I. The Rate of Hydration of beta-Methylcrotonaldehyde; the Equilibrium between beta-Methylcrotonaldehyde and beta-Hydroxyisovaleraldehyde in Dilute Aqueous Solution.

II. Esters of Alginic Acid.

III. Oxidation of Alginic Acid by Periodic Acid.

IV. A Chromatographic Study of Lignite.

Thesis by

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In Partial Fulfilment of the Requirements

for the Degree of Doctor of Philosophy

California Institute of Technology

Pasadena, California

1941

Acknowledgment

The author expresses his appreciation and sincere thanks to Professor H. J. Lucas and Dr. L. Zechmeister whose direction and encouragement have made these researches possible. The helpful advice of Dr. Carl Niemann and Dr. David Pressman is gratefully acknowledged.

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The Rate of Hydration of beta-Methylcrotonaldehyde; the Equilibrium between beta-Methylcrotonaldehyde and beta-Hydroxyisovaleraldehyde in Dilute Aqueous Solution

The Rate of Hydration of beta-Methylcrotonaldehyde; the Equilibrium between beta-Methylcrotonaldehyde and beta-Hydroxyisovaleraldehyde in Dilute Aqueous Solution

Introduction

This study is one of a series[1,2,3,4,5] undertaken to determine the effect of various substituents on the heat of hydration of the ethylenic double bond. Data have been collected in an endeavor to correlate structural effects with hydration and dehydration reaction velocities, activation energies and heats of hydration.

Since the heats of hydration of crotonaldehyde[3], crotonic acid[4] and β-methylcrotonic acid[5] have been determined, it was desirable to study the hydration of β-methylcrotonaldehyde and its equilibrium with β-hydroxyisovaleraldehyde. No reference has been found regarding the hydration of β-methylcrotonaldehyde nor has the hydrated compound, β-hydroxyisovaleraldehyde, been reported in the literature.

The heat of hydration was conveniently determined from equilibria between the hydrated and dehydrated compounds in dilute aqueous nitric acid at several different temperatures. Activation energies were obtained from the equilibrium and kinetic constants of the hydration and dehydration reactions.

Experimental

β-Methylcrotonaldehyde.--This unsaturated aldehyde was prepared according to the method of Fischer, Ertel and Lowenberg[6] which involves bromination of isovaleraldehyde, conversion of the brominated product to the diethyl-

acetal, loss of hydrobromic acid on heating with potassium hydroxide and hydrolysis of the resulting ethylacetal of β-methyl crotonaldehyde. Since β-methylcrotonaldehyde ethylacetal was found to be quite stable toward oxidation and polymerization, and, furthermore, could be converted readily to the free aldehyde, it served as convenient source of the free aldehyde. The use of the acetal form of the aldehyde in the preparation of reaction mixtures, as discussed under Preparation of Reaction Mixtures, necessitated its careful purification. This was accomplished through the use of a total reflux distillation column of the Weston type. The fraction, b.p. 89.5-90.0° at 60 mm., $n_D^{21°}$ = 1.4200, 1.00 double bonds per mole by bromine absorption, was set aside in sealed ampoules and used for the hydration studies.

Hydration Solutions.--The use of perchloric and hydrohalic acid solutions in the hydration studies was excluded, since excessive polymerization occurred in the presence of those reagents. Hydration was found to proceed in aqueous nitric acid with no interference from polymerization. However, the oxidizing action of nitric acid at elevated temperatures (ca. 45°C.) necessarily limited the temperatures at which the hydration could be studied. Solutions of known acidity were prepared and adjusted to any desired ionic strength and acidity by addition of water and/or standard sodium or potassium nitrate solutions.

Preparation of Reaction Mixtures.--Preparations of β-methylcrotonaldehyde deteriorated rapidly on standing even when stored under an inert gas.

This fact necessitated the use of a freshly distilled preparation in a hydration experiment. This difficulty was circumvented by preparing reaction mixtures directly from the acetal compound which could be stored unchanged for a considerable time. To justify this procedure it was necessary to determine the rate at which the acetal hydrolyzed to the free aldehyde. Two-tenths ml. of the acetal were added to ten ml. of pure water and the resulting mixture was thoroughly mixed by shaking. The acetal was not appreciably soluble in water and therefore separated as a separate upper phase. After acidification with one drop of 6 N sulfuric acid the mixture was shaken for several seconds. The organic phase immediately disappeared and a water-clear solution of the free aldehyde resulted. Since the hydrolysis was practically instantaneous, solutions of the free aldehyde could be prepared rapidly and conveniently from the acetal with no complications due to rate effects. This procedure was followed in all experiments. Measured amounts of the acetal were added to hydration solutions at the desired temperature, acidity and ionic strength. The resulting mixtures were shaken for ten seconds and placed in a thermostat controlled within 0.05° ± of the temperature desired. Samples were immediately withdrawn and analyzed for unsaturation following the method described under Analysis. The course of the hydration was followed by determining the decrease in unsaturation as the reaction proceeded.

Analysis.--Reaction samples were analyzed by a quantitative bromination method. The procedure employed in previous studies[3,4,5] was not applicable

in the analysis of β-methylcrotonaldehyde as it yielded erroneous results. The method used previously involved addition of a reaction sample to an excess of standard bromate-bromide solution followed by liberation of bromine on the addition of sulfuric acid. After a suitable bromination period, aqueous potassium iodide solution was added and the liberated iodine titrated with sodium thiosulfate solution. This method gave quantitative results in the analysis of crotonaldehyde, crotonic acid and β-methylcrotonic acid but failed when applied in the analysis of β-methylcrotonaldehyde. When the above procedure was followed in the analysis of known amounts of β-methylcrotonaldehyde, the results varied considerably from the theoretical values and depended on the excess of bromate-bromide solution, the length of the bromination period and the lapse of time from the addition of potassium iodide to the titration with sodium thiosulfate. In addition, the end-point of the titration was indefinite, since the liberation of iodine continued over a period of twenty to thirty minutes after the addition of potassium iodide. In a typical series of analyses with a 35% excess of bromate-bromide solution the amount of bromine absorption was 1% greater than the theoretical value, for a ten-second bromination period, 8% greater for a five-minute bromination period, 10% for a ten-minute bromination period and 13% for a thirty-minute bromination period. The drift of the end points in all cases was in a direction corresponding to approach to theoretical bromination. These observations were best explained on the basis of a reversible substitution of bromine as well as addition at the double bond. Variations of concentrations, temperature

light intensity and duration of bromination period did not improve the results. This fact necessitated a modification of the method.

The use of mercury salts in the analysis of olefins has been extensively employed[7]. In general, it has been found that the bromination of unsaturated compounds is catalyzed by mercuric salts. In the development of a satisfactory method of analysis for β-methylcrotonaldehyde, it was reasoned that the presence of a mercuric salt in an analysis mixture might influence substitution of bromine. Quantitative addition with no substitution was found to occur when the bromination was carried out in the presence of mercuric sulfate, and a quantitative procedure was developed that was both convenient and rapid.

The analysis technique employed required the use of a special vacuum flask. Twenty-five ml. (an excess) of 0.05 N bromate-bromide solution were measured into a 300-ml. Erlenmeyer flask with a ground glass neck; a ground glass plug, fitted with a stopcock, was inserted in the neck of the flask and the system was evacuated. Ten ml. of 6 N sulfuric acid were introduced into the flask and the mixture was allowed to stand for five minutes to insure complete liberation of bromine. After the addition of 75 ml. of 0.75 N mercuric sulfate solution (2 N in sulfuric acid), the flask was covered with a black cloth to exclude light and 25 ml. of the analysis sample were introduced. The bromination was allowed to proceed for one and one-half minutes when it was stopped by the consecutive addition of 20 ml. of 2 N sodium chloride and 10 ml. of 60% potassium iodide solutions. Titration of the liberated iodine was carried out with 0.025 N sodium thiosulfate solution. In general,

there was a negligible variation of results with bromination periods varying from one and a half to five minutes. The end points were not subject to drift with time and the analysis of known amounts of β-methylcrotonaldehyde gave theoretical results.

Data and Discussion

The hydration is found to be first order with respect to β-methylcrotonaldehyde since plots of $\log_{10} \varepsilon / (\varepsilon - x)$ against time, where x represents the fraction of original β-methylcrotonaldehyde hydrated at time t and ε denotes the fraction hydrated at equilibrium, are linear as required for a first order reaction. The initial slopes of the plots of (1 - x) against t when the hydrogen ion concentrations are 1.04 and 0.52 molar indicate the hydration is first order with respect to hydrogen ion concentration (Figure 1). The slope at the higher concentration corresponds to a rate constant of 0.0124, a value approximately twice the rate constant of 0.006 for the lower concentration. Hydrogen ion is a catalyst for the reaction and its concentration remains constant throughout any one run.

Equilibrium is reached when approximately 39% of the β-methylcrotonaldehyde is converted to β-hydroxyisovaleraldehyde at 15°, 36% at 20°, 31% at 25° and 26% at 35°. The equilibrium lies more in the direction of unsaturation than does any equilibrium reported in the series of studies. It is to be expected that β-methylcrotonaldehyde would hydrate to a lesser extent than crotonaldehyde, since β-methylcrotonic acid hydrates to a lesser extent than crotonic acid.

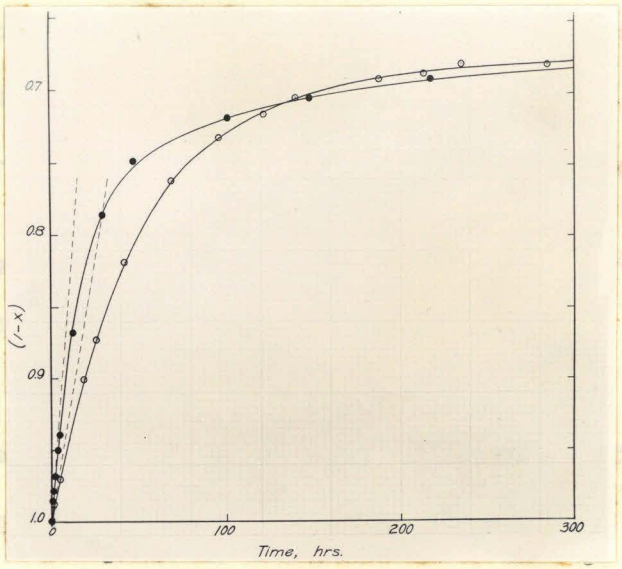

Figure 1

Plot of (1 - x) against time for the hydration of β-methylcroton-aldehyde at 25°

β-Methylcrotonaldehyde	○	●
C_o molal	0.021	0.021
(H^+) molal	.520	1.040
molal	1.040	1.040

Crotonic acid hydrates to the extent of 76% at 111.3°, β-methylcrotonic acid to the extent of 70.8% at 111.85° and crotonaldehyde to the extent of 47% at 25°. The value of 31% at 25° for β-methylcrotonaldehyde is consistent with these values. That the equilibrium is essentially independent of the hydrogen ion concentration is indicated in Figure 1. Here the equilibrium values of the β-methylcrotonaldehyde concentration differ only a few percent for a twofold variation in hydrogen ion concentration. The reaction may be symbolized by the following equation

$$(CH_3)_2C{=}C{-}CHO + H_2O + H_3O^+ \underset{k_2}{\overset{k_1}{\rightleftharpoons}} (CH_3)_2CHOHCH_2CHO + H_3O^+ \qquad (1)$$

where k_1 and k_2 are the specific rate constants for the forward and reverse reactions respectively. During the course of a hydration experiment the amount of unsaturation was observed to decrease rapidly as the reaction approached equilibrium and then to drift slowly downward for a long period of time. In few cases did the amount of unsaturation attain a final constant value. A correction for this drift was made by a graphical method. In the treatment of the data of any one run, the value of ε was varied so as to yield a straight line in a plot of $\log_{10} \varepsilon / (\varepsilon - x)$ against time. The data for a typical run are listed in Table I, and the effect of variation of ε on the plot of $\log_{10} \varepsilon / (\varepsilon - x)$ against time is indicated in Figure 2. Values of x close to equilibrium are discarded since slight titration errors in the determination of β-methylcrotonaldehyde near the equilibrium would cause considerable errors in the value of $\log_{10} \varepsilon / (\varepsilon - x)$. This graphical treatment of the data was carried out in all

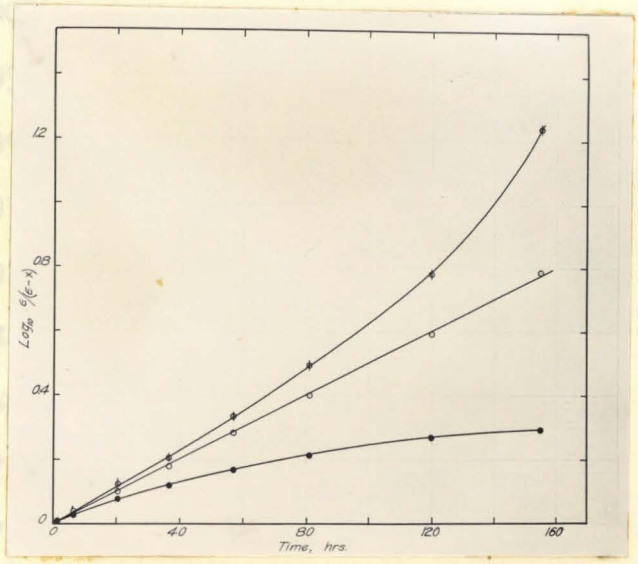

Figure 2

Effect of variation of ε on plot of $\log_{10} \varepsilon / \varepsilon - x$ against time: initial concentration of β-methylcrotonaldehyde, C_o, 0.019; (H^+), 0.52 molal; T, 20°: ϕ , ε = 0.32; ○ , ε = 0.358; ● , ε = 0.400.

Table I

Effect of Variation of ε on $\log_{10} \varepsilon / (\varepsilon - x)$ in the Hydration of β-Methylcrotonaldehyde at 20° and 0.52 N Nitric Acid.

Time, hrs.	(1 - x)	ε = 0.32 $\log_{10} \frac{\varepsilon}{\varepsilon - x}$	ε = 0.358 $\log_{10} \frac{\varepsilon}{\varepsilon - x}$	ε = 0.400 $\log_{10} \frac{\varepsilon}{\varepsilon - x}$
0.0	1.000			
0.6	.996	0.005	0.005	0.004
1.6	.994	.008	.007	.006
6.7	.972	.040	.035	.028
21.4	.920	.125	.109	.080
37.0	.880	.204	.177	.120
57.6	.828	.335	.284	.172
81.1	.784	.488	.402	.216
120.0	.733	.781	.595	.267
155.0	.703	1.243	.769	.297

cases with the exception of the studies at 15° C. Due to the prohibitive amount of time involved it was impractical to carry out a complete hydration study at 15°; instead reaction mixtures were allowed to proceed close to equilibrium at 25°, the temperature was lowered to 15° and the system allowed to come to equilibrium at that temperature. The drift effect was negligible at this temperature for after establishment of equilibrium there was no observable change in two hundred and forty hours.

The values of k_1 and k_2, the specific velocity constants for the forward and reverse reactions, were calculated from the equilibrium constant and the integrated expression for a unimolecular reaction approaching equilibrium.

$$K = \frac{k_1}{k_2} = \frac{\varepsilon}{1 - \varepsilon} \tag{2}$$

$$\log_{10} \frac{\varepsilon}{\varepsilon - x} = \frac{(k_1 + k_2)t}{2.303} \tag{3}$$

The slope of the straight line obtained when $\log_{10} \varepsilon /(\varepsilon - x)$ is plotted against time is $(k_1 + k_2)/2.303$. In Table II, values of k_1 and k_2 and $k_1/(H^+)$ and $k_2/(H^+)$ are found along with values of the equilibrium constant, K.

Salt Effect.--The values of ε tabulated in Table III are observed to decrease with increasing ionic strength. It is further observed that the salt effects of hydrogen ions and sodium or potassium ions are not equivalent, for replacement of hydrogen ions by sodium or potassium ions is accompanied by a considerable increase in values of ε. The same salt effects have been noted in the hydration studies of crotonaldehyde, crotonic acid and β-methylcrotonic acid.

Thermochemistry.--Thermal data for the hydration and dehydration reactions are listed in Table III. The heats of activation, Q_1 and Q_2, of the forward and reverse reactions were calculated from values of the specific

Table II

Data on the Hydration of β-Methylcrotonaldehyde at Various Temperatures

	Temp. °C.	(HNO_3)	(KNO_3)	$(NaNO_3)$	(μ), M	Initial (C_5H_8O), M	(ε)	k_1	k_2	$k_1/(H^+)$	$k_2/(H^+)$	K
1	15	0.520	-----	-----	0.52	0.018	0.411	-----	-----	-----	-----	0.669
2	15	1.040	-----	-----	1.04	.018	.376	-----	-----	-----	-----	.603
3	15	0.520	0.52	-----	1.04	.021	.388	-----	-----	-----	-----	.635
4	15	0.520	-----	0.52	1.04	.020	.381	-----	-----	-----	-----	.616
5	15	0.922	0.96	-----	1.88	.018	.368	-----	-----	-----	-----	.583
6	20	1.040	-----	-----	1.04	.018	.325	0.0075	0.0156	0.0072	0.0149	.482
7	20	0.52	0.52	-----	1.04	.019	.358	.0044	.0078	.0084	.0149	.558
8	25	0.520	-----	-----	0.52	.021	.320	.0061	.0133	.0117	.0255	.461
9	25	1.040	-----	-----	1.04	.021	.290	.0124	.0303	.0119	.0291	.408
10	25	0.520	0.52	-----	1.04	.021	.310	.0065	.0144	.0124	.0276	.449
11	25	0.520	-----	0.52	1.04	.021	.300	.0065	.0152	.0125	.0292	.428
12	25	0.922	0.96	-----	1.88	.022	.285	.0102	.0256	.0111	.0278	.399
13	35	0.520	-----	-----	0.52	.018	.265	.0179	.0496	.0344	.0954	.361
14	35	1.040	-----	-----	1.04	.018	.240	.0345	.1091	.0332	.1049	.316
15	35	0.520	0.52	-----	1.04	.017	.258	.0195	.0560	.0375	.1077	.348
16	35	0.520	-----	0.52	1.04	.017	.250	.0199	.0595	.0383	.1144	.334
17	35	0.922	0.96	-----	1.88	.020	.235	.0330	.1075	.0357	.1165	.307

velocity constants at 25° and 35°. The difference of these activation energies is the heat of hydration. However, the values so obtained are not as accurate as the values calculated from the equilibrium constants at 15°, 20°, 25° and 35°. Plots of $\log_{10}K$ against the reciprocal of the absolute temperature are shown in Figure 3. The slopes of these straight lines are practically identical and from them the heat of reaction is readily calculated by the relation

$$\Delta H = \text{slope} \times 2.303 \times R.$$

Table III

Thermal Data Concerning the Hydration of β-Methylcrotonaldehyde

(HNO_3)	($NaNO_3$)	(KNO_3)	(μ), M	Q_1 kcal.	Q_2 kcal.	ΔH^a (from heats of activation)	ΔH^b (from Equilibria)
0.520	-----	----	0.52	24.1	29.5	-5.4	-5.42
1.040	-----	----	1.04	23.4	28.6	-5.1	-5.53
.520	-----	0.52	1.04	24.8	30.5	-5.7	-5.34
.520	0.52	----	1.04	25.0	30.4	-5.4	-5.42
.922	----	0.96	1.88	26.2	32.1	-5.9	-5.57
			Mean	24.7	30.2	-5.5	05.46

a. Calculated from specific velocity constants at 25° and 35°.

b. Calculated from equilibrium constants at 15°, 20°, 25° and 35°.

Energies of activation were calculated from the slopes of the plots of $\log_{10} k_1$ and $\log_{10} k_2$ against the reciprocal of the absolute temperature.

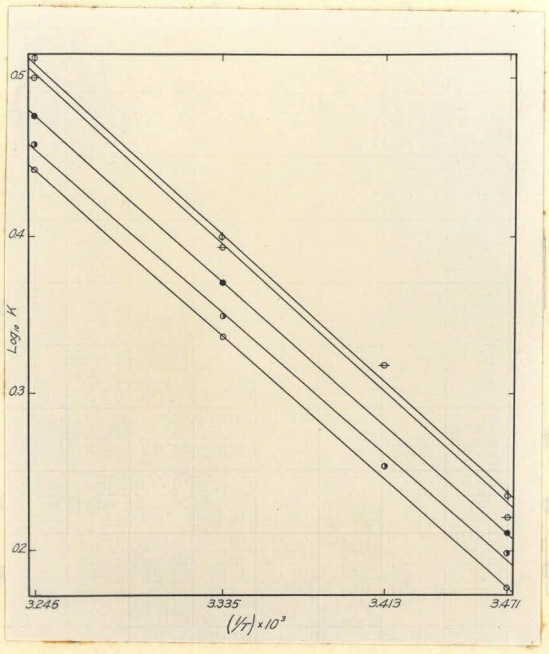

Figure 3

Plots of $\log_{10}$ K against the reciprocal of the absolute temperature.

	(HNO_3)	$(NaNO_3)$	(KNO_3)	μ, molar
○	0.520	-----	-----	0.52
◑	0.520	-----	0.520	1.04
●	0.520	0.520	-----	1.04
⊖	1.040	-----	-----	1.04
ϕ	.922	-----	.960	1.88

The heats of activations, Q_1 and Q_2, for the hydration and dehydration reactions are consistent with those reported in the studies of crotonaldehyde[3], crotonic acid[4], and β-methylcrotonic acid[5] (Table IV).

Table IV

	Q_1 kcal.	Q_2 kcal.	ΔH kcal.
Crotonic acid	20.2	25.5	-5.4
β-Methylcrotonic acid	26.9	35.0	-8.1
Crotonaldehyde	18.2	24.5	-6.3
β-Methylcrotonaldehyde	24.7	30.2	-5.5

Thus it is to be expected that the heats of activation in the case of β-methylcrotonaldehyde be higher than those for crotonaldehyde since the same relationship exists for the heats of activation of the respective acids.

Summary

The reversible hydration of β-methylcrotonaldehyde to β-hydroxyisovaleraldehyde in dilute aqueous nitric acid has been investigated at 15°, 20°, 25° and 35°. Hydration is first order with respect to β-methylcrotonaldehyde and hydrogen ion; dehydration is first order with respect to β-hydroxyisovaleraldehyde and hydrogen ion.

The values of the equilibrium constant is 0.621 at 15°, 0.520 at 20°, 0.429 at 25° and 0.334 at 35°; these values correspond to a $-\Delta H$ of 5.46 kcals. for the hydration reaction. The heat of activation is 24.7 kcal. for the hydration and 30.2 kcal. for the dehydration.

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Esters of Alginic Acid

[Reprinted from the Journal of the American Chemical Society, 62, 1070 (1940).]

[Contribution from the Gates and Crellin Laboratories of Chemistry, California Institute of Technology, No. 728]

Esters of Alginic Acid

By H. J. Lucas and W. T. Stewart[1]

Since alginic acid is a polyuronide,[2] it would be expected to form two types of esters, *viz.*, alkyl esters and acyl esters, the former because of one carboxyl group per mannuronic residue, the latter because of one or two free hydroxyl groups per mannuronic residue. Of course, the terminal residue will have one additional hydroxyl group. If all of the carboxyl groups are free or are bound as in a salt or in an ester, there would then be two free hydroxyl groups per residue (except for the terminal ones), and dialkyl esters should be possible. However, if the carboxyl groups are bound in lactone formation, then only one hydroxyl group per residue would be free. Therefore the degree of esterification which can be attained presumably would depend, at least in part, upon the extent to which the mannuronic residues of the alginic acid molecule are in the acid or in the lactone form.

Alginic acid might be expected to undergo esterification and etherification of the hydroxyl groups, much as pectin does. The latter can be formylated, acetylated and nitrated without difficulty.[3,4] Methylation of pectin fragments or of pectin which has suffered partial degradation has been accomplished by heating the silver salt with methyl iodide under pressure,[5] by dimethyl sulfate[6] and by methyl iodide and thallium ethoxide.[7] Pectin acid has been exhaustively methylated.[8] Alkylation of the carboxyl group of alginic acid has been effected by heating with methanolic hydrogen chloride.[9,10]

Acetates of alginic acid have been prepared using acetic anhydride and aqueous hydriodic acid as the acetylating agent.[11] A dimethyl ether has been obtained from alginic acid methylglycoside methyl ester (from partially degraded alginic acid and methanolic hydrogen chloride) by the use of methyl iodide and thallium ethoxide.[10]

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Experimental

Alginic Acid.—This was supplied by the Kelco Company, of San Diego, who manufactured it from *Macrocystis pyrifera* by a process involving digestion of the kelp with hot aqueous sodium carbonate, precipitation of calcium alginate by the addition of aqueous calcium chloride and conversion of this to alginic acid by means of hydrochloric acid.[12] Alginic acid is a wet fibrous product of about 12% solids. The dried product leaves about 2.5% ash on ignition. For the nitration water was extracted by agitating with several portions of ethyl alcohol which were poured off. The alcohol left finally was allowed to evaporate. The alginic acid remained as a rather compact mass, water content 15%. Another portion was dissolved in aqueous sodium carbonate. The acid was precipitated from the solution by the addition of dilute hydrochloric acid. After filtration through cloth, washing twice with water and then with aqueous methanol until free of chloride, washing well with alcohol, finally with ether, and drying over sulfuric acid, the alginic acid was left as a light, fluffy mass. The ash was reduced to 2.1%. This product was used in methylation as well as in some of the nitration experiments. The presence of methanol (25%) in the water prevented peptization of alginic acid and yet allowed removal of chloride.

Nitration.—This was accomplished with a mixture of nitric acid (d. 1.47) and concentrated sulfuric acid (d. 1.84). The mixture was kept cold during the mixing of the acids and placed in a refrigerator at 3 to 5°. The product was recovered by adding ice and water and centrifuging. Mineral acid was removed by shaking the residue with ice and water and centrifuging. This was done eight times. At first, two or three preparations were worked up by filtering the nitration mixture through a sintered glass funnel, followed by washing with water. Centrifuging was found to be more satisfactory and convenient, however. Next, the nitrated product was taken up in acetone and precipitated by the addition of ether. After this was done five times, the product was placed in a vacuum desiccator over calcium chloride. The dried weight is the one recorded in Table I.

For analysis all samples were dried at 60° and 30 mm. over phosphorus pentoxide. Nitrogen was determined by the micro Dumas method. The analytical results are given in Table I.

TABLE I

NITRATION OF ALGINIC ACID AT 3 TO 5°

Expt.	HNO_3, d. = 1.47, ml.	H_2SO_4, d. = 1.84, ml.	Alginic acid, g.	Moles HNO_3/moles $C_6H_8O_6$[c]	Time of standing, hr.	Product, g.	Nitrogen, %	$NO_3/C_6H_6O_6$[d]
1[a]	45	45	15	13	60	3.8	3.83	0.50[e]
2[a]	90	90	15	25	48	2.2	3.81	.49
3[b]	30	30	5	30	72	1.7	7.27	1.08
4[a]	67.5	22.5	15	19	1	12.0	5.09	0.69
5[a]	67.5	22.5	15	19	60	2.6	5.92	.83
6[a]	22.5	7.5	5	19	150	1.6	7.69	1.18
7[b]	67.5	22.5	15	23	12	9.8	5.23	0.71
8[b]	67.5	22.5	15	23	60	3.0	6.09	.85
9[a]	135	45	15	38	48	5.4	5.23	.71[f]
10[b]	45	15	5	45	48	1.8	6.94	1.01
11[b]	45	15	5	45	48	2.0	6.12	0.86

[a] Reprecipitated alginic acid, alcohol and ether washed, dried over sulfuric acid to a light, fluffy product, ash, 2.1%. [b] Alginic acid, alcohol washed, dried to a compact mass, ash, 2.5%, H_2O, 15%. [c] As free acid. [d] As lactone. [e] Ash, 0.6%. [f] Ash, 0.5%.

Methylation.—Several attempts were made to methylate alginic acid with dimethyl sulfate and aqueous sodium hydroxide. It was found necessary to work at a temperature of 60° and to repeat the methylation twice with fresh reagents. An opalescent solution resulted. The product was recovered by acidifying with hydrochloric acid cold, centrifuging, and washing the precipitate four times with water, centrifuging each time. The product was dried by washing with ether, in which the methylated product was insoluble. Less than one methoxyl group per mannuronic residue was introduced. The methoxyl content of these and other preparations was determined by the method of Viebock and Schwappach[13] after drying at 60° and 30 mm. over phosphorus pentoxide (Table II).

TABLE II

METHYLATION OF ALGINIC ACID

Experiment		1	2	3	4
Alginic cpd.		Acid	Acid	Acid	Ag salt
Taken, g.		17.6	1.76	56.5	1.45
Reagent	Cpd.	Me_2SO_4 + NaOH	0.2 *M* CH_2N_2 in Et_2O		MeI
	g.	[a]	35.7[b]	2485	7.25
Methylated product (A)	g.	9.8	1.6	51	0.9
	MeO, %	9.78	18.2	22.4	2.44
Ammonolyzed product (B)	MeO, %	...	5.08	7.96[c]	..
MeO per C_6 unit	A	0.58	1.13	1.40	0.14
	B	...	0.29	0.48	..

[a] Me_2SO_4, 75.6 g.; NaOH, 30%, 80 g. Three treatments at 60°. [b] For eight hours. [c] After forty-eight hours; MeO was 8.16% after twenty-four hours.

Best results were obtained with diazomethane. The alginic acid used in the methylations with diazomethane was the fluffy reprecipitated product. It was not freed of ether but was treated directly with diazomethane. Seven portions of 500 ml. (355 g.) of 0.2 *M* diazomethane in ether were added over a period of two hundred and forty hours to the acid (56.5 g. of the original dry acid). The final volume was 3.5 liters. The yellow color after addition of the first and second portions largely disappeared in a few hours, but after subsequent additions diminished but little on standing. The methylated alginic acid was recovered by drawing off most of the ether through a filter stick. The rest was removed by suction filtration under a rubber dam and by vacuum drying over concd. sulfuric acid.

Silver alginate was prepared by adding aqueous silver nitrate to aqueous ammonium alginate. The precipitate, after filtering and washing with methanol, was shaken with a solution of methyl iodide in methanol for ten hours, and the mixture allowed to stand in the dark, with occasional shaking, for two months. The product was recovered by first removing the liquid through filtration, then adding aqueous sodium cyanide to the solid and precipitating

(12) Thornley and Walsh, U. S. Patent 1,814,981 (July 14, 1931).

(13) Viebock and Schwappach, *Ber.*, **63B**, 2818 (1930); *J. Assoc. Official Agr. Chem.*, **15**, 136 (1932).

TABLE III

METHYLATION OF NITRATED ALGINIC ACID AT 25°

Experiment		1	2	3
Nitrated acid	Table I	3	5	10
	N, %	7.27	5.92	6.94
	NO_3/ $C_6H_5O_5$	1.08	0.83	1.01
	Grams	0.50	2.0	0.50
Reagents		2.4 g. HCl in 15 ml. MeOH	1.25 g. Me_2SO_4 0.4 g. NaOH 2 g. H_2O	9.1 g. MeI 0.13 g. Ag_2O 5 ml. Me_2CO
Time, hours		48	0.5	72
Product	Grams	0.4	1.5	0.4
	N, %	7.20	5.50	6.32
	MeO, %	10.3	3.26	6.28
Per C_6 unit	NO_3	1.18	0.78	0.97
	MeO	0.79	.21	.44

the sodium salt of partially methylated alginic acid by the addition of ethyl alcohol to the cyanide solution. After centrifuging and washing the resulting gelatinous precipitate with small amounts of water, dilute hydrochloric acid was added. The gelatinous material was centrifuged, washed with a small amount of water, then with ether, and dried over concd. sulfuric acid at 30 mm.

Methylation of different specimens of nitrated alginic acid was carried out in three different ways as shown in Table III. The product from methanolic hydrogen chloride was precipitated by the addition of ether, thoroughly washed with ether and dried at 30 mm. over solid sodium hydroxide and concd. sulfuric acid. The aqueous solution from dimethyl sulfate treatment was acidified and the residue remaining after centrifuging was washed with 25% ethanol–water and dried at 30 mm. over sulfuric acid. The acetone solution from the treatment with silver oxide and methyl iodide was diluted with methanol, precipitated with ether and dried as above.

Ammonolysis.—Ammonolysis of methylated alginic acid was carried out by allowing the material to stand with concd. ammonium hydroxide for twenty-four hours. The resulting viscous liquid was acidified, the precipitated acid was washed several times with 50% ethanol–water, then with ether, and dried as usual. The recovery was approximately 60%. The methoxyl content was determined as before.[13] The results are shown in Table II. That the reaction was essentially complete is shown by the small drop in methoxyl content on a second twenty-four-hour treatment (8.16 to 7.96% OCH_3).

Other Derivatives.—Attempts to prepare products soluble in acetone by the action of benzoyl chloride, benzyl chloride and phenacyl bromide on sodium alginate were not successful.

Optical Rotations.—Optical rotations were observed on alginic acid, on two different specimens of nitrated alginic acid and on two specimens of methylated alginic acid (Table IV). The materials had been dried at 60° and 30 mm. over phosphorus pentoxide for twenty-four hours. The alginic acid, the nitrated alginic acid and the material methylated by means of dimethyl sulfate and sodium hydroxide were dissolved in aqueous sodium hydroxide, using just enough base to effect solution. The product obtained by the use of diazomethane was dispersed in water. Observations were limited to very dilute solutions, 0.3%, because at higher concentrations the Tyndall effect was so pronounced that satisfactory readings could not be made.

Titration.—The neutralization equivalent of nitrated alginic acid dried at 60° and 30 mm. over concd. sulfuric acid and solid caustic was determined by titrating a solution of the sample in acetone, obtained by shaking for about two hours, with standard base to a phenolphthalein

TABLE IV

Material	Description Table	No.	Solvent	Concn., %	Rotation α	$[\alpha]^{25}D$	$[M]^{25}D \times 10^{-3}$
Alginic acid			Aq. NaOH	0.300	−0.41	−137°	−22
Nitrated product	I	5	Aq. NaOH	.300	.34	−113	−22
Nitrated product	I	10	Aq. NaOH	.300	.33	−110	−22
Methylated product	II	1	Aq. NaOH	.300	.29	− 97	−16
Methylated product	II	3	H_2O	.300	.35	−117	−23
Methylated product	II	3	H_2O	.300	.35	−117	−23

end-point. The combined neutralization–saponification equivalent was obtained by heating the sample with an excess (10%) of aqueous standard base for four hours on a steam-bath, and back titrating. The equivalent weights are shown in Table V. The calculations were made thus:

Neutralization equivalent = $158 + (NO_3/C_6 \times 45)$

Combined neutralization–saponification equivalent = neut. equiv./$(1 + NO_3/C_6)$.

TABLE V

TITRATION OF NITRATED ALGINIC ACID

From Table I		No. 8	No. 11
N, %		6.09	6.12
Neut. equiv.	Calcd.	197	198
	Found	198	200
Combined neut.–sapon. equiv.	Calcd.	107	107
	Found	104	109
Ash, %		0.3	0.1

Discussion

The various specimens of nitrated alginic acids are much alike in properties. They dissolve in aqueous sodium carbonate, from which they are precipitated on acidification. They are readily soluble in acetone, methanol and ethanol, less readily in 1-propanol, dioxane and methyl iodide, and but slightly soluble in acetic anhydride and glacial acetic acid. They are insoluble in ethyl ether, isopropyl ether and chloroform. These last serve to precipitate the nitrated alginic acids from their solutions in other solvents.

The extent to which nitration took place varied from 0.49 to 1.18 nitrate groups per mannuronic unit, calculated as the lactone (Table I). Addi-

tional purification by dissolving once more in acetone and precipitating with ether left the material essentially unchanged, as shown in experiments 3 and 10, for the nitrogen content in the first case changed from 7.27 to 7.02 and in the second case from 6.94 to 7.21%. The best yield was obtained in experiment 4, where the time was shortened to one hour. The extent of nitration, however, was low. When the time was lengthened the yield was less, and in a general way was lower the longer the period of standing. On the other hand, the degree of nitration usually increased as the time was lengthened.

The recovery of a small amount (2%) of material from the first acetone–ether liquor (Expt. 8, Table I) indicates that precipitation of a nitrated alginic acid from its acetone solution by the addition of ether is essentially quantitative. The recovered material analyzed 8.05% N (1.26 NO_3 per C_6 unit).

Since the maximum number of nitrate groups in the main products was essentially 1 per C_6 residue, the assumption is justified that the alginic acid while undergoing nitration was essentially in the lactone form unless the second free hydroxyl group would not nitrate under these conditions, even in the presence of a good excess of the nitrating mixture. This must be regarded as a possibility in view of the fact that with diazomethane less than one hydroxyl group was methylated (Table II). However, lactonization of alginic acid presumably would be as likely as that of nitrated alginic acid which was shown by titration to be lactonized when dried. Alginic acid being nitrated may have been lactonized during drying, or when put into the nitrating mixture.

The recovery of a small amount of higher nitrated product from the acetone–ether mother liquor (Expt. 8) would seem to indicate that higher nitration products are formed to some extent.

The various specimens of methylated alginic acid are alike in that they peptize in water but in solutions containing 25% methanol or ethanol they do not peptize. They are alike also in that they are slightly soluble in methanol and in acetone, insoluble in ethyl ether and in chloroform. They differ in that the diazomethane preparations are less soluble and do not reduce Fehling solution. The product obtained by the action of methyl sulfate and hot sodium hydroxide reduced Fehling solution. Thus the drastic conditions involved in methylating with dimethyl sulfate bring about degradation of the alginic acid molecule whereas this is not the case with diazomethane.

Ammonolysis of alginic acid methylated by diazomethane showed that in the two cases approximately 74 and 66%, respectively, of the total methylation had taken place on carboxyl groups, and 26 and 34%, respectively, on alcoholic hydroxyl groups (Table II). It showed also that in the first case 84% and in the second case 92% of the carboxyl groups had been methylated. These results are based on the assumption that only the carbomethoxy groups react with ammonia.

Methylation of specimens of nitrated alginic acid did not change the ratio of nitrate groups per C_6 unit. There is thus no replacement of these groups by methoxyl under the conditions employed (Table III). The calculation of nitrate and methoxyl content in the methylated product is made on the basis that the extent of lactone ring opening is equivalent to the degree of methylation.

From the optical data equivalent rotations $[M]^{25}D$ were calculated on the assumption that the dried samples of alginic acid, of nitrated alginic acid and of the alginic acid methylated by means of dimethyl sulfate were lactonized. However, in the solutions of these as examined the materials were lactone free since they were in the form of salts. The equivalent rotation of the product methylated with diazomethane agrees well with the rotations of the sodium salts of alginic acid and of nitrated alginic acid. Thus in the substances which essentially were lactone free (the data of Table II indicate that 92% of the carboxyl groups were methylated) and which had not suffered degradation, the equivalent rotations were in agreement.

The low equivalent rotation for the methylated product resulting from dimethyl sulfate treatment can be ascribed to degradation, for this was the only material which reduced Fehling solution. The drop in rotation may be the result of isomeric changes in the terminal mannuronic residues of the smaller degraded molecules. At one end the configuration about C-1 might change and at the other end a furanose structure might change over to a pyranose structure.[14]

From the data in Table V it is evident that when thoroughly dried, specimens of nitrated alginic acid go over to the lactone form.

(14) Levene, Meyer and Kuna believe that in pectic acid some of the galacturonic units have the furanose structure.

This research was made possible by a grant from the Kelco Company of San Diego, California.

Summary

Alginic acid is converted into a nitrated alginic acid when in contact with nitric–sulfuric acid mixture. The ratio of nitrate groups per mannuronic unit, which varies from 0.49 to 1.2, largely depends upon the excess of nitric acid taken and upon the time of standing.

The failure to obtain higher nitration products is believed to be due to lactonization of the mannuronic units as the result either of drying the acid or of its coming in contact with concd. sulfuric acid.

When nitrated alginic acid is thoroughly dried lactonization of the carboxyl groups takes place.

Methylation of alginic acid by means of dimethyl sulfate and aqueous sodium hydroxide at 60° is not satisfactory since less than one methyl group per mannuronic unit is introduced, and degradation takes place.

Diazomethane is satisfactory as a methylating agent in that little or no degradation takes place. While the carboxyl group is undergoing methylation some methylation takes place on the hydroxyl group.

Nitrated alginic acid can be partially methylated but there is no replacement of nitrate by methoxyl groups.

PASADENA, CALIFORNIA RECEIVED FEBRUARY 6, 1940

Oxidation of Alginic Acid by Periodic Acid

[Reprinted from the Journal of the American Chemical Society, **62,** 1792 (1940).]

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, NO. 775]

Oxidation of Alginic Acid by Periodic Acid

BY H. J. LUCAS AND W. T. STEWART[1]

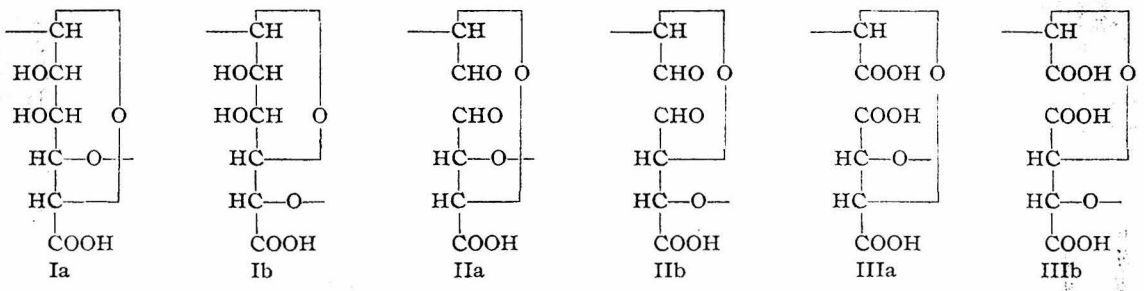
Evidence in regard to the structure and mode of linkage of the mannuronic acid units in alginic acid[2] has been obtained recently by Hirst, Jones and Jones.[3] They subjected alginic acid to partial degradative methanolysis by means of methanolic hydrogen chloride and completely methylated the partially degraded alginic acid. This under drastic treatment with methanolic hydrogen chloride gave the methyl ester of 2,3-dimethyl-methyl-*d*-mannuronide which was then hydrolyzed to 2,3-dimethyl-*d*-mannuronic acid The last was oxidized to 2,3-dimethyl-*d*-mannosaccharic acid. From this and other evidence they concluded that in the mannuronic units of alginic acid hydroxyl groups are attached to C_2 and C_3, while bridge and ring linkages are attached to C_4 and C_5. Although the evidence did not permit a decision between pyranose and furanose structures, the former was favored in view of the resistance of alginic acid toward hydrolysis, and its large negative rotation.

Independent evidence from the oxidation of alginic acid is desirable. Periodic acid would be expected to convert the mannuronic units of al-

(1) Kelco Company Fellow, 1938–1939.

(2) Nelson and Cretcher, THIS JOURNAL, **51,** 1914 (1929); **52,** 2130 (1930); **54,** 3409 (1932); Bird and Haas, *Biochem. J.*, **25,** 403 (1931); Schoeffel and Link, *J. Biol. Chem.*, **100,** 397 (1933).

(3) Hirst, Jones and Jones, *Nature*, **143**, 857 (1939); *J. Chem. Soc.*, 1880 (1939).



ginic acid, of structure Ia or Ib, to the corresponding dialdehyde unit IIa or IIb, which when oxidized by bromine would be expected to yield the corresponding tricarboxylic acid unit IIIa or IIIb. Hydrolysis of II would yield glyoxal and *d*-erythuronic acid while hydrolysis of III would yield glyoxylic and *meso*-tartaric acids.

The isolation of glyoxal would be especially significant, for it can arise only by the scission of the bond between C_2 and C_3. The isolation of *meso*-tartaric acid from III is desirable also as a confirmation. However, its isolation would not be as conclusive as that of glyoxal, for *meso*-tartaric acid could result from the dismutation of erythuronic acid, which would be formed if the scission took place between C_4 and C_5 rather than between C_2 and C_3. The structure which would permit this to happen, *i. e.*, bridge and ring formation involving C_2 and C_3, appears to be quite improbable, as pointed out by Hirst, Jones and Jones.[3] It is of interest to note that the scission of 2,3-dimethyl-*d*-mannosaccharic acid to dimethyl-*meso*-tartaric acid, which was accomplished by these authors with periodic acid, took place between C_4 and C_5. Thus the isolation by them of dimethyl-*meso*-tartaric acid is of value as regards configurations at C_2 and C_3, while the isolation here of *meso*-tartaric acid as an oxidation product of alginic acid itself would be important as regards configurations at C_4 and C_5.

The isolation of glyoxal in 42% yield from the hydrolysis of the oxidation product II, and of *meso*-tartaric acid in 25% yield from the hydrolysis of oxidation product III corroborates the findings of Hirst, Jones and Jones[3] that the mannuronic units in alginic acid are correctly represented by structure Ia or structure Ib. No decision between these two structures can be made on this new evidence.

Confirmation that C_2 and C_3 hold hydroxyl groups was obtained from the periodic acid oxidation of methyl alginate, prepared from alginic acid and diazomethane. The intermediate oxidation products are the methyl esters of the compounds of structure II and III. Hydrolysis of these would yield the same products as in the case of alginic acid itself. Glyoxal was obtained in 30% yield by the hydrolysis of the first oxidation product. However, no *meso*-tartaric acid was isolated from the product corresponding to III.

The methods described by others for the recovery of reaction products resulting from the oxidation of carbohydrates with periodic acid[4] were followed in the main. However, an improvement in the separation of reaction product II from iodic and periodic acids was realized by adding tertiary butyl alcohol to the reaction mixture. This threw down the reaction product while the inorganic materials remained in solution.

This investigation was made possible by a grant from the Kelco Company of San Diego. The authors express their appreciation for this aid, and also their thanks to Professor C. Niemann for helpful advice.

Experimental

Alginic Acid.—This was material obtained from *Macrocystis pyrifera*, similar to that used previously.[5] It was precipitated from its solution in dilute aqueous sodium hydroxide by the addition of dilute hydrochloric acid in slight excess, centrifuged and washed with 50% aqueous alcohol until free of inorganic chloride. The product, a fine white powder, was dried at 30 mm. over calcium chloride. The ash was reduced to 0.7 from 2.5% by this treatment. This drying probably was not powerful enough to convert the acid to the lactone.[5]

Methyl Alginate.—This was prepared by the action of an ether solution of diazomethane upon freshly precipitated alginic acid.[5] The methoxyl content of 18.2% corresponds to 1.1 MeO per C-6 unit. But part of this (5.08% or 0.3 MeO per C-6 unit) was ether methoxyl, as shown by ammonolysis.

Oxidation of Methyl Alginate.—A mixture of 5 g. (0.0259 equiv.) of the above methylated alginic acid and 77 ml. of 0.499 *M* periodic acid (0.038 mole) was stirred until thoroughly dispersed and then allowed to stand for

(4) Jackson and Hudson, THIS JOURNAL, **59**, 2049 (1937); **60**, 989 (1938); Levene and Kreider, *J. Biol. Chem.*, **120**, 593 (1937).

(5) Lucas and Stewart, THIS JOURNAL, **62**, 1070 (1940).

twenty hours, by which time 1.1 mole of periodic acid per equiv. of alginic acid had disappeared.[6]

After iodate and periodate ions had been removed with 5 g. of barium carbonate (stirring and filtering), 6 ml. of 7 *N* sulfuric acid was added and the solution was diluted to 250 ml. After heating on a steam-bath for sixteen hours, sulfate ions were removed by the addition of 5 g. of barium carbonate, and the solution was decolorized with Norit A.

Glyoxal from Oxidation of Methyl Alginate.—A mixture of 225 ml. of the above hydrolyzate (90% of the total), 30 ml. of alcohol and 7.8 g. of phenylhydrazine was heated for one hour on a steam-bath. The precipitated yellowish glyoxalphenylosazone after drying weighed 1.66 g. (0.007 mole), a 30% yield of crude material. After two crystallizations from alcohol and one from benzene this melted at 169–170° to a red-brown liquid.[7]

Anal. Calcd. for $C_{14}H_{14}N_4$: N, 23.5. Found: N, 23.6.

To the remaining 25 ml. of solution was added a solution of 1.2 g. of 2,4-dinitrophenylhydrazine in 50 ml. of glacial acetic acid and the mixture was heated for one hour on a steam-bath. After cooling, the orange precipitate was collected on a sintered glass filter plate, washed once with 5 ml. of hot glacial acetic acid, twice with 5 ml. of alcohol and once with 10 ml. of ether. The dry weight of 0.267 g. (0.00064 mole) represents a yield of 25%. The solid melted at 321° with decomposition,[8] and after crystallization from nitrobenzene, at 323°.

Anal. Calcd. for $C_{14}H_{10}O_8N_8$: C, 40.2; H, 2.4; N, 26.8. Found: C, 40.5; H, 2.6; N, 26.4.

Bromine Oxidation of Oxidized Methyl Alginate.—A mixture of 10 g. (0.0518 mole) of methyl alginate and 154 ml. of 0.499 *M* periodic acid (0.076 mole) was stirred until dispersed, then allowed to stand for twenty hours. The subsequent steps of oxidation to stage III and hydrolysis involved removal of iodic and periodic acids with barium carbonate, oxidation with bromine for twenty-four hours in presence of barium carbonate, removal of bromine by aeration, removal of barium ions by sulfuric acid, removal of bromide ions by silver carbonate, removal of silver ions by hydrogen sulfide and then hydrolysis by heating for sixteen hours. Stirring with bromine for twenty-four hours in the presence of barium carbonate followed by removal of barium ions with sulfuric acid was expected to form oxalic and *meso*-tartaric acids. Two methods of separation were attempted, (1) precipitating oxalic acid from the solution of oxalic and tartaric acid by means of silver oxide, and (2) heating the residue after evaporation with benzoyl chloride to form dibenzoxysuccinic anhydride from the tartaric acid. In the first case no tartaric acid could be isolated from the filtrate after removal of silver ions by hydrogen sulfide, and in the second, no crystalline material could be isolated.

(6) The change in periodate concentration was followed by titration with standard sodium arsenite, Willard and Greathouse, THIS JOURNAL, **60**, 2869 (1938). This is simpler than the method of Fleury and Lange, *J. pharm. chim.*, **17**, 107 (1903), which requires standard solutions of arsenite and iodine.

(7) Literature, 169–170°: Fischer, *Ber.*, **17**, 575 (1884); Hess and Uibrig, *ibid.*, **50**, 367 (1917); 170–171°, Jackson and Hudson.[4]

(8) Lucas and Prater, THIS JOURNAL, **57**, 725 (1935), report 322°, with decomposition; Gladstone and Hickling, *J. Chem. Soc.*, 824 (1936), report 330° (cor.).

Separation of *meso*-Tartaric Acid from Oxalic Acid.—When oxalic acid dihydrate and *meso*-tartaric acid monohydrate are heated separately with excess benzoyl chloride at 100–150°, the former is decomposed while the latter is converted into *meso*-dibenzoxysuccinic anhydride. When a mixture of 0.126 g. (0.001 mole) of oxalic acid dihydrate and 0.15 g. (0.0009 mole) of *meso*-tartaric acid monohydrate is heated with 10.4 g. (0.074 mole) of benzoyl chloride the anhydride can be isolated from the reaction mixture by first extracting it and benzoyl chloride from the solid acids with ether and then diluting this with low boiling petroleum ether, which precipitates the anhydride. This is purified by dissolving in ethyl ether and adding petroleum ether (b. p. 60–70°). The solid is finally washed with a little water to remove unchanged acid; recovery, 0.15 g. (*ca.* 50%); m. p.[9] 207°.

The failure of this method as applied to the oxidation product from methyl alginate may have been due to the presence of other oxidation products or to oxidation of the tartaric acid by bromine in diffused light.[10]

When 0.700 g. (0.0028 mole) of $CuSO_4 \cdot 5H_2O$ in 12 ml. of water was added to a solution of 0.1500 g. (0.001 mole) of anhydrous *meso*-tartaric acid (dried at 100° over phosphorus pentoxide) and 0.1260 g. (0.001 mole) of oxalic acid dihydrate in 30 ml. of water, and the *p*H was adjusted to 2.0 by the addition of a few drops of sulfuric acid, 0.1520 g. (0.00095 mole) of copper oxalate hemihydrate (95% recovery) separated during two hours of standing. After precipitation of copper ions from the solution with hydrogen sulfide, and of sulfate ions with barium hydroxide, evaporation of the solution gave 0.1600 g. (95% recovery) of *meso*-tartaric acid monohydrate which, after conversion to the anhydrous acid, melted at 140°. This method of separation was used in the subsequent work.

Brucine *meso*-Tartrate.—This was prepared by dissolving 0.1000 g. (0.000595 mole) of *meso*-tartaric acid monohydrate in a solution of 0.7800 g. (0.00167 mole) of brucine tetrahydrate in 10 ml. of alcohol and 30 ml. of water. After heating for three hours on a steam-bath, excess brucine was removed by shaking with chloroform. The solution was concentrated to 20 ml. and the brucine tartrate which separated was crystallized twice from water, washed with absolute alcohol and dried at 100° and 30 mm.; m. p. (uncor.) 259° with decomposition; $[\alpha]^{30}D$ −23°; (α^{30} −0.23°; l = 2 dm.; c = 0.5%).

Anal. Calcd. for $(C_{23}H_{26}N_2O_4)_2C_4H_6O_6$: C, 63.3; H, 5.53; N, 5.96; MeO, 13.22. Calcd. for $C_{23}H_{26}N_2O_4 \cdot C_4H_6O_6$: C, 59.54; H, 5.94; N, 5.15; MeO, 11.40. Found: C, 59.72; H, 5.77; N, 5.60; MeO, 11.48.

These analyses indicate that this salt, even when prepared with an excess of brucine, is a monobrucine tartrate. Confirmation is given by the recovery of brucine from the salt by heating with barium hydroxide solution, centrifuging, extracting brucine from the filtrate and washings with chloroform, from the precipitated barium salts with alcohol, and evaporating the solutions.

Recovery from 0.5918 g., calcd. for monobrucine salt: 0.4317 g.; for dibrucine salt: 0.4967 g. Found: 0.4272 g.

(9) Brigl and Gruner, *Ber.*, **65**, 641 (1932), report the m. p. as 207°. The anhydride is difficult to purify.

(10) In direct sunlight *d*-tartaric acid is oxidized by bromine; Ciusa and Piergallini, *Atti accad. Lincei*, **23**, I, 821 (1914).

Brucine tartrate was prepared as above also from 0.001 mole of each of the reactants, 0.466 g. of brucine tetrahydrate and 0.168 g. of *meso*-tartaric acid monohydrate. The unreacted brucine, obtained by extracting the solution with chloroform and the solid with alcohol, was recovered by evaporating the extracts. The unreacted acid was recovered by evaporating the aqueous phase. The results summarized in Table I and the analysis of the salt show that it is monobrucine tartrate. Thus the same salt is formed when the unreacted brucine is extracted by chloroform, no matter what excess of brucine is taken initially.

Anal. Found: C, 60.41; H, 6.00; N, 5.37; MeO, 11.35.

TABLE I

	Actual wt., g.	Weight expected, if salt is: Monobrucine tartrate, g.	Dibrucine tartrate, g.
Brucine tartrate	0.442	0.544	0.469
Unreacted brucine recovered	.106	.088	.027
Unreacted acid recovered	.029	.031	.089

Oxidation of Alginic Acid with Periodic Acid.—Dried alginic acid, 15 g. (0.085 equiv.) was stirred vigorously with 425 ml. of 0.380 *M* periodic acid until peptized, which required about one and one-half hours. The mixture stood at room temperature for twenty to twenty-four hours during which time periodic acid was reduced (1.1 mole per equivalent of alginic acid). The oxidation proceeded comparatively rapidly during the first two hours (Fig. 1). Addition of 1600 ml. of tertiary butyl alcohol threw down an amorphous precipitate. This was centrifuged down and washed four times with 50 ml. of aqueous tertiary butyl alcohol (1 part of alcohol to 3 of water). After drying at 30 mm. over sulfuric acid the resulting fluffy white residue, which corresponds to reaction product II, weighed 13 g.

This material is easily peptized when added to water and the aqueous solution reduces Fehling solution; $[\alpha]^{25}D$ +36.7 (α^{25} +0.11°; l = 1 dm.; c = 0.3%).

Hydrolysis of II and Isolation of Glyoxal.—Two grams (0.0115 equiv.) of II was heated with 100 ml. of water on a steam-bath for two hours to effect solution. The hot solution was filtered, acidified with 2.5 ml. of 10 *N* hydrochloric acid and diluted to 200 ml. This was heated on a steam-bath until the optical rotation became constant (α +0.06) after sixteen hours. The hydrolyzate was neutralized with sodium hydroxide and divided into two equal portions. One-half was heated with 2.7 g. of phenylhydrazine at 100° for two hours, the other with a solution of 4.5 g. of 2,4-dinitrophenylhydrazine in 175 ml. of hot glacial acetic acid. The former half yielded 0.580 g. (0.00244 mole) of yellow glyoxal phenylosazone, melting after purification at 169–170°.

Anal. Found: N, 23.7.

The latter half yielded 0.660 g. (0.00158 mole) of orange-red glyoxal dinitrophenylosazone, melting at 317° with decomposition, and at 323° after purification as before.

Anal. Found: C, 40.6; H, 2.5; N, 26.4.

The yield of glyoxal in the form of osazones is 42 and 27%, respectively.

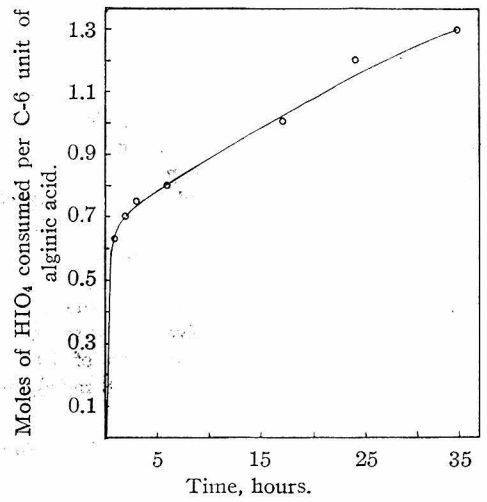


Fig. 1.

Bromine Oxidation of II to III and Isolation of Brucine *meso*-Tartrate.—Ten grams (0.0575 equiv.) of II dispersed in 475 ml. of water, 12 ml. of bromine and 43.4 g. of barium carbonate were stirred mechanically for twenty-four hours. Bromine was removed by aeration and the solution freed from barium ions by the addition of an equivalent amount of sulfuric acid. The solution was diluted to 900 ml. and then heated on the steam-bath until hydrolysis was complete (zero rotation after sixteen hours). The hydrolyzate was concentrated to 400 ml., made slightly alkaline while hot with barium hydroxide and kept hot for three hours. During this time glyoxalic acid was converted in part to oxalic and glycolic acids. The precipitated barium salts, *viz.*, barium oxalate and barium tartrate (barium glycolate is slightly soluble in water), were centrifuged down and washed twice with 40 ml. of hot water. Barium ion was precipitated by the careful addition of sulfuric acid to a suspension of the salts in 150 ml. of water. At this point the solution contained oxalic and *meso*-tartaric acids.

The solution was concentrated at 40 mm. to 30 ml., 4 g. of $CuSO_4 \cdot 5H_2O$ was added, and the *p*H was adjusted to 2.0 with sulfuric acid. The precipitated copper oxalate was removed by filtration, the filtrate diluted to 70 ml., cupric ion was precipitated with hydrogen sulfide and sulfate ion with barium hydroxide. On the addition of 45 ml. of water, 50 ml. of alcohol and 20 g. (an excess) of brucine, followed by heating for three hours on the steam-bath, removal of excess brucine with chloroform and concentration of the aqueous solution to 30 ml. at 40 mm., 8.0 g. (0.0147 mole) of monobrucine *meso*-tartrate was recovered in 25% yield. After two crystallizations from water this melted at 259° with decomposition: $[\alpha]^{30}D$ −22° (α −0.11°; l = 1 dm.; c = 0.5%).

Anal. Calcd. for $C_{23}H_{26}N_2O_4(C_4H_6O_6)$: C, 59.5; H, 5.9; N, 5.15; MeO, 11.4. Found: C, 60.0; H, 6.0; N, 5.4; MeO, 11.4.

Dibenzoxysuccinic Anhydride from Hydrolyzate.—When 0.669 g. (0.00123 mole) of the above brucine salt was heated with 22 ml. of 0.1202 *M* (0.0026 mole) barium hydroxide for one hour on the steam-bath, an insoluble barium salt separated. This was centrifuged down.

washed with 10 ml. of water and 20 ml. of alcohol, and dried. After quantitatively freeing this of barium ions with sulfuric acid and removing barium sulfate, the solution was taken to dryness in a vacuum desiccator. The residue of 0.196 g. (0.0012 mole as tartaric acid monohydrate) was heated slowly with 0.85 g. (0.0059 mole) of benzoyl chloride to 150° over a period of three hours. The reaction mixture was extracted with 30 ml. of ether, the clear extract concentrated to 4 ml., and 10 ml. of petroleum ether, b. p. 60–70°, was added. Crystallization of dibenzoxysuccinic anhydride was promoted by scratching the sides of the vessel. The supernatant liquid was decanted, the solid was dissolved in 10 ml. of ether and then thrown down by the addition of 10 ml. of petroleum ether; recovery 0.23 g. (0.00068 mole), m. p., 206°.

Anal. Calcd. for $C_{18}H_{12}O_7$: saponification equiv., 85.0. Found: sapon. equiv., 86.1.

Summary

Alginic acid has been oxidized by means of periodic acid to a substance, presumably the corresponding polymeric dialdehyde acid, which undergoes hydrolysis in dilute acid. From the hydrolyzate glyoxal has been obtained in 42% yield.

The polymeric dialdehyde acid has been oxidized by bromine water. From the hydrolyzate of the resulting acid, *meso*-tartaric acid has been isolated in 25% yield.

Methyl alginate has been subjected to oxidation by periodic acid. Glyoxal in 30% yield has been isolated.

These results show that the scission of the mannuronic acid units of alginic acid takes place between the second and third carbon atoms. Therefore, hydroxyl groups are attached to C_2 and C_3, while bridge and ring linkages are attached to C_4 and C_5. The presence of other structural units in small amount is not excluded.

No conclusion has been drawn in regard to furanoside or pyranoside structure, or to alpha or beta configuration at C_1.

PASADENA, CALIFORNIA RECEIVED APRIL 15, 1940

A Chromatographic Study of Lignite

A Chromatographic Study of Lignite*

Chromatographic analysis has been employed extensively in the isolation of compounds present in natural products. In many cases the isolation of pure substances from complex mixtures has succeeded only through the use of the Tswett method.

The organic compounds present in lignite have not been investigated to any great extent. Since lignite is an extremely complex mixture, the isolation of any constituent in a pure state is rather difficult. Recently L. Zechmeister and O. Frehden[1] carried out a partial chromatographic analysis of a Hungarian lignite and succeeded in isolating two crystalline compounds, one a triterpene, the other the potassium salt of an organic acid. This latter compound possessed a surprisingly high reducing power toward iodine, silver nitrate, selenic acid and dichlorophenol-indophenol. Their method involved extraction of raw lignite with light petroleum and a partial adsorption of the dissolved substances on calcium hydroxide. The resulting chromatogram was cut under an ultraviolet lamp and the adsorbed substances were eluted from the calcium hydroxide with alcohol and light petroleum. Such a technique of adsorption and examination under ultraviolet light had not been used previously in the study of lignite. Many of the substances present in lignite exhibit an intense fluorescence on illumination with ultraviolet light and a separation of layers of different fluorescent intensity and color can be effected readily.

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* The lignite used in this investigation was kindly supplied by the University of North Dakota.

It was desirable to investigate an American lignite and compare its chromatographic behavior with that of Hungarian lignite. When the procedure described was followed exactly only amorphous powders could be isolated from the American lignite. The two crystalline compounds described by the Hungarian authors could not be isolated in the course of the present investigation. Extraction solvents other than light petroleum were tried and an acetone-water mixture (9 : 1) was found to give the best results. The aqueous acetone extract was diluted with water and extracted with light petroleum ether. This served to effect a partial separation of the constituents present in the original extract. The light petroleum solution was then chromatographed on calcium hydroxide. The course of the development was followed by observation under the ultraviolet lamp. The resulting chromatogram consisted of a great number of layers of varying fluorescent intensity and color. A representative chromatogram is shown in Figure I. Individual zones showing intense fluorescence were cut out under ultraviolet light and re-chromatographed several times. After the final chromatographic purification the fluorescent substances were crystallized once from light petroleum and three times from a mixture of benzene and alcohol. In this way six different and well crystallized substances were isolated.

Experimental

Twenty-five kilograms of lignite divided into five portions were shaken with twenty liters of 90% acetone for one hour. The lignite was allowed to stand overnight in contact with the acetone and the

resulting extracts were drained off and filtered clear. Filtrates from each of the 5 kilogram portions were concentrated to 2 liters, diluted to 4 liters with water and extracted three times with 600 ml. of light petroleum, b.p. 60-70°. The three ligroin extracts were combined and concentrated to 75 ml. and chromatographed on a calcium hydroxide column (20 x 5 cm.). The chromatogram was developed with 400 ml. of light petroleum and cut under the ultraviolet lamp into six zones as indicated in Figure I. The separate zones were eluted with 200 ml. of a mixture of alcohol and light petroleum (1:1) and eluates of corresponding zones from all of the chromatograms prepared from the 5 kg. portions were combined and washed free of alcohol in a separatory funnel. The resulting light petroleum solutions were dried with sodium sulfate and re-chromatographed. Again the strongly fluorescing zones were cut out and eluted as before. After this preliminary division into zones, each zone was chromatographed again on a calcium hydroxide column (20 x 3 cm.), developed with 200 ml. of light petroleum and divided further into sections consisting of several narrow layers or of a single broad layer (Figure I). The individual layers were eluted with a mixture of ligroin and alcohol. The alcohol was removed by washing and each of the ligroin solutions was dried with sodium sulfate and concentrated to a volume of about 0.5 ml. Small colorless glittering crystals were deposited from several of these concentrates, and after standing for several days in the cold room the mother liquors were decanted. The crystals were washed with a small amount of cold light petroleum and recrystallized three times from a mixture of alcohol and benzene (1:1). Some properties of these compounds are summarized in Table 1.

Table 1

Some Properties of Crystalline Compounds Isolated from Lignite

Substance	Amount isolated from 25 kg. of lignite--mg.	Fluorescence in solid state	Fluorescence adsorbed on lime from ligroin	Melting Point (corrected)[b] ° C.	Liebermann and Burkhardt's sterol reaction[c]
I	2-1/2	lavender	dull green	261-264	negative
II	2	yellow (weak)	greenish gray	317-319	"
III	3-1/2	blue white	greenish gray	273-274	"
IV	4	blue white (strong)	sky blue	238-240	"
V	2	lavender	yellow	297-298	"
VI	2	yellow	dull greenish gray	249-251	"

a. Crude crystalline material before recrystallization from alcohol-benzene mixture.

b. The melting points were determined in a copper block as designed by E. Berl. Melting was accompanied by a slight darkening. On re-solidifying and re-melting the substances melted 3-4° lower.

c. A chloroform solution of the substance to be tested is mixed with one drop of acetic anhydride and a few drops of concentrated sulfuric acid are added. The presence of a sterol is indicated by typical colors in the upper and lower layers.

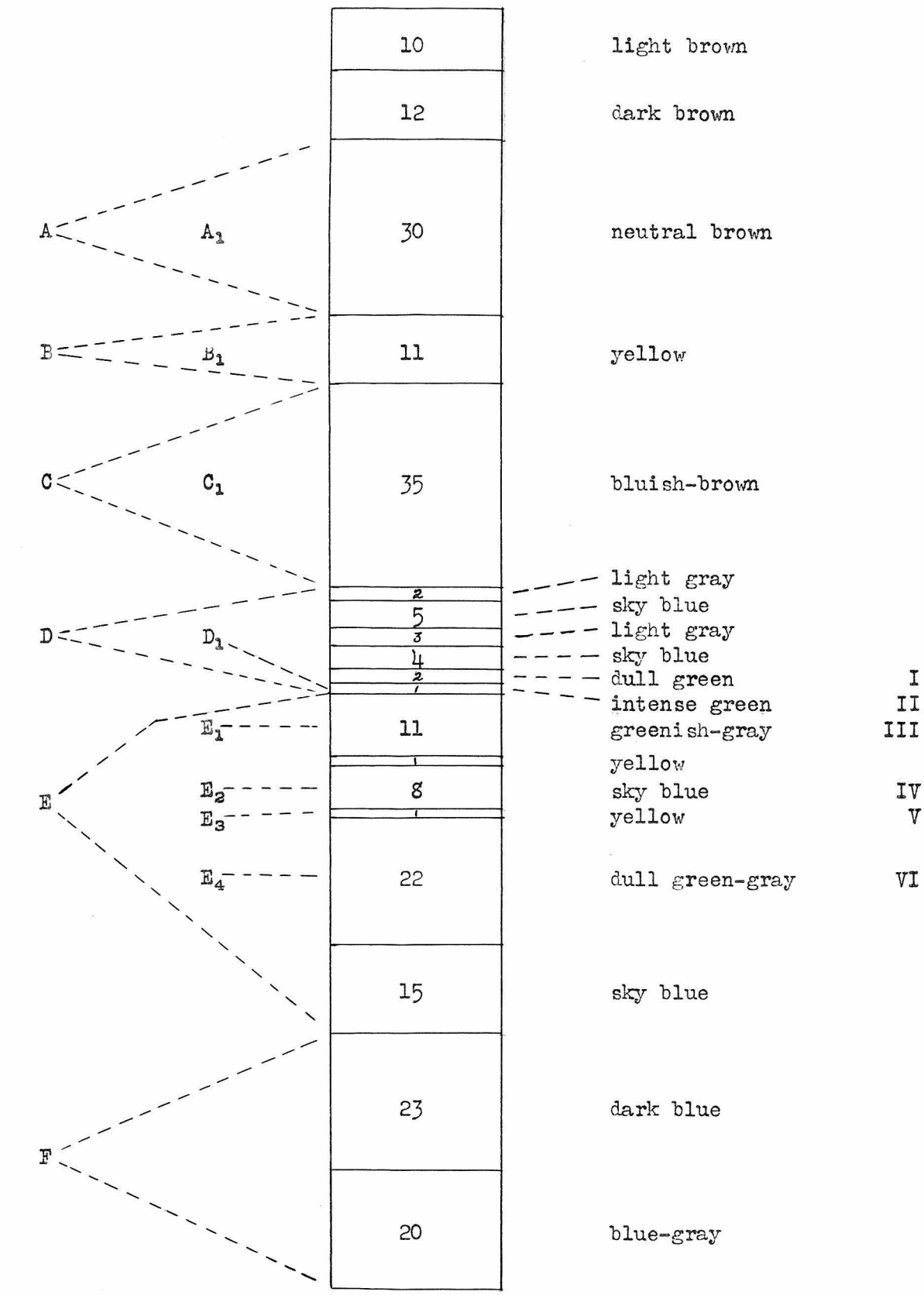

Figure I

A typical fluorescent chromatogram of an extract of lignite

The substances are very soluble in chloroform and benzene, moderately in light petroleum, slightly in absolute alcohol and insoluble in water.

An attempt was made to determine the molecular weights of the crystalline compounds by the micro-method of Giral[2]. This procedure, which is a modification of the Rast[3] camphor method, employs Exaltone (cyclopentadecanone) as a solvent. It was found, however, that the substances were insoluble in molten Exaltone and the method accordingly could not be used. This insolubility is somewhat surprising since Exaltone is an excellent solvent for a great number of high molecular weight organic compounds, e.g. sterols, carotenoids, etc.

Summary

A systematic chromatographic fractionation of aqueous acetone extracts of North Dakota lignite was carried out and six crystalline colorless substances were isolated in small quantities. Their melting points range from 238-240 to 317-319°. All compounds exhibit fluorescence under ultraviolet light. Two crystalline substances found in Hungarian lignite by earlier investigators could not be isolated from North Dakota lignite.

Appendix

Preliminary experiments suggest that the method of fluorescence chromatography may be useful in the investigation of crude petroleum. A sample of California crude petroleum was diluted with benzene and chromatographed on a calcium hydroxide column. The chromatogram consisted of a series of more or less strongly fluorescing layers. The sharp definition of the borders of these zones indicates the possibility of further fractionation and purification based upon the Tswett method.

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1. L. Zechmeister and O. Frehden, Nature, 144, 331 (1939).

2. F. Giral, Anales de la Sociedad Espanola de Fisica y Quimica, 33, 438 (1935).

3. K. Rast, Berichte, 55, 1051 (1922).

Summary

The reversible hydration of β-methylcrotonaldehyde to β-hydroxyisovaleraldehyde in dilute aqueous nitric acid has been investigated at several temperatures. The specific velocity constants and heats of activation of the hydration and dehydration reactions have been calculated. The heat of reaction has been determined from equilibrium constants at different temperatures.

Alginic acid has been nitrated with a mixture of nitric and sulfuric acids to yield nitrated products containing from 0.49 to 1.2 nitrate groups per mannuronic unit. Methylation of alginic acid is best accomplished with diazomethane. While the carboxyl group is undergoing methylation some methylation takes place in the hydroxyl group.

Evidence regarding the structure of alginic acid has been obtained from the oxidation of alginic acid and methyl alginate by means of periodic acid. It can be concluded that hydroxyl groups are attached to C_2 and C_3, while bridge and ring linkages are attached to C_4 and C_5. No conclusion can be drawn in regard to furanoside or pyranoside structure, or to alpha or beta configuration at C_1.

The chromatographic adsorption method in combination with fluorescence analysis has been employed in a study of North Dakota lignite. Several highly fluorescing crystalline compounds have been isolated and some of their properties determined.

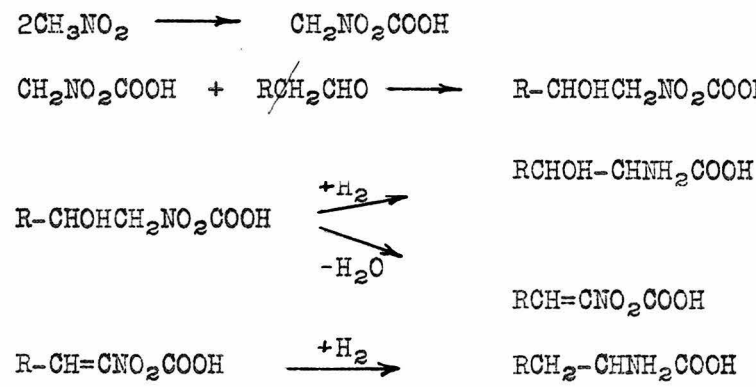
Propositions

Propositions

1. The formation of either alpha- or beta-alkylcellobioside heptaacetates from alpha-bromocellobiose heptaacetate and alcohols in the presence of mercury salts can be explained on the basis of two competing reactions, one involving a direct replacement of the bromine atom by an alkoxy group, the other involving a replacement of the bromine atom by an acyl group with subsequent replacement of the acyl group by an alkoxy group.

   Zemplen and Gerecs, Ber. 63, 2720 (1930).

2. A synthesis of a number of amino acids starting with nitromethane and various aldehydes is proposed as follows:



   B. M. Vanderbilt and H. B. Hass, J. Ind. and Eng. Chem. 33, 65 (1941)
   Welkendorf and Trenel, Ber. 56, 611 (1923); Ber. 57, 306 (1924).

3. A comparison of the heats of vaporization and heats of combustion (calculated for the gaseous state) of 1,2-propanediol and 1,3-propanediol indicates the existence of intramolecular hydrogen bonding in the 1,2-propanediol molecule.

   Louguinine, Compt. rend. 91, 297 (1880)
   J. Nef, Annalen 335, 203 (1904)
   L. Henry, Chemisches Zentralblatt 68, 741 (1897).

4. Recent evidence indicates that the decomposition of acetates of nitro-alcohols to nitroölefins in aqueous solution probably occurs through a base catalyzed cleavage of the ester molecule rather than hydrolysis with subsequent dehydration.

   B. M. Vanderbilt and H. B. Hass, J. Ind. and Eng. Chem. 32, 34 (1940)

   J. B. Tindal, ibid. 33, 65 (1941).

5. The acid catalyzed oxygen exchange between a carboxylic acid and water may involve the approach of a hydronium ion to a carboxylate ion.

   M. Cohn and H. C. Urey, J. Am. Chem. Soc. 60, 679 (1938)

   M. Senkers and W. Brown, Jour. of Org. Chem. Vol. II, 569 (1938).

6. A quantitative procedure for the separation and analysis of a mixture of oxalic acid and meso-tartaric acid is proposed. This procedure involves the following steps:

   a. Quantitative separation of the copper salts of the two acids.

   b. Conversion of the copper salts to the free acids.

   c. Quantitative estimation of meso-tartaric acid by oxidation with potassium permanganate.

   d. Quantitative estimation of oxalic acid by oxidation with potassium permanganate or with bromine in the presence of mercuric ion.

   H. J. Lucas and W. T. Stewart, J.Am. Chem. Soc. 62, 1794 (1940)

   K. J. Goering, Master's Thesis, California Institute of Technology,(1938).
   Mestrezat, Chemisches Zentralblatt, 78, II, 185 (1907).

7. Both d- and ℓ-tartaric acids form dibrucine salts in the presence of an excess of brucine, whereas, meso-tartaric acid forms only a mono-brucine salt. The failure of meso-tartaric acid to form a dibrucine salt can be attributed to internal hydrogen bond formation and steric hindrance

   H. J. Lucas and W. T. Stewart, J. Am. Chem. Soc. 62, 1794 (1940).

8. A compilation of a table of constants indicative of the relative rates of descent of substances in a Tswett column is very desirable.

9. Micro-biological assays for a number of vitamins excel over chemical and physical methods from the standpoint of specificity, sensitivity and accuracy.

10. Lundegardh's calculations of the efficiency of ion absorption by plant tissues are subject to question. Contrary to his conclusion, calculations based upon recent work indicate that ion absorption may be a reasonably efficient process.

H. Lundegardh, The Annals of the Agricultural College of Sweden, 8, 320 (1940).

F. C. Steward and W. E. Berry, Journal of Experimental Biology, 11, 103 (1934).