ± 0.29	55.3 ± 0.82
Mean gain during depletion, gms.	70.2 ± 0.87	74.6 ± 2.00
Mean days to depletion, days	36.0 ± 0.33	39.0 ± 0.46
Mean basal diet eaten daily, gms.	8.66 ± 0.06	10.67 ± 0.12
Mean calories eaten daily, calories	40.7	38.4

[1]Original Thesis submitted July, 1931.

itial weights, mean gains in weight, mean days to depletion and mean daily food intake during the depletion period of vitamin A test animals from these three colonies were found to be significant. These data show that the results of a study using animals of one colony could not be applied directly to the data of another colony without first testing the homogeneity of the two groups of animals.

A statistical treatment of the data of the vitamin A depletion period of 123 standard test animals from the Wistar colony was made and upon the basis of the knowledge thus gained an attempt was made to control the weight-gains of the animals by controlling the amount of basal diet they consumed. The method used was designed to produce animals whose weights at the end of the depletion period approach more closely the mean than the weights of animals fed the basal diet ad libitum.

The means and probable errors for the depletion period of these 123 animals used for vitamin A tests in this laboratory are:

Mean intitial weight ..... 51.9 ± 0.29

Mean gain ..... 70.2 ± 0.87

Mean days to depletion ..... 35.9 ± 0.33

Mean basal diet eaten daily ..... 8.66 ± 0.06

The correlations between the variables are:

Initial weight and total gain ..... 0.24 ± 0.06

Initial weight and days to depletion ..... 0.53 ± 0.04

Initial weight and mean basal diet eaten daily ..... 0.27 ± 0.06

Total gain and days to depletion ..... 0.31 ± 0.05

Total gain and mean basal diet eaten daily ..... 0.64 ± 0.04

Days to depletion and mean basal diet eaten daily ..... 0.09 ± 0.06

The largest and most significant of these correlations is that between the total gain and the mean basal diet eaten daily, 0.64 ± 0.04. A multiple correlation coefficient (R), and a regression equation were calculated. The initial weight (A), total gain (B), and days to depletion (C), were used as the independent variables and the mean daily food intake ($\overline{X}$) as the dependent variable. The multiple correlation coefficient (R) and the regression equation for this group of 123 animals are:

$$R = 0.77 \pm 0.04$$

$$\overline{X} = 0.07A + 0.05B - 0.09C + 4.56$$

By substituting in the regression equation an estimate of the amount of food to be fed to each animal was made in advance and the animal fed according to a food intake curve based upon the food consumption records of the original 123 animals.

Five groups of twenty rats each were fed. The first group was fed the basal vitamin A free diet ad libitum to serve as a control group. The

second and third groups were fed the estimated mean amount of food, one group being fed for four weeks and the other for five weeks. To determine the quantity of food to be fed to the fourth group the standard deviations of the weekly food intake records of the original group of 123 rats were calculated and the food intake curve lowered the distance of one standard deviation. The fourth group of rats was fed on the basis of this curve. They then received daily a quantity of food equivalent to the original mean minus the standard deviation. In the fifth trial the food intake curve was dropped one and five-tenths standard deviations and the animals fed on the basis of this curve. They received a quantity of food equivalent to the original mean minus 1.5 standard deviations. The mean initial weights, mean weight-gains, mean days to depletion, and the mean daily food intake for each of these five groups of test rats are shown in table 2.

TABLE 2

	Mean initial weight	Mean weight gains	Mean number of days to depletion	Estimated mean daily food intake	Actual mean daily food intake
Rats fed basal diet ad libitum	51.4 ± 1.18	49.1 ± 1.79	34.8 ± 0.55		7.23 ± 0.14
Rats fed estimated mean for four weeks	47.2 ± 1.12	46.7 ± 1.94	33.2 ± 0.68	8.46 ± 0.43	7.15 ± 0.13
Rats fed estimated mean for five weeks	59.2 ± 0.76	57.8 ± 1.82	34.8 ± 0.55	8.96 ± 0.43	8.02 ± 0.12
Rats fed estimated mean minus 1.0	57.9 ± 0.75	43.4 ± 1.51	35.4 ± 0.51	7.68 ± 0.43	7.05 ± 0.08
Rats fed estimated mean minus 1.5	54.2 ± 0.39	49.2 ± 1.74	35.8 ± 0.42	6.79 ± 0.43	6.58 ± 0.04

It was hoped that the limitation of the food intake by this method of feeding would lower the variability in the weight-gains made during the depletion period. The differences in the variance of the weight-gains of the five groups of rats were found to be quite insignificant.

In analyzing the data to discern reasons for the failure of these 100 animals to react positively to the test it was discovered that the test animals and the 123 animals upon which the regression equation was based did not belong to the same population even though both groups were offspring of the highly inbred Wistar stock colony. A study of litter-size within the two groups of animals showed a distribution of different sized litters that was practically the same for both groups. Litter size then was not the factor responsible for the change in the population observed. To determine if the seasonal variation was responsible the original 123 animals were divided into two groups, the animals started on experiment in January, February and March of 1929 making one group and those started in May and June of 1929 a second group. The test animals were started during the succeeding winter and form a third group. The mean differences in the

measured variables and the standard deviations of the differences for these three groups are found recorded in table 3.

TABLE 3

	Initial weight		Gain in weight		Days to depletion		Mean daily food intake	
	Diff.	σ	Diff.	σ	Diff.	σ	Diff.	σ
Winter 1929 vs. Summer 1929	2.4	1.6	8.7	2.2	3.1	2.1	1.25	0.22
Summer 1929 vs. Winter 1930	1.1	1.7	13.8	2.0	2.8	1.9	0.39	0.18
Winter 1929 vs. Winter 1930	1.3	1.3	22.5	2.5	0.33	1.06	1.6	0.22

The differences in the mean initial weights and mean days to depletion are not significant. The difference in the mean weight-gains are all significant. Two of the differences in food consumption are significant; the third difference, i. e., that between the rats fed during the summer of 1929 and the winter of 1930, approaches significance. These differences led to the conclusion that the seasonal variation was not the factor responsible for the change in the population. Rather there seems to be a progressive change within the colony itself.

This change in the population of the colony is exceedingly interesting and important. It emphasizes the necessity of standardizing vitamin A test animals in regard to these two factors and shows that until more uniform reactions can be ascertained comparative vitamin tests must be run simultaneously in order to prevent such changes in the population from vitiating the results of the experiment.

THE VOLATILE ACIDS FORMED FROM CITRIC AND LACTIC ACIDS BY STREPTOCOCCUS CITROVORUS AND STREPTOCOCCUS PARACITROVORUS

MICHAEL B. MICHAELIAN

From the Department of Dairy Industry, Iowa State College

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A butter culture of good quality contains two types of organisms, one of which *(Streptococcus lactis)* produces primarily lactic acid when grown in milk, while the other *(Streptococcus citrovorus* or *Streptococcus paracitrovorus)* ferments the citric acid with the production of volatile acid and possibly diacetyl. The addition of a small amount of citric acid to milk inoculated with one of the citric acid fermenters results in a pronounced increase in the volatile acid formed, while the addition of lactic acid also results in an increase, but a much smaller one than is secured with the citric acid (1).

The work herein reported involves the study of the amounts and types of volatile products formed in media to which various acids, particularly citric acid or lactic acid, had been added.

The addition of citric acid to phosphate-yeast-beef-infusion bouillon or fermented milk (free from citric acid) (2), inoculated with one of the citric acid fermenters, always gave large increases in the volatile acidities, while the addition of lactic, beta-hydroxy propionic, tartaric, succinic, malic or glycollic acid did not give significant increases.

The addition of citric acid to sterile fresh milk inoculated with one of the citric acid fermenters always gave greater increases in the volatile acid production than when lactic or tartaric acid was added. These latter acids gave appreciable increases in volatile acidity with *S. citrovorus,* while with *S. paracitrovorus* the increases were rather insignificant. The addition of sulfuric or phosphoric acid to sterile fresh milk, inoculated with one of the organisms, gave essentially the same amounts of volatile acids as the addition of lactic or tartaric acid. Apparently, sulfuric and phosphoric acids function in milk cultures in the same manner as lactic or tartaric acid. Since inorganic acids presumably cannot be changed into volatile acids, it appears probable that citric acid is the actual source of volatile acids formed by the citric acid fermenters in milk, and that these organisms are not capable of fermenting lactic acid. The lactic acid may change the citrates naturally present in milk into a form from which volatile acid is readily produced. The fact that *S. paracitrovorus* is capable of producing a small amount of lactic acid in milk, while *S. citrovorus* is not (1), partially explains the reason for the greater volatile acid production in milk by the former than by the latter organism. The availability of the citric acid may have a bearing on the types of volatile acids formed. *S. citrovorus*

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[1]Original Thesis submitted June, 1931.

cultures in milk, with no added acid, produced acetic acid plus a large amount of propionic acid, while *S .paracitrovorus* cultures produced primarily acetic acid, with a trace or a small amount of propionic acid. The type of volatile acids secured with the addition of organic or inorganic acids to milk cultures consisted primarily of acetic acid with a trace (seldom a small amount) of propionic acid.

None of the citric acid fermenters produced diacetyl in fresh milk, while only a few of them produced it when non-volatile organic or inorganic acids had been added to the milk. A greater production of diacetyl resulted from the addition of sterile citric acid to cultures than from the addition of other organic or inorganic acids. An increased amount of volatile acidity was not necessarily accompanied by an increase in the diacetyl production, however. It seems probable that citric acid is the actual source of diacetyl formed. Furthermore, since the addition of increased amounts of organic or inorganic acids (in concentrations which did not inhibit the growth of organisms) to the cultures nearly always increased the amount of diacetyl present, it appears probable that a certain range of pH, variable with different organisms, is an essential factor in the maximum production of diacetyl by the citric acid fermenters. This agrees with the idea held by certain investigators, that a high aroma and flavor in butter cultures cannot be secured without considerable total acidity.

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THE FERMENTATION OF LEVULOSE BY SOME BACTERIA OF THE GENUS AEROBACILLUS[1, 2]

ROGER PATRICK

From the Section of Bacteriology, Iowa Agricultural Experiment Station

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A study of the products of dissimilation of levulose by organisms of the genus Aerobacillus is reported and a classification of species of this genus developed.

The genus Aerobacillus was proposed by Donker (4) to include the aerobic (facultative anaerobic) sporulating rods producing gas from carbohydrates. These forms have previously been included in the genus Bacillus.

Although levulose has been shown by a few investigators to be fermented by species of Aerobacillus, relatively few quantitative investigations have been made of the products of dissimilation.

Bacillus acetoethylicus (Aerobacillus acetoethylicus) has been reported by Northrop, Ashe and Senior (8); Northrop, Ashe and Morgan (7); Peterson and Fred (10; Arzberger, Peterson and Fred (1); Speakman (13); Bakonyi (3); and Donker (4) to yield acetone, acetoin, ethyl alcohol, formic, acetic, lactic and succinic acids; hydrogen and carbon dioxide from carbohydrate media. Northrop, Ashe and Morgan (7) were the only members of this group who worked with a purified levulose. Their interest was in acetone production.

Aerobacillus polymyxa was employed by Donker (4) to ferment glucose. He identified carbon dioxide, hydrogen, acetone, acetoin, ethyl alcohol, formic acid, acetic acid, and 2,3-butylene glycol as products.

McFall (5) made a systematic study of the organisms of the genus Aerobacillus; no attempt was made to determine the products of dissimilation.

The analytical methods used in this study are for the most part standard and only a brief outline is given.

A. CO_2 produced during fermentation. Absorbed in 40 per cent KOH.

2A. Unfermented sugar. Analytical Methods of Assn. of Off. Agr. Chem. (2).

3A. Medium acidified. H_2SO_4, acid to Congo Red.)

b. CO_2 (combined and dissolved).
Absorbed in 40 per cent KOH by heating and aerating, using reflux condenser. Combined with A, precipitated by BaOH and total CO_2 determined gravimetrically.

2b. Distilled

c. Volatile acids and neutral products in distillate. Neutralize with NaOH and distill.

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[1]Original Thesis submitted September, 1931.

[2]Journal Paper No. B67 of the Iowa Agricultural Experiment Station.

d. Volatile products in distillate.

e. Quantitative tests applied for

f. Acetoin, diacetyl. Add peptone solution, $FeCl_3$ and 10 per cent KOH. Copper color shows test for acetoin or diacetyl. Werkman (15).

2f. Add $(NH_4)_2SO_4$ and $Na_2Fe(CN)_6NO$ and NH_4OH. Blue color shows the precence of acetone.

3f. Ethyl alcohol. Add 2,4-dinitrophenyl hydrazine to the solution, precipitate ketone bodies (Van Niel (14), filter and distill. To filtrate add iodine and KOH, iodoform test.

2e. Quantitative tests applied for

f. Total volatile acids produced by oxidation of neutral products. Take aliquot with $K_2Cr_4O_7$ and H_3PO_4, heat, distill, and run partition method to determine the kind and amount of acids (Osburn and Werkman (9). Checked by production of paratoluidine derivatives.

2f. Ethyl alcohol. Remove ketone bodies from aliquot by precipitating with 2,4-dinitrophenylhydrazine and filter. Neutralize the filtrate with NaOH, distill, oxidize distillate with $K_2Cr_4O_7$, distill, determine volatile acids. Osborne and Werkman (9).

3f. Ketone bodies. The amount of acid found under 2f is subtracted from that found under f, the difference is the amount of acid produced by oxidation of the ketone bodies: acetoin, acetone and diacetyl.

2d. Non-volatile residue (Salts of volatile acids). Acidify with H_2SO_4 and distill.

e. Formic acid. To distillate add an excess of $HgCl_2$, reflux one hour, filter precipitate, dry, weigh and calculate formic acid.

2e. Other volatile acids. Distill filtrate of e and use partition method for determination of other volatile fatty acids.

2c. Residue. Non-volatile acids and non-volatile products. Mix with Na_2SO_4 and dry. Extract with ether, evaporate ether, make to volume with distilled water (CO_2) free.

d. Lactic acid. To aliquot add excess $KMnO_4$, make alkaline with NaOH, heat, add alcohol, centrifuge, decant supernatent fluid Add HCl to decanted fluid Heat to drive off

CO_2. Make alkaline (NH_4OH). Add $CaCl_2$, let stand to precipitate calcium oxalate. Filter. Add H_2SO_4 and water to precipitate, heat and titrate with 0.1 N, $KMnO_4$. Calculate lactic acid.

2d. Succinic acid. To an aliquot add $Ba(Cl)_2$ free of CO_2 Filter. Add to filtrate alcohol to 85 per cent. Let stand to precipitate. Filter and determine succinic acid by weighing.

Two strains of *Aerobacillus acetoethylicus,* two strains of *Aero.polymyxa,* and one strainn of *Areo. asterosporus* were employed in this study. The organisms were grown in a levulose medium at 30°C. with and without aeration. Dextrose was substituted in the medium for comparison.

The same products of dissimilation were found with dextrose as with levulose: ethyl alcohol, acetone, acetoin, acetic acid, formic acid, succinic acid, hydrogen and carbon dioxide.

Ethyl alcohol. In aerated cultures maintained at approximately pH 7.0, ethyl alcohol was not formed by *Aero. acetoethylicus* and *Aero. asterosporus;* but was formed in variable amounts by *Aero. polymyxa.* In unaerated cultures, with conditions similar otherwise, ethyl alcohol was always found in cultures of *Aero. acetoethylicus* and *Aero. asterosporus;* however, the production was not constant and the yield was variable in cultures of *Aero. polymyxa.*

Acetoin. Aerated cultures of *Aero. acetoethylicus,* kept approximately at pH 7.0, were found to produce acetoin consistently; but in cultures of *Aero. asterosporus* and *Aero. polymyxa* the production was variable. When the same cultures were not aerated the production of acetoin was variable.

Acetone Aero. acetoethylicus did not produce acetone in aerated cultures (pH 7.0); while *Aero. asterosporus* and *Aero. polymyxa* produced acetone in variable amounts. Unaerated cultures of these three species did not always produce acetone. *Aero. polymyxa* showed the greatest tendency to be constant in the production of acetone. The gas from the fermentation flasks was forced through a solution of 2, 4-dinitrophenylhydrazine hydrochloride to prove that acetone and acetoin did not pass from the aerated fermentation flask with the gas. If these substances were escaping in this way, a hydrazone would have been found; at no time was a precipitate detected.

Acetic acid. Aeration of the culture medium resulted in an unusually high yield of acetic acid as compared with unaerated cultures. The results show considerable correlation between increased production of acetic acid and diminished yields of ethyl alcohol. The species showed no differences in this respect.

Formic acid. Formic acid was always produced; but the yield was variable. There was a greater amount of formic acid produced per gram of sugar fermented in the aerated than in the unaerated cultures.

Succinic acid. The yield of succinic acid was usually small. It was noted that aerated fermentations yielded greater amounts of the acid than the unaerated formentations.

Lactic acid. The yield of lactic acid in aerated and unaerated fermentations was not strikingly different. Lactic acid was present as an end product in both types of fermentations.

Gas. Carbon dioxide and hydrogen were the only gases produced from levulose and dextrose. The amount of carbon dioxide produced from aerated fermentations varied. Hydrogen was not determined quantitatively.

2,3-butylene glycol. The non-volatile residue from aerated and unaerated fermentations was tested for 2,3-butylene glycol, but none was found.

Methyl-glyoxal. The work of Neuberg and Kobel (6) was repeated with the species of Aerobacillus in levulose media. No methyl-glyoxal could be isolated.

Acetaldehyde. Acetaldehyde was detected as an intermediate product when the fermentation was blocked with sodium bisulfite (1.0 per cent). Acetaldehyde was detected qualitatively by the use of Schiff's reagent. Quantitative determinations were not made.

These studies indicate the extreme sensitiveness of yields of products of fermentation to environmental influences. Duplicate flasks showed markd quantitative differences in the yields of end products and even qualitative differences were apparent in the case of such products as acetoin.

Cultural studies of strains of *Aerobacillus asterosporus, Aero. polymyxa* and *Aero acetoethylicus* did not yield results which justify recognition of three species. It is felt that one species, *Aerobacillus polymyxa,* should be recognized to include *Aero. asterosporus* and *Aero. acetoethylicus.*

SUMMARY

The dissimilation of seven strains, representing three species of the genus Aerobacillus was studied. Ethyl alcohol, acetone, acetoin, carbon dioxide, hydrogen, lactic acid, succinic acid, acetic acid and formic acid were formed by these organisms from both dextrose and levulose media. Acetaldehyde was determined as an intermediate product. No 2,3-butylene glycol was isolated. The production of methyl-glyoxal could not be determined.

A study has been made of the cultural characteristics of these species and the conclusions drawn that *Aerobacillus acetoethylicus* and *Aero. asterosporus* should be included in the species, *Aero. polymyxa.*

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SOME DIETARY FACTORS AFFECTING LACTATION IN THE ALBINO RAT[1]

LOUISE JENISON PEET

From the Department of Foods and Nutrition, Iowa State College

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The period of lactation is a critical time in the life of a mother. Since this function requires a reserve of energy which is dependent upon the maintenance of optimum nutrition, the ability to wean vigorous, healthy offspring may be regarded as an index of the adequacy of the mother's diet.

In this investigation, a meat diet, adequate for growth and reproduction in albino rats, had to be supplemented during lactation if optimum and consistent results were to be secured. A well-balanced synthetic ration containing pressure cooked lean beef-round as the chief source of protein was used as the basal diet. To this basal diet were added during the latter part of pregnancy and throughout lactation the dietary modifications which had been recommended by previous investigators as being specifically connected with milk production. These modifications were 15 per cent dried brewery yeast, three times the amount used for maintenance: 6.5 per cent of autoclaved yeast to supply the G factor of vitamin B; 4 drops of tikitiki daily to supply the antineuritic factor of vitamin B; 12 per cent of lemon juice in the drinking water to provide vitamin C; 4 drops of ether extracted wheat germ oil daily to supply vitamin E; increased meat protein, obtained by drying a given weight of the meat to half and using it in the original recipe; increased meat protein and 15 per cent yeast. Each modification was fed to a separate group of rats.

The study was carried through three generations of albino rats, after the parent generation, with which the experiment started. The parent generation was rejected because it had not been previously fed a meat diet. In each generation the group of rats was fed the same supplementary diet during pregnancy and lactation as the parents of the previous generation had received. Accordingly each supplementary factor was carried through one family group from generation to generation. Two females in a generation were remated until each had borne five litters, if possible. In addition to the seven groups of animals on the modified diets, two groups were fed as controls; one group on the basal diet without modification and a second on a Steenbock grain diet.

Environmental conditions were as constant as could be maintained in the nutrition laboratory. The albino rats were kept in all metal cages with false bottoms to prevent access to feces; during the growing and mating period in cages 9″x12″x18″, during pregnancy in individual cages.

As a basis for evaluating the success of the diets in promoting lactation, the following criteria were considered: (1) the age of the mothers when the first litter to be successfully raised was born; (2) the gain or loss in weight of the mothers during the lactation period; (3) the number of young successfully weaned by the mothers on a given diet; (4) the weight and physiological condition of the young at time of weaning; (5) the

[1]Original Thesis submitted June, 1929.

percentage of concentration of the hemoglobin of rats on the ration; (6) the alveolar condition of the mammary glands of the mothers.

Protocols of animals from each group through the three generations are given in tables 1 and 2. All the diets, except the basal, apparently enabled the mothers to bear litters at the normal age of about three months. In this case the average age of the mothers at the birth of the first litters raised was 211.6 days. In some individual cases increasing the meat protein of the diet also seemed to delay the onset of pregnancy.

TABLE 1. *Age of mothers at birth of first litters raised and their gain or loss in weight during the lactation period*

Diet	Average age of mothers at birth of first litter raised (days)	Average gain in weight of mothers during lactation (gms.)	Average loss in weight of mothers during lactation (gms.)
Basal meat	211.6	....	28.2
A 15% yeast	127.8	7.1	....
B Autoclaved yeast	113.4	8.3	....
C 4 drops tikitiki	137.5	....	16.5
D 12% lemon juice	109.4	....	5.5
E 5 drops wheat germ oil	109.8	....	21.2
F Increased meat	114.8	....	6.3
G Increased meat and 15% yeast	119.0	....	3.2
Steenbock stock (control)	132.0	3.0	....

The diets modified by the additions of tikitiki and wheat germ oil and the control basal diet were least efficient in maintaining the weight of the mothers during lactation. It is generally agreed that a diet is adequate for lactation if the mother's weight remains about constant during the period, or shows only slight gain or loss.

Mothers on the increased meat diet plus 15 per cent yeast weaned the largest percentage of the young born in the three generations. But in the first and second generations an even larger percentage was reared by the mothers fed the basal diet with the autoclaved yeast addition.

The growth response of the young was greatest on the diet with increased meat protein plus 15 per cent yeast. Several diets seemed to cause affections of the skin. The young in some litters showed loss of hair, in others ringed tails with a tendency for part of the tail to drop off. This was especially true on the diets containing tikitiki and wheat germ oil.

The hemoglobin concentration per 100 cc. of blood was determined in duplicate for 82 young selected at random from the second and third gen-

TABLE 2. *Percentage of young reared in the three generations and their weights at 21 days*

Diets	Mother rats (no.)	Litters born (no.)	Litters raised (no.)	Young reared on each diet (per cent)	Average weight of young at 21 days (gm.)
Basal meat	5	21	5	24.7	34.9
A 15 per cent yeast	10	44	19	42.4	37.3
B Autoclaved yeast	7	27	17	62.5	33.2
C 4 drops tikitiki	8	22	11	32.0	32.0
D 12 per cent lemon juice	9	27	13	53.3	34.5
E 5 drops wheat germ oil	7	26	13	62.2	33.3
F Increased meat	8	25	14	57.1	37.3
G Increased meat and 15 per cent yeast	8	26	16	71.9	42.3
Steenbock stock (control)	9	19	10	66.6	34.2

erations of the albino rats on the various diets. That the percentages might be comparable, young rats were used at approximatly the age of weaning, between 21 and 30 days of age. The diets which contained, during the suckling period, an increased percentage of yeast, autoclaved yeast, tikitiki, lemon juice and wheat germ oil led to significantly greater hemoglobin formation in the young than the diets containing increased protein.

A comparison was made of the status of the non-functioning mammary gland and of the normally lactating gland with the status of the resting gland.

In the resting gland a small amount of secretion was occasionally found in the alveoli, but most of the alveoli and ducts were empty and separated by a large quantity of connective tissue. The non-functioning tissue, from rats apparently unable to nurse their young, showed a beginning in the development of glandular tissue and some colostrum in the alveoli, indicating some preparation for lactation. Evidently the dietary constituents for building the mammary tissue were not sufficient.

The connective tissues from rats lactating normally were slight in amount so that the groups of alveoli were almost contiguous. The lumina of the alveoli were filled with milk. The blood supply was abundant. This rich blood supply may account in part for the superior lactation obtained.

PREPARATION OF THE LOWER CHLORIDES OF SILICON[1]

JOSEPH BRADLEY QUIG

From the Department of Chemistry, Iowa State College

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Although silicon tetrachloride is well known and easily prepared[2], the lower chlorides such as Si_2Cl_6 and Si_3Cl_8 are difficult to prepare and the yields are low. They are obtained along with $SiCl_4$ during the chlorination of either silicon or ferrosilicon with chlorine gas.

One of the assumptions[3] made as to the mechanism of the reaction during their preparation was that the $SiCl_4$ was first formed and then this reacted further with silicon to form Si_2Cl_6. This latter reaction was favored by high temperature. Martin[4] has shown that the latter reaction does not take place below 144°C. (the boiling point of $SiCl_4$) and that it is slow below 300°C. It has been shown that the yield of lower chlorides is higher at lower temperatures. Martin assumes therefore that the lower chlorides are formed first and that these are further chlorinated to the tetrachloride.

A study was made of the conditions which affect the yields of the lower chlorides using either pure silicon or ferrosilicon (50 per cent). A portion of this work was published[5] in which it was shown that the yields of lower chlorides could be increased materially by mixing vapor of $SiCl_4$ with the chlorine gas. The data obtained are shown in table 1.

TABLE 1. *Effect of mixing $SiCl_4$ vapor with chlorine in the preparation of the lower chlorides of silicon*

Temp. of $SiCl_4$	0°	25°	25°	25°	25°	0°C
Ferrosilicon used	102	76	140	130	105	127
$SiCl_4$ aspirated	83.4	108.9	372.6	370.9	343.8	564.0
$SiCl_4$ per min.	0.1158	0.2016	0.4968	0.5152	1.2813	2.092
Weight of lower chloride, formed	49.4	58.9	114	108	66.4	67.1
Lower chloride, equiv. to Si used	242.7	180.8	331.1	309.3	249.8	302.2
Percentage lower chloride	20.3	32.6	34.2	34.9	26.6	22.3

[1]Original Thesis submitted August, 1926.

[2]Friedel, compt. rend., **73**, 1011 (1871). Troost and Hantefenille, ann. chim. phys., (5) **7**, 453 (1876). Besson and Fournier, compt. rend., **148**, 839 (1909); **149**, 34 (1909). Stock, Brandt and Fischer, Ber., **58**, 648 (1925).

[3]Gatterman and Weinlig, Ber., **27**, 1943 (1894).

[4]Martin, J. Chem. Soc., **105**, 2836 (1914).

[5]Quig with Wilkinson, J. Am. Chem. Soc., **48**, 902 (1920).

The preparation of the lower chlorides depends on the suppression of the two reactions which tend to destroy them.

(a) $Si_2Cl_6 + Cl_2 = 2\ SiCl_4$

(2) $2\ Si_2Cl_6 = 3\ SiCl_4 + Si$

There are two factors which are important in this suppression, namely the chlorine concentration and the temperature. The two are closely related because with increasing chlorine concentration the reaction is more rapid and therefore develops more heat and the temperature of the furnace is raised. To overcome these effects the $SiCl_4$ was added to the chlorine. The effect may be due to any of the following:

(a) The $SiCl_4$ acts as an inert gas to sweep out the lower chlorides as fast as they are formed and before they have had time to decompose.

(b) The $SiCl_4$ acts as a dilutent of the chlorine and thus decreases the rate of the reaction so that the temperature is not raised high enough to decompose the lower chlorides.

(c) The $SiCl_4$ being a product of the final chlorination will tend to prevent the reaction taking place.

The concentration of the $SiCl_4$ was varied by passing the chlorine gas through a flask containing pure $SiCl_4$ and by varying the temperature of the $SiCl_4$ the vapor pressure could be increased and thus the fraction of $SiCl_4$ in the chlorine could be easily regulated.

It is shown that the increase of $SiCl_4$ up to a certain point increases the yield of lower chlorides, but beyond that the yield decreases again. In order to show that it was not because $SiCl_4$ was the end product of the reaction, CCl_4 was substituted for $SiCl_4$ and almost the same yield of Si_2Cl_6 was obtained. This indicates that the effect of the $SiCl_4$ is due to the dilution of the chlorine and the sweeping effect of the dilutent.

It is known that better yields were obtained using 50 per cent ferrosilicon than with pure silicon. This is due to the lower temperature at which the chlorination takes place. It is not due to any catalytic effect of the $FeCl_3$ formeded because the addition of $FeCl_3$ to pure silicon did not increase the yield of lower chlorides. The addition of wood charcoal also had no effect on the yield.

SUMMARY

In the chlorination of silicon or ferrosilicon the formation of lower chlorides takes place first and these are then oxidized to $SiCl_4$. The yields of lower chlorides may be increased by preventing the second reaction. This may be done by keeping the temperature low and diluting the chlorine gas with $SiCl_4$ vapor. The $SiCl_4$ acts as a dilutent only and may be replaced by CCl_4. The ease of chlorination of ferrosilicon compared to pure silicon is due to the lower temperature at which the reaction takes place and is not due to any catalytic effect of the $FeCl_3$ formed.

THE DEVELOPMENT OF SYNTHETIC LUMBER FROM CORNSTALKS

ROGER W. RICHARDSON

From the Department of Chemical Engineering, Iowa State College

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SMALL SCALE EXPERIMENTS

The Method: (1) Subjecting the shredded stalks to a water or chemical digestion or to a mechanical defibering treatment or to both; (2) forming a mat of pulp from the water suspension by removing the water in a suction mold; (3) applying pressure to the wet mat, and (4) drying in a shelf dryer. After a proper seasoning period, the boards were tested.

Sizing. Three per cent was found to be the optimum pulp consistency, but in commercial practice this factor would depend somewhat on the board-forming machine used. From 40 to 60°C. was found to be the best temperature range for sizing and for the formation of good board. Different kinds of water had little effect upon sizing.

Effect of vacuum. The vacuum for best board formation was found to be from 12 to 18 inches of mercury.

Relation of various properties. The strength of the boards was almost directly proportional to a decrease in freeness of the pulp, and to an increase in the density. The water absorption did not vary with the freeness. It would be expected that the modulus of rupture increased with the increase in pressure applied in forming the board, but this relationship is not sharply defined. The stronger and denser boards were slightly more resistant to water penetration.

Drying experiments. The range of best drying temperature was found to be 340-360°F.

Miscellaneous experiments. Thermal conductivity of both mechanical and cooked pulp insulating board was found to vary with density; an almost straight line relationship existing. Boards made of several types of raw material showed widely different coefficients of thermal conductivity.

Old stalks gave yields of pulp from four to six per cent higher than freshly harvested stalks, the stored stalks having lost soluble materials during storage. The loss of cooking for three hours in water at 45 pounds pressure was found to be about 20 per cent.

SEMI-COMMERCIAL SCALE WORK

Process Developed: Baled stalks were broken open and dry-shredded with an ensilage cutter. The shredded material was cooked in water (rotary digester) for two hours at 90 pounds steam pressure, refined in a rod-mill, followed by washing in a trommel washer, and further refined in a Claflin refiner. Rosin size was added before the Claflin, and alum immediately after. The mat was formed on a forming machine and pressed between three sets of press rolls. The board was dried in a Coe roller dryer.

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[1]Original Thesis submitted June, 1930.

A yield of 55 per cent based on bone dry weight of stalks may be expected by the above process. A yield of as high as 77 per cent was obtained by a strictly mechanical process.

Pulp cooked with chemicals was found to be less desirable than water cooked pulp. The rod-mill-Claflin combination was the best means of pulp refining investigated.

Board made with a mixture of mechanical and cooked pulp resembled mechanical rather than cooked pulp board, although as much as six per cent of scrap and trim may be added to the pulp without causing a noticeable decrease in quality. Up to ten per cent of newsprint mixed with mechanical pulp improved the board flexibility. Added to cooked pulp, it resulted in a mixture difficult to handle on a forming machine. Up to 50 per cent of straw pulp can be satisfactorily used with cooked cornstalk pulp. Broom corn may also be used, but requires more strenuous cooking. Fair types of board can be made from artichoke plants, milk weeds and flax straw.

Overcooking of stalks results in a lowering of the quality of product; undercooking in a product resmbling mechanical pulp. Allowing cooked pulp to age up to a period of three days improves the quality very slightly. There would be some advantage in forming boards at a stock temperature of from 40 to 60°C.

It is possible to use up to one per cent of sulphuric acid in sizing to reduce alum costs. It is best to add the acid before the rosin, so that the alum does the actual precipitating of the emulsified size.

Properties of finished board were found to be independent of the precooking shredding treatment.

Semi-commercial cornstalk boards were produced which compared favorably with the best commercial boards in appearance, strength, insulating value, water resistance, resistance to humidity, expansion and general applicability.

PRODUCTION OF YEAST GROWTH STIMULANTS BY MOLDS ON VARIOUS MEDIA[1]

H. H. SCHOPMEYER

From the Department of Chemistry, Iowa State College

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It had been noticed in studying the growth of yeast in synthetic media that a considerably enhanced growth of yeast was obtained in those flasks which had been accidentally contaminated by molds. A systematic study of this phenomenon was undertaken, using several molds in different synthetic media. The molds employed were: *Aspergillus niger, Aspergillus clavatus, Trichoderma lignorum, Rhizophus nigricans* (plus and minus), and *Pencillium roqueforti.* The medium used for the growth of the yeast (Saccharomyces cereviseae, No. 4226 Amer. Type Culture Collection) contained per 100 cc.: 0.188 gram of ammonium chloride, 0.10 gm. of dipotassium phosphate, and 10 gm. of sucrose. This is the Medium *C* of Fulmer, Nelson and Sherwood (1921). In the preliminary experiments two media were employed (Schopmeyer and Fulmer (1921)) for the growth of the mold, one identical with the medium described above, and the other containing per 100 cc., 0.05 gm. of magnesium sulfate, 0.01 gm. of dipotassium phosphate, 0.05 gm. of potassium chloride, 0.001 gm. of ferrous sulfate, 0.53 gm. of ammonium chloride and 10 gm. of glycerine. This medium is that developed by Naylor, Weisbrodt-Smith and Collins (1930) for the growth of *Penicillium roqueforti* except for the use of glycerol in the place of sucrose.

After the molds had grown for two weeks on these media at 25°, the mold felts were removed and the filtrates divided into two portions, one set being sterilized by steam and the other by filtration through Berkefeld filters. These media were tested for the presence of yeast growth stimulants. All the molds produced considerable quantities of the stimulants.

In order to study this phenomenon quantitatively, a synthetic medium was developed in order to obtain the best growth and chemical activity of the mold. This work was done using *Aspergillus niger.* The concentration of the components of the medium and pH were varied for optimum results. The growth was estimated by the weight of dry mold produced and the chemical activity by the titrable acidity developed. The best medium developed contained per 100 cc.: 0.07-0.1 gm. of magnesium sulfate, 0.10-0.15 gm. of dipotassium phosphate, 0.005-0.015 gm. of ferrous sulfate, 0.005-0.015 gm. of zinc sulfate, 1.75-2.25 gm. of ammonium chlorate, and the appropriate substrate.

Using the appropriate media studies were made as to the nature of the extra-cellular and intra-cellular stimulant produced by the molds *Aspergillus niger* and *Aspergillus clavatus.* Glycerol was used as a substrate since the sucrose remaining in the medium rendered fractionation and purification difficult. Moreover, the use of pure glycerol removed the objection that the stimulants might be present in the sucrose as impurities.

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[1]Original Thesis submitted December, 1931.

The stimulant is non-volatile and stable at boiling temperatures and is not appreciably affected by boiling for fifteen minutes with 10 per cent KOH or with HCl of equivalent strength. Eight liters of filtrate from a medium which had supported the growth of *Aspergillus niger* were evaporated to a small volume on a steam plate and then almost to complete dryness on a water bath. The resulting 50 gm. mass of dark gummy material was extracted with 95 per cent alcohol. The stimulant was found concentrated in the alcohol soluble fraction. Ether was added to the alcoholic solution to remove salts. About 7 gm. of precipitate were formed, 6 gm. remaining in the solution together with practically all of the stimulant. This material was boiled with norite to remove the coloring matter and evaporated. A clear sirupy material considerably more viscous than glycerol remained. About 1.5 gm. of this fraction were obtained and it represented practically all of the potency of the original filtrate. A 1 gm. sample of the purified material was dissolved in a very little water and 200 cc. of absolute alcohol were added. About 0.7 gm. of a granular material separated and upon evaporation 0.25 gm. of whitish crystalline material remained. The material insoluble in the alcohol contained practically all of the stimulant.

The material soluble in the ether alcohol solution was stable toward hot acid and hot alkali, which is not in accord with the properties of Bios as described by Wildiers (1901). The optimum concentration of this fraction was 80 mgm. per 100 cc., at this concentration the yeast crop was tenfold that obtained without the material. The stimulative materials produced by *Aspergillus nigar* and *Aspergillus clavatus* are similar.

The properties of these stimulative materials were compared to those of Bios I and Bios II of Miller (Lucas, G. H. W. (1924), Eastcott, E. V. (1928)). The stimulants could not be separated into the fractions of Miller, nor did inositol enhance their effect. The stimulative materials were not identical with the bioses described by Fulmer, Nelson and Duecker (1924).

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THE SOLUBILITY OF ROCK PHOSPHATE AS INFLUENCED BY SULFUR AND GYPSUM[1]

WINFIELD SCOTT

From the Department of Farm Crops and Soils, Iowa State College

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Experiments were carried out in the field and greenhouse to study the effects of elemental sulfur and of gypsum alone or with rock phosphate or rock phosphate and limestone, on crop growth, on various bacteriological activities and on the content of available phosphorus and other plant foods in the Waukesha silt loam and the Miami silt loam, two important soil types in Iowa.

The data secured from eight samplings on the Miami silt loam in the greenhouse test show that the nitrate content of the soil was depressed by the application of sulfur at the rate of 500 pounds per acre. Rock phosphate applied at the rate of one ton per acre and gypsum at the rate of 500 pounds per acre, alone or in combination, had little or no effect on the nitrate content of the soil at the different samplings. The limestone applied at the rate of one ton per acre stimulated nitrate production materially in this soil, but when rock phosphate was applied with the limestone, no greater effect was shown and when the sulfur or the gypsum was used wtih the limestone the nitrate content was reduced.

The sulfur treatment depressed the nitrifying power of the soil as measured by the tumbler method, using ammonium sulfate; tests being made at eight sampling dates. Gypsum had little effect on the nitrification process, sometimes showing slight increases and at other samplings having no effect. Rock phosphate when applied either alone or in combination had no influence on the nitrification process. Limestone brought about a distinct increase in the process.

The sulfate content of the variously treated soils at the different samplings was not influenced by any of the treatments except the sulfur and the gypsum, the former showing a pronounced and consistently stimulative effect.

The sulfofying power of the soils at the various samplings was determined by the tumbler method, adding sulfur, incubating two weeks and determining the sulfate content by the photometric method. The sulfur added to the soils in the greenhouse greatly increased the sulfofying power of the soils, according to the laboratory tests at the different samplings. Neither gypsum nor rock phosphate had any influence on the sulfofication process, but limestone brought about a very definite reduction in the process.

The amount of neutral ammonium citrate soluble phosphorus was determined in the soils at the various samplings and while the results varied somewhat it was evident that sulfur alone or in combination did increase the amount of available phosphorus in the soils. The gypsum and rock phosphate had no effect, but limestone brought about increases at most samplings. The limited effects of the treatments on available phosphorus in this soil is believed to be due to the low content of organic matter in the soil.

[1]Original Thesis submitted August, 1926.

The determinations of water-soluble phosphorus in the soils at the various samplings did not show any very definite effects of the treatments tested. Some increases were noted from the different fertilizers, but they were small and not of great significance.

The hydrogen ion concentration was determined colorimetrically at all samplings and it was found that sulfur increased the acidity or hydrogen ion concentration of the soil, whether it was applied alone or in combination with other fertilizers. Gypsum had no influence on the soil reaction and no effect was shown by the rock phosphate. The additions of limestone showed the usual normally large effects in reducing acidity.

The yields of soybeans on the Waukesha silt loam were secured for three years in the field tests and increases in crop yields were obtained from some of the treatments. The largest effects were shown by the treatment with rock phosphate plus limestone plus sulfur. In many cases the yields were so variable that conclusions were difficult to draw.

The yields of soybeans on the variously treated pots of Miami silt loam in the greenhouse showed more definite effects from the treatments. The sulfur reduced the yield in all cases whether used alone or in combination. The least depressive effect occurred when the sulfur was used with rock phosphate and limestone. Limestone had little or no effect on the soybean growth on this soil. Neither rock phosphate nor gypsum alone or in combination had any appreciable influence on the crop grown. The soil used in this work was only slightly acid, hence the limestone would not be expected to exert as great an effect as would be shown on a more acid soil. The sulfur treatment greatly reduced the nodule formation on the soybeans and this may be attributed to the increased acidity. This indicates that on more acid soils soybeans would be benefited to a much greater extent by limestone additions and that the crop would be better inoculated and hence more valuable from the protein standpoint.

The lack of effects from the rock phosphate and gypsum treatments on these soils from the crop standpoint and also from the bacteriological and chemical standpoints is quite striking and indicates that these natural materials may have little effect on some soils when applied as they were in these tests. Sulfur applied with rock phosphate may increase the availability of the phosphorus in the phosphate, but there is no large increase as might be expected from the increased acidity brought about by the sulfur. In fact, the limestone additions were also found to bring about some increases in the available phosphorus so that the reaction of the soil is evidently not the sole factor influencing phosphorus availability. In soils high in organic matter the effects of both the sulfur and the limestone might be quite different. Gypsum apparently does not affect the availability of the phosphorus in the rock phosphate.

A close correlation between the pH of the soils and the nitrifying power was shown, the latter increasing with treatments which decreased the hydrogen ion concentration of the soil. The influence of limestone in increasing the nitrifying power of the soils was definitely shown whether the limestone was applied alone or in combination. The depressing effect of sulfur treatments on nitrification was also correlated with increases in hydrogen ion in concentration.

In general, it appears that no large nor striking effects of any of the treatments on the availability of the phosphorus in rock phosphate were brought about in these soils.

BAND SPECTRA PRODUCED BY CERTAIN EXPLOSION MIXTURES[1]

HARLEY A. WILHELM

From the Department of Chemistry, Iowa State College

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This paper deals with the method of exciting molecular spectra by the combustion of mixtures of solids. Some observations on the spectra of MgS and PbS are given. A quantum analysis of a system of bands of MgS is included.

The work on molecular spectra was begun with the idea of studying emission spectra of intermetallic compounds. Failure to observe such spectra in the arc and spark between electrodes of the compounds led to a study of methods of exciting spectra of less metallic compounds. The aim of this part of the work was to proceed from these compounds to the more metallic compounds. In the study of excitation which followed, it was observed that flash-light powders produce band spectra. Out of this observation, the method of exciting band spectra by explosion mixtures has developed.

THEORETICAL

When a change in electronic energy occurs in a gaseous diatomic molecule as it vibrates along the line joining the centers of the two nuclei and rotates on an axis perpendicular to this line, the total change in energy either by absorption or emission is

$$\Delta E = \Delta E_e + \Delta E_v + \Delta E_r.$$

ΔE_e is the energy change due to electron shift, ΔE_v is the change connected with the change in vibrational state and ΔE_r is that part of the change assigned to the change in rotational energy. ΔE_r is small for the molecules studied in this work, and it will be neglected here since its effect is not resolvable on the spectograph used.

From quantum mechanics,

$$\Delta E_v = hc[\omega_o'(v' + \tfrac{1}{2}) - \omega_o'x'(v' + \tfrac{1}{2})^2] - hc[\omega_o''(v'' + \tfrac{1}{2}) - \omega_o''x''(v'' + \tfrac{1}{2})^2]$$

The (′)'s refer to the upper electronic state while the (″)'s refer to the lower electronic state. In this equasion h is Planck's constant, $c\omega_o$ per second is the frequency of vibration of infinitesimal amplitude, v is the quantum number for the vibrational state and x is a small constant.

If the energy shange is expressed as a wave number ν, in (cm^{-1}) units,

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[1]Original Thesis submitted December, 1931.

$$\nu = \nu_e + \nu_v$$

$$\nu = \nu_e + [\omega_o'(v' + \tfrac{1}{2}) - \omega_o'x'(v' + \tfrac{1}{2})^2] - [\omega_o''(v'' + \tfrac{1}{2}) - \omega_o''x'' (v'' + \tfrac{1}{2})^2]$$

All the constants and variables in this equation can be given values from the data by arranging the wave numbers for the bands of a system in a table for v' v'' assignments[2].

APPARATUS AND MANIPULATIONS

The spectra were photographed with a Hilger El quartz spectograph. The Wratten and Wainwright panchromatic photographic plates which were used were sufficiently fast to register bands up to 6500 A with very short exposures.

For general use, a one-fourth inch carbon rod having a cone shaped cup drilled in one end was used to support the charge. This rod was used in an upright position with the cup in the upper end and placed before a lens in such a way that the light from the reacting material was focused on the slit of the spectograph. To produce the MgS bands for final measurement, a magnesium rod was used to support the charge. This charge was a mixture of magnesium and magnesium sulfate.

In order to ignite the mixture an arc was struck on the side of the supporting rod in the vicinity of the cup. This method provided heat for the mixtures which required higher temperatures for their reactions to take place. The supporting rod was connected to the positive pole of a 100 volt storage battery.

Wave lengths of the band heads were determined by comparison with the iron arc spectrum. Wave lengths of the iron lines were plotted against micrometer readings on a large graph. The micrometer reading for a band head then gave from the graph the corresponding wave length in air.

The graininess of the photographic plate, which ordinarily makes weak lines very difficult to measure, was eliminated by an attachment which fits on the microscope of the Societie Genevoise measuring machine. This machine can be read to .0001 centimeter. The attachment, designed here, consists of a small metallic cube which rotates on one axis and has thin glass plates fastened over the two ends of each of two large holes which were drilled through the cube along the other two axes. The attachment is supported from the microscope and allows the small cube to rotate on an axis perpendicular to the axis of the microscope and between the object and the objective. The cube is housed and an adjustment, for turning the axis of rotation perpendicular to the lines on the plate, is provided for. A small aperture is left in the housing between the cube and the object. Over this aperture a small correcting lens may be placed, but such a lens was not necessary with the magnification of the microscope used in this work. As the cube is rotated the image of each grain travels in one direction across the field. With a speed of four or five revolutions per second the grains which make up a spectrum line are fused into a solid, well defined line. The field can be easily examined for spots by stopping the rotating cube. The cube can be rotated by a belt from a small motor to a pulley on the axis shaft extended outside the housing.

[2]Meggers and Wheeler, B. S. Jour. Research, 6, 239 (1931).

A Monroe calculating machine was used to carry out the calculations in the work.

EXPERIMENTAL

In the course of the work it was observed that a flash-light powder made of magnesium and barium peroxide showed the BaO bands. The PbO and MnO bands were found in the spectra produced by mixtures of magnesium with lead dioxide and manganese dioxide, respectively. When other substances such as sulfur were added to the mixtures new bands appeared in their spectra.

In the work, following the above observations, certain advantages and disadvantages of the method of exciting molecular spectra by combustion mixtures have been found.

The advantages of this method as compared with some other methods are:

(a) The intensities of the atomic spectra are very small when compared to the intensities of the molecular spectra produced by explosion mixtures.

(b) Systems are excited which are difficult to excite otherwise.

(c) A small amount of equipment is necessary.

The disadvantages so far encountered in using the mixtures are:

(a) The spectra are weak in the ultra-violet.

(b) A weak continuous background may appear in the spectrum.

(c) The large number of elements involved in the combustion of a mixture makes the selection of the carrier of a system less certain.

(d) The time necessary to photograph a spectrum is long unless very fast photographic plates are used.

The work was narrowed for the present to the excitation of spectra of the sulfides of the metals. By the method of elimination, used to find the carrier of a band spectrum produced by the combustion of a mixture, spectra have been assigned to MgS, PbS and CuS.

RESULTS

Several bands throughout the blue and violet have been shown to be due to MgS. These bands make up more than one system. A system, which is well isolated in the blue and made up of bands which degrade to the red, has been measured and the wave numbers set up in a table for v' v'' assignments. From this table, the approximate equation

$$\nu = 23{,}055.8 + [495.3(v' + \tfrac{1}{2}) - 2.8(v' + \tfrac{1}{2})^2] - [525.2(v'' + \tfrac{1}{2}) - 2.93(v'' + \tfrac{1}{2})^2]$$

has been worked out to represent the system.

This system is produced by an electron shift between two levels sep-

arated by 2.85 volts. If the $v-\omega_v$ graph[4] is asumed to give a straight line to $\omega_v = 0$, the energy of dissociation in the lower electronic level is about 67,000 g.-cal per g.-mole.

A spectrum consisting of more than 100 bands between 6600 and 4100 A is produced during the combustion of a mixture of aluminum, lead dioxide and sulfur. These bands degrade to the red and show no fine structure in the dispersion used. The spectrum resembles the PbO spectrum[3], but the two when photographed through a Hartmann diaphragm show that the mixture produces bands which with other evidences can be assigned to PbS. A quantum vitbrational analysis for these bands has not yet been completed.

Bands carried by CuS have been observed in the yellow region of the spectrum. They make up about five sequences which degrade to the red. The spectrum has been excited by the copper arc in an atmosphere of sulfur vapor, and by the explosion of a mixture of magnesium and copper sulfate. Further measurements and work are to be done on this spectrum.

SUMMARY

1. The method of exciting band spectra by explosion mixtures has wide applicability.

2. The advantages of the explosion method outweigh the disadvantages in the production of many molecular spectra.

3. The spectra of some metallic sulfides have been excited by the combustion of mixtures.

4. A quantum vibrational analysis has been given for one band system carried by MgS.

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[3] Bloomenthal, Phys. Rev., **35**, 34 (1930).

[4] Birge and Sponer, Phys. Rev., **28**, 259 (1926).

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