[CONTRIBUTION FROM THE DIVISION OF ORGANIC CHEMISTRY OF THE STATE UNIVERSITY OF IOWA]

THE STRUCTURE OF METHYLATED SUGARS. II[1]

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RECEIVED DECEMBER 14, 1929 PUBLISHED APRIL 7, 1930

On looking over the literature on the methylation of simple sugars, it is seen that the so-called gamma or active or unstable derivatives have been obtained from solutions at comparatively low temperature and low concentration of acid. Except in the first paper of this series,[3] the active monomethyl derivative has never been made by the methyl sulfate method. It has had to be isolated from other preparations before further methylation.

Since low temperatures and nearly neutral solutions appear to be the least drastic and most normal conditions under which methylation can take place, it seemed desirable to determine whether the product obtained by methylating simple sugars under these conditions is a stable or unstable form.

Mannose was selected for the present investigation because previous work by one of us[4] indicated that under conditions similar to those here considered, mannose formed a product which could not be completely methylated by the methyl sulfate method.

One of the most characteristic properties of the methyl derivatives of unstable sugars is the speed of reaction with potassium permanganate. There is considerable confusion in the use of this test because some writers specify alkaline permanganate solution, while others specify neutral permanganate and still others do not specify whether the solution is alkaline, neutral or acid. The test is not generally used in a way that is even semi-quantitative.

A method has been developed[5] for determining the rate of oxidation of various sugars and hexosides by means of potassium permanganate in an acid buffered solution. This method seems not to have been applied previously to alkyl derivatives of either stable or unstable sugars and it seemed desirable to apply it to these derivatives.

Apparatus

The two pieces of apparatus deserving particular description are the methylator and the vacuum distilling apparatus.

[1] This paper is taken from a thesis submitted by John E. Milbery in partial fulfilment of the requirements for the degree of Master of Science in the Department of Chemistry in the Graduate College of the State University of Iowa.

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[3] Whitnah, THIS JOURNAL, **51**, 3490 (1929).

[4] C. H. Whitnah, "Studies in the Structure of the Lactones of Sugar Acids," University of Nebraska Thesis, **1925**, p. 9.

[5] Kuhn and Wagner-Jauregg, *Ber.*, **58**, 1441 (1925).

The reaction flask of the methylator was an ordinary 2-liter pyrex balloon flask (A). This was fitted with a rubber stopper through which extended one end of a condenser (L), electrodes of antimony and platinum (Sb, Pt) and the two buret tips (M,N). There was also a small hole in the stopper used in removing the samples used for indicator tests.

This flask was immersed to about half its depth in a simple constant temperature bath made from an ordinary pneumatic trough. The bath was heated by an electric lamp which was controlled by a mercury thermoregulator and relay. The bath was agitated by jets of air from a glass tube in which several small openings had been drawn out. The temperature variation was too small to be detected by an ordinary thermometer graduated in single degrees.

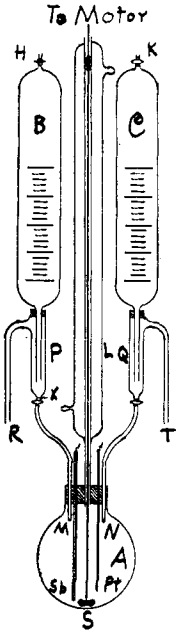

Fig. 1.—Methylator.

The methylator was originally constructed for handling larger quantities of sugar at higher temperatures where the reaction was rapid. For this reason the burets (B,C) were large and were so arranged that a comparatively constant head of liquid might be maintained even though the volume of liquid was decreasing. These burets were filled by placing the tubes (R) and (T), respectively, in a flask of the reagent and applying suction at (H) or (K). Before the end of the work here reported, the sodium hydroxide buret was replaced by an ordinary 50-cc. buret which was sealed to the bent tube at the point (X).

The stirrer (S) was passed through the condenser to a motor mounted above.

The electrode (Sb) was made by pouring molten antimony into a sealed glass tube and then dipping a copper wire into it. When the antimony had become solid, the glass was broken from the end of it, leaving a rod of antimony sealed into the glass tubing. The electrode (Pt) consisted of a platinum wire welded to a copper wire and sealed through a glass tube.

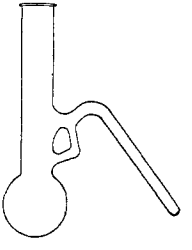
Fig. 2.

The arrangement of methylator and wiring for thermostat and potentiometer is indicated by Fig. 1.

The vacuum distillation apparatus consisted of an oil-sealed pump, a mercury manometer of the U type, a two-pronged adapter, which made it possible to collect two fractions without disturbing the vacuum, a short water-cooled condenser and a special 25-cc. distilling flask (Fig. 2) which prevented the liquid from bumping over into the condenser.

Experimental Part

The mannose used in this work was prepared from ivory nut shavings by a slight modification of Harding's method,[6] the shavings being supplied by the Rochester Button Co. The crystalline product used for methylation melted at 125–126° uncorrected and gave a specific rotation of 16.6° five minutes after solution. The product was found to be free from both barium and sulfate. The crystals were white and dissolved in water to form a transparent colorless solution.

[6] Harding, *Sugar*, **25**, 583 (1923).

Methylation of Mannose. All methylations were carried out in the general manner now to be described. The particulars of each significant run are shown in Table I.

The bath was adjusted to the desired temperature and the burets were filled. Then the sugar was dissolved and poured into the flask through the hole from which samples for titration were withdrawn. A little dimethyl sulfate was then added and the solution adjusted to the desired alkalinity by the addition of 30% sodium hydroxide. The alkalinity was tested by adding a drop of solution to a drop of indicator on a spot plate and then adding drops of solution and dilute acid as necessary. Since relatively large quantities of indicator had to be used, enough sodium hydroxide was added to the phenolphthalein solution to give it the faint pink color of the end-point. Each drop of brom thymol blue, which is insoluble in neutral solution, was neutralized on the spot plate.

When the alkalinity had been adjusted, it was kept near this point by adding a drop of alkali from time to time as required. The alkalinity was also checked with some success by means of the electrode–potentiometer arrangement. The potentiometer was so set that there was no deflection of the galvanometer when the mixture was at the desired alkalinity. Alkali was added as soon as the needle swung to the acid side. If the electrical control worked at all, it was much more sensitive and convenient than the indicator method. Occasional checking by the indicator method was always necessary, however, and at times the electrical control was very erratic.

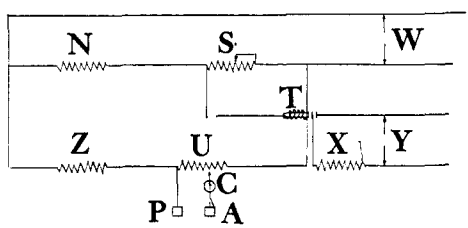

Fig. 3.—Wiring diagram. N, 30 c.p. 110 v. lamp; S, 30 ohms; W, 110 v. d.c.; T, thermostat relay; Z, 20 c.p. 220 v. lamp; U, 6 ohms; X, heating lamp; Y, 110 v. a.c.; C, galvanometer key; P, platinum; A, antimony.

The reaction was considered to be practically complete when a marked decrease occurred in the rate at which sodium hydroxide was required. The last traces of methyl sulfate were then removed either by heating or by long continued action of alkali at the temperature of methylation.

When constant alkalinity showed the destruction of methyl sulfate to be complete, the solution was cooled if necessary and extracted twice with 75-cc. portions of chloroform. The aqueous residue was then made faintly acid with dilute sulfuric acid and extracted twice more.

The combined chloroform extracts were dried by shaking with lumps of calcium chloride, decanted from the drying agent and the chloroform was distilled off on a water-bath. The sirup which remained was transferred to the special distilling flask with the aid of a little chloroform.

The last traces of chloroform were then removed by heating to about 100° in an oil-bath and applying suction from a water pump. The suction was then changed with the aid of a three-way cock to an oil-sealed mechanical pump. After the pressure became suitably reduced, the temperature of the oil-bath was gradually raised until the product distilled at the temperature and pressure shown in Table I.

In Table I the following abbreviations are used: Pot. = readings on arbitrary scale of potentiometer; PP = reaction toward phenolphthalein; BTB = reaction toward brom thymol blue; NaOH = number of cc. of 30% sodium hydroxide added; Me_2SO_4 = number of cc. of dimethyl sulfate added; under the columns headed PP and BTB, N = neutral, N–A =

TABLE I

METHYLATION OF MANNOSE

Run no.	2			5					8					9				10			
Reagents	50 cc. H_2O; 20 g. sirup			50 cc. H_2O; 20 g. crystals					30 cc. H_2O; 10 g. crystals					50 cc. H_2O; 20 g. crystals				50 cc. H_2O 20 g. crystals			
Temp. 1 (during reaction)	50° for 2 hrs.			30° for 14 hrs.					30° for 17 hrs. before reagents added; 30–40° in 1 hr.; 40° 6.5 hrs.					30° for 18 hrs.				35° for 11 hrs.			
2 (final)	100° 0.5 hr.			30° until titer constant					40° until titer const.					30° until titer const.				35° until titer const.			
Yield	10.4 g., 49.3% CH_3O			3.5 g., 52.7% CH_3O					Fr. I, 2.8 g., 52.5% CH_3O					Fr. I, 1.9 g., 52.7% CH_3O				Fr. I, 3.9 g., 54.2% CH_3O			
°C./mm.	116–128/2			122–124/1					95–105/0.5					115/1–7				95–101/0.5			
									Fr. II, 1.3 g., 50.0% CH_3O					Fr. II, 5.7 g., 52.5% CH_3O				Fr. II, 7.7 g., 52.5% CH_3O			
									105–115/0.5					99–100/1				101–115/0.5			
Form	Stable			Unstable					Stable					Unstable				Stable			
Time	P. P.	NaOH	Me_2SO_4	Pot.	P. P.	BTB	NaOH	Me_2SO_4	Pot.	P. P.	BTB	NaOH	Me_2SO_4	P. P.	BTB	NaOH	Me_2SO_4	P. P.	BTB	NaOH	Me_2SO_4
0:00	1/2	0	0		A	B	0	0		N	B	0.0	0	B	B	0.0	0	A	A	0.0	0
0:14	1/1	7	5		A	N-B	6	45		N-A	B	0.4	6	B	B	3.7	53	A	B	5.1	58
0:22	1/1	19	15							A	N	0.6		A	B	3.7		A	B	5.3	
0:30	1/2	26	24	120	A	B	7			A	B	0.8		A	B	4.0		A	B	6.2	
1:00	2/1	54	47							N	B	1.5		A	B	5.1		A	B	7.9	
1:30	N	86	82	107	A	B	10	49	110	N	B	4.8	17	A	B	5.8		A	B	10.0	
2:00	2/1	114 end	100	105	A	B	11		91	N-A	B	9.0		A	B	6.6		N-A	B	11.9	
2:30				99	A	N-B			91	A	B	11.0		A	N	7.3		A	B	14.8	74
3:00				103	A	B	11.5		91	A	B	17	31	A	B	8.5		A	B	17.9	
3:30				103	A	B	14		88	A	N	20	32	A	B	9.3		A	N	21.1	
4:00				97	A	B	16		88	A	B	25	34	A	B	10.0	93	N	B	24.8	
5:00				97	A	B	17		85	N		40	46	A	B	14.0		N-A		32.8	
6:00				97	A	B	20		94	1/3		56	52	A	N	16.0		N		39.7	
7:00									112	N		83	76	A	B	18.0		1/3		48.6	94
7:45				97	A	N	24		112	1/3		95		A	B	19.5		N		67.6	114
8:35				107	A	N	29							A	B	21.5		1/4		89.7	
9:00				103		N-B	30			1/2		96 end		A	B	22.6		N		101.0	140
10:00				97		N-B	34							N-A		26.6		N		138.0	
10:45				93	N-B		36							N		30.6					
11:30				87	1/1		45							1/2		37.2		1/1		178.0 end	
13:05				95	1/1		98	89						N-B		55.5					
13:35				95	1/1		127	131						1/2		66.6					
13:55					1/1		144 end							N-B		72.9	114				
16:11														1/4		134.0	139				
17:53														1/2		166 end					

neutral to acidic, A = acidic, N–B = neutral to basic and B = basic. The denominators of the fractions in these columns indicate the number of drops of solution required to neutralize the number of drops of 0.025 *N* hydrochloric acid indicated by the numerators. Hence the larger the value of the fraction, the more alkaline the solution.

Runs 6 and 7, which are mentioned in the report of oxidations (Table II) but are not included in Table I, were on crystalline mannose at 50 and 40°, respectively. Run 10 shows that they were not significant to the present problem.

It must be understood that the alkalinity required testing very frequently and that the observations recorded are typical instances.

The unstable products are distinguished from the stable by speed of reaction with neutral permanganate, speed of reaction with buffered acid permanganate, greater mobility when fresh, greater discoloration and much greater viscosity after standing for a few weeks.

Oxidation Experiments.—The modifications in the method of Kuhn and Jauregg were mainly for the purpose of adapting the method to smaller quantities of material.

The calibration of volumetric apparatus did not seem justified in the present work. The same instruments, however, were used in all runs and the buret used in thiosulfate titrations was filled to the zero mark each time.

An approximately 0.1 *N* solution of potassium permanganate was allowed to stand until its concentration became sensibly constant and then standardized against sodium oxalate as 0.1026 *N*. This solution was used in all runs.

Approximately 0.02 *N* solutions of sodium thiosulfate were made and titrated against the permanganate solution in buffered potassium iodide, the exact procedure being followed that was used in oxidation experiments.

The phosphate buffer was made up so as to be approximately *M*/15 with respect to both phosphoric acid and primary potassium phosphate. The actual quantities were 5.0 cc. of H_3PO_4 (85%) and 9 g. of KH_2PO_4 per liter of solution. The P_H of this solution after being diluted five times, as was the case in the oxidations, was determined colorimetrically to be 2.3. No difference could be observed in the P_H of a solution in which oxidation had been allowed to continue until the permanganate had been destroyed.

A 10% solution of potassium iodide was made up by weight and replaced whenever it became slightly yellow.

The experimental procedure was as follows: 20 cc. of the buffer solution was run from a buret into a 100-cc. volumetric flask and placed in a thermostat at 30°. The permanganate was then added from a pipet. When this mixture had come to temperature, the substance to be tested was added and the solution quickly made up to volume with water at 30°. When studying the effect of time in solution on rate of oxidation, 5 g. of substance was weighed out and made up to 250 cc. of solution. This solution was kept in the bath at 30° and 50 cc. used for each oxidation. When the initial rate only was desired, exactly 1 g. of substance was dissolved and then added to the oxidizing solution.

At definite time intervals after starting the oxidation 10-cc. samples were removed with a pipet and run into flasks containing 15 cc. of buffer solution and 1 cc. of potassium iodide solution. The liberated iodine was immediately titrated with thiosulfate, starch indicator being used near the end-point.

TABLE II

OXIDATION OF SUGARS IN BUFFERED POTASSIUM PERMANGANATE

Substances oxidized	Minutes of oxidation	$KMnO_4$ added, cc.	1	2	3	6	7	10	12	20	30	40	50	60	70
			Volume in cc. of 0.1026 *N* $KMnO_4$ decomposed in minutes shown above												
1.1027 g. glucose		10						0.66		3.14	6.48	6.64	6.82	6.86	
1.0209 g. glucose		10						.32		1.60	4.10	6.60	6.71	6.82	
Glucose; 2:55 hr.		10						.44		0.78	1.06	1.36	1.76	2.12	2.40
Glucose; 23:05 hr.		10						.30		.46	0.68	0.86	1.14	1.44	1.72
Glucose; 40:05 hr.		10						.38		.56	0.70	0.86	1.10	1.42	1.72
Mannose; 0:15 hr.		10						.48		.84	1.42	1.80	2.40	3.04	3.66
Mannose; 42:05 hr.		10						.42		.64	1.00	1.42	1.80	2.36	2.80
Product of Run 2		10						.84		1.22	1.48	1.74	1.94	2.20	2.36
Same; 30:10 hr.		10						.84		1.24	1.54	1.80	2.04	2.28	2.50
Same; 47:40 hr.		10						.80		1.22	1.54	1.80	2.00	2.26	2.52
Fraction I, Run 6		10						.40		0.50	0.62	0.78	0.88	1.04	1.12
Fraction I, Run 7		10						.36		.46	.58	.76	.88	1.02	1.14
Fraction I, Run 8		10						.42		.54	.64	.76		1.04	
Fraction I, Run 10		10						.44		.58	.68	.76	.90	1.06	
Fraction II, Run 6		10						.26		.46	.54	.76	.84	0.94	1.14
Fraction II, Run 7		10						.26		.44	.56	.70	.80	.94	
Fraction II, Run 10		10						.20		.40	.54	.72	.82	.92	
Product of Run 5		10			9.46		9.72	..	9.92						
Same; 0.1044 g.		10				2.86	2.91	3.02	3.10	3.34	3.55	3.65	3.74	3.86	3.95
Fraction II, Run 9		20	17.30			18.26		..	18.64						
Same (2d distillation)		20		14.24			15.44	..	15.76	16.08					

The volume of permanganate reduced by the substance was then calculated and is shown in Table II.

The first two determinations on glucose were made without the buffer and clearly show the variable results obtained without buffer.

Summary and Conclusions

The character of the products obtained by methylating mannose with dimethyl sulfate and sodium hydroxide at temperatures between 30 and 50° has been studied.

A sirup having the analysis and properties of trimethyl-γ-methyl-mannoside was formed in a solution between P_H 7.0 and 8.5 at a temperature of 30°. The product formed on similar treatment at 35° had no gamma properties and was a mixture of sugar methylated to varying degrees.

The method of Kuhn and Jauregg for studying the rate of oxidation of sugars by permanganate was applied to the oxidation of polymethyl methyl mannosides. The results showed clearly the very great difference in the behavior of stable and unstable forms toward potassium permanganate.

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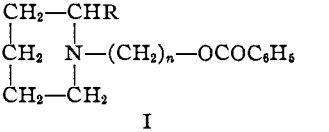
[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

PIPERIDINE DERIVATIVES. VIII. SUBSTITUTED PIPERIDINO-ALKYL BENZOATES

BY C. F. BAILEY AND S. M. MCELVAIN

RECEIVED DECEMBER 20, 1929 PUBLISHED APRIL 7, 1930

In a previous communication[1] from this Laboratory a number of substituted piperidino-alkyl benzoates (Type I) were reported. It was found that when a methyl group substituted the 2- or 3-position of the piperidine nucleus, a much more effective local anesthetic was produced than when the piperidine nucleus remained unsubstituted or was substituted by a *n*-propyl group or a carbethoxy group. Other types of structure which have been found to produce quite pronounced local anesthetic effect are the 1-alkyl-3-carbethoxy-4-piperidyl benzoates (Type II)[2] and the 1-alkyl-4-piperidyl benzoates (Type III).[3] In those cases in which the R of Types II and III is a phenylethyl group extraordinary local anesthetic action is shown.



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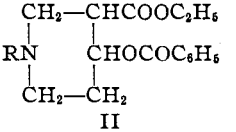

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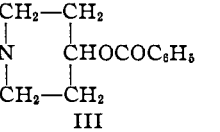

III

[1] McElvain, THIS JOURNAL, **49**, 2835 (1927).

[2] McElvain, *ibid.*, **48**, 2179 (1926); Thayer and McElvain, *ibid.*, **49**, 2862 (1927).

[3] Bolyard and McElvain, *ibid.*, **51**, 922 (1929).