2-Benzoylfluorene was prepared by Perrier's[10] modification of the Friedel–Crafts reaction. It melted at 122°.

Potassium 2-benzoyl-9-aci-nitrofluorene was obtained by the method of Ray and Palinchak.[6]

2-Benzoyl-9-fluorenoneoxime.—Three and one-half grams of potassium 2-benzoyl-9-*aci*-nitrofluorene, 3.5 g. of benzyl chloride and 40 cc. of 95% alcohol were refluxed for eight hours. Potassium chloride slowly separated and the orange color faded to yellow. The solution was filtered and treated with water. An oil separated which solidified on stirring. After three recrystallizations from alcohol the melting point was 207–208° (β-form).

As this material was somewhat difficult to purify we condensed 27 g. of 2-benzoylfluorene with 13.7 cc. of isoamyl nitrite in the presence of 5.5 g. of potassium in 30 cc. of absolute methyl alcohol, 100 cc. of anhydrous ether and 200 cc. of pure benzene.

The product that precipitated weighed 16.1 g. It was separated, acidified and recrystallized from alcohol. This α-form of the oxime was obtained as a pale yellow powder melting at 213–214°.

Anal. Calcd. for $C_{20}H_{13}O_2N$: N, 4.67. Found: N, 4.66.

The acetyl derivative was prepared with acetyl chloride in pyridine–ether solution. Greenish-yellow fibers were obtained that melted at 144–145°.

Anal. Calcd. for $C_{22}H_{15}O_3N$: N, 4.11. Found: N, 4.31.

(10) Perrier, *Bull. soc. chim.*, [3] **31**, 859 (1904).

β-Oxime.—The filtrate from the α-potassium salt was evaporated to 50 cc. and 500 cc. of ether was added. The precipitate weighing 12.4 g. was separated, acidified and recrystallized three times from alcohol. Long, bright yellow crystals were obtained melting at 207–208°. These were identical with those prepared by Fortner's[4] method.

Anal. Calcd. for $C_{20}H_{13}O_2N$: N, 4.67. Found: N, 4.63.

This acetyl derivative crystallized in brilliant yellow plates that melted at 150–151°.

Anal. Calcd. for $C_{22}H_{15}O_3N$: N, 4.11. Found: N, 4.33.

Summary

The following new compounds of fluorene have been prepared: 2-fluoryl isocyanate; methyl, ethyl and *n*-propyl 2-fluorylcarbamate; 2-fluorylurea; *sym*-phenyl-2-fluorylurea; *sym*-di-2-fluorylurea; and the potassium salts and the acetyl derivatives of the α- and β-9-oximes of 2-benzoylfluorenone, as well as the free α- and β-oximes.

The compound obtained by the reaction between 2-benzoylfluorenone and hydroxyl amine reported by Fortner[4] is shown to have been a mixture of the α- and β-forms of the 9-oxime.

CINCINNATI, OHIO RECEIVED SEPTEMBER 2, 1938

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

The Analytical Separation of Various Classes of Sugars

BY CHARLES D. HURD AND SIDNEY M. CANTOR[1]

The purpose of this investigation was to develop a method for the separation of various classes of sugars, such as monosaccharoses from di- or trisaccharoses, or pentoses from hexoses. It was hoped that the method would achieve not only separation but also analysis of the mixture as well. The importance of a direct method of analysis is obvious, in view of the fact that most of the analytical methods which have been suggested for sugars are empirical and depend largely on a preëxisting knowledge of the identity of the sugar. Methods based on the reducing action of copper salts or on the preferential fermentation by specific microörganisms are obviously limited in scope.

Methylation and fractional distillation of sugar mixtures appeared to be the most promising plan of attack for several reasons: (1) the methyl ethers are known to distil *in vacuo* without decomposition, (2) sizable boiling point differences exist between the methyl ethers of the sugar classes, (3) the methylation procedure would be satisfactory on a wide variety of sugars since it brings about no serious structural changes, (4) the fact that physical constants of a large number of methylated sugars are known would be of help in subsequent identifications.

Preliminary experiments indicated the feasibility of the plan but they also demonstrated the inapplicability of the customary procedure of direct methylation of the sugar by Haworth's method. For example, glucose, lactose and sucrose were methylated by means of methyl sulfate and alkali and the methylated derivatives distilled. The yields, respectively, were 50, 45 and 46%. These results seemed acceptable but it was found that no such uniformity in yields was at-

(1) Corn Products Refining Company Fellow, 1932–1936.

tainable when mixtures were taken at the outset. A 50:50 mixture of glucose and lactose gave an apparent per cent. value of 63:37. Such a preferential methylation of the monosaccharose demonstrated the inapplicability of Haworth's method of direct methylation as an analytical procedure. Although the yields in methylation reactions are far from quantitative, equivalence in the yields is the only requirement for a satisfactory analytical separation.

Two other disturbing features were revealed in these preliminary experiments. One was the fact that the glucose derivative, even upon repeated methylation, did not show the correct specific rotation for β-methyl tetramethylglucoside. A mixture containing about one-fifth of the α-isomer seemed to be indicated. A similar observation is known to occur with xylose[2] but glucose seems not to have been tested before. The specific rotation of the methylated glucose fractions, therefore, would have been useless for purposes of identification.

The other item concerned the methylation of sucrose. During this process there was found to be an appreciable hydrolysis, for which a correction factor would need to be applied to interpret the results on an unknown mixture containing sucrose. The yields, respectively, of methylated mono- and disaccharoses were 6 and 46%.

Attention was next directed to experiments on direct acetalization with a view to subsequent direct methylation of the methyl glycosides thus produced. The hemi-acetal hydroxyl of the reducing sugars seemed to promote the non-uniformity observed when Haworth's method was applied directly.

Glucose and methanol, when treated with 1–3% hydrogen chloride by Fischer's method, gave rise to 45% of crystalline α-methyl glucoside (A) and a sirup (B). Methylation by Haworth's method of A and B yielded, respectively, 60 and 67% of α-methyl tetramethylglucoside. It was evident, therefore, that separation of A from B was not necessary for the subsequent methylation.

Trouble was encountered with maltose, however, because some hydrolysis was noticed. α-Methylglucoside was isolated from the reaction mixture. This was substantiated later when a mixture (57:43) of glucose and maltose was methylated first by Fischer's method, then by Haworth's method. The products isolated on distillation indicated an apparent composition of 67:33. Therefore, this procedure also was poor from an analytical viewpoint.

Methylation in liquid ammonia[3] was tried and abandoned because the method required methyl glycosides or other derivatives wherein the hemiacetal hydroxyl was protected. The free sugars could not be used.

Feist[4] observed that methyl dibromoformal, $Br_2C(OCH_3)_2$, was a methylating agent toward sodium phenoxide or sodium acetate, yielding anisole and methyl acetate, respectively. It was thought that it might convert the monosodium derivative of glucose (prepared by glucose and sodium methoxide in methanol) into methyl glucoside but such a reaction was not observed.

Likewise, there was non-reaction when glucose, suspended in ether, was treated with diazomethane, although Schmid[5] has reported the methylation of starch in this way. Water or aluminum ethoxide[6] were not effective in catalyzing the reaction between diazomethane and glucose.

The plan of direct methylation, therefore, was changed to that of indirect methylation. Acetylation of the sugar by acetic anhydride and pyridine at 0° was the first step in a successful procedure. In the acetylation of glucose, Hudson and Dale[7] obtained an 88% yield of pure glucose pentaacetate. Maltose has not been acetylated in this manner, but in the present work it was found that a 90% yield of β-maltose octaacetate was isolable. One may infer that the acetylation reaction gives rise to practically quantitative yields of the acetyl sugars.

The problem, therefore, was to substitute uniformly the acetyl groups by methyl groups. To do this, the following steps were visualized and found to be satisfactory: replacement of the acylal[8] group by chlorine, substitution of this chlorine by methoxyl, hydrolysis of the ester groups without disturbing the acetal function, methylation of the alcoholic hydroxyl groups and separation by fractional vacuum distillation.

(2) Phelps and Purves, THIS JOURNAL, **51**, 2443 (1929).

(3) Muskat, *ibid.*, **56**, 693, 2449 (1934).

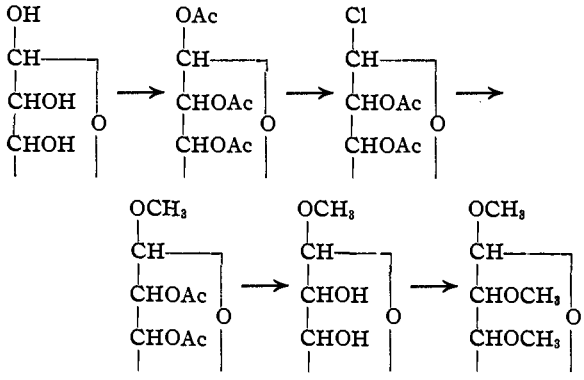
(4) Feist, *Z. angew. Chem.*, **35**, 489 (1922).

(5) Schmid, *Ber.*, **58**, 1964 (1925).

(6) Meerwein and Hinz, *Ann.*, **484**, 1 (1931).

(7) Hudson and Dale, THIS JOURNAL, **37**, 1264 (1915).

(8) If C, C′ and C″ represent, respectively, the states of oxidation of alcohol, aldehyde and acid, then C–O–C, C–O–C′, C–O–C″, C″–O–C″ represent the familiar functional groups of ether, acetal, ester and acid anhydride. No term exists for C′–O–C″ or C′–O–C′. The name "acylal" is proposed for the former and "aldal" for the latter.



Each of these steps will be discussed in turn.

Glucose pentaacetate may be converted into acetobromoglucose by the use of liquid hydrogen bromide[9] or by a mixture of hydrogen bromide in acetic acid.[10] It may be changed into acetochloroglucose by liquid hydrogen chloride, or phosphorus pentachloride[9] or by a mixture of phosphorus pentachloride and aluminum chloride[11] (but this method may bring about structural changes), or by titanium tetrachloride[12] in chloroform. The last method was far better than the others because of high yields and because of its freedom from causing structural changes. Pacsu's directions were followed for the preparation of tetraacetylglucosyl chloride with a resulting yield of 90%. In an analogous experiment with β-maltose octaacetate, not studied by Pacsu, heptaacetyl-α-maltosyl chloride was obtained in 86% yield. Also it was established that under comparable conditions there was no action between titanium tetrachloride and sucrose octaacetate or levoglucosan triacetate, neither of which contained acylal groups.

Maltose.—Five grams of β-maltose octaacetate, m. p. 157–158°, was dissolved in 35 g. of dry chloroform in a flask equipped with a reflux condenser and a dropping funnel. To the solution was added gradually 3 g. of titanium tetrachloride dissolved in 25 g. of chloroform. The reaction was carried out on a water-bath at 50° for two hours, then at 70° for one hour, at which time the original yellow, mushy precipitate had changed to a clear yellow solution. Crystallization of the product from ether and petroleum ether netted 2.2 g. of crystalline solid, and 2 g. of a sirup which was desiccated to a white powder. The crystals melted at 77–80°, and the specific rotation in chloroform, $[\alpha]^{20}D$, was 151.8°. The rotation of the powder was 142.1°. From the viewpoint of an analytical procedure, the important item is the high yield of isolated product (86.5%) which suggests that the reaction itself was practically quantitative.

The melting point of heptaacetylmaltosyl chloride prepared by other methods is variously listed in the literature[13] as 88, 119, 125°. Both Foerg and Brauns reported 159.5° as the value of the specific rotation.

Sucrose.—Ten grams of sucrose octaacetate, m. p. 68°, dissolved in 150 cc. of dry chloroform was added to a solution of 5.6 g. of titanium tetrachloride in 50 cc. of chloroform. After three hours of refluxing and twelve more hours of standing it was possible to recover 9.6 g. of recrystallized sucrose octaacetate (m. p. and mixed m. p., 68°).

Levoglucosan.—Two grams of levoglucosan was prepared by pyrolysis of starch.[14] It was placed with 10 cc. of pyridine and 15 cc. of acetic anhydride and left at 0° for twenty-four hours. The solution was then poured into chloroform, washed thoroughly, first with acid then with sodium carbonate solution, then water, after which it was dried and the chloroform removed by distillation. The residual sirup was taken up in alcohol and water added until the acetylated material precipitated as a gum. Extraction of the gum with ether and decolorization of the solution finally yielded 0.2 g. of white crystals melting sharply at 108° and showing $[\alpha]^{20}D$ −58.6° in absolute ethyl alcohol. Pictet gives as the melting point 110°, while the rotation as determined by Vongerichten and Müller[15] is −45.5° in alcohol, a value which may be questioned in view of the present work.

Rotation: sample, 0.0449 g.; ang. rotn., −0.20°; tube length, 1.89 dm.; volume solution, 25.0 cc.; $[\alpha]^{20}D$ −58.6°.

A portion of this crystalline material, 0.138 g., was dissolved in chloroform and made up to exactly 25.0 cc. The rotation was then determined in a 2.00-dm. tube: ang. rotn., −0.70°; $[\alpha]^{20}D$ −63.6°. To the chloroform solution was added approximately 0.5 g. of titanium tetrachloride in 5 cc. of chloroform. The resulting yellow solution was kept at 60–70° for ninety minutes. It was then poured into water, and the chloroform layer washed in the usual manner. After drying, it was made up again to 25.0 cc. and the rotation redetermined in the 2.00-dm. tube: ang. rotn., −0.68°; $[\alpha]^{20}D$ −61.8°. The chloroform was then removed and the residue taken up in ether. On cooling, 0.1 g. of crystals, m. p. 107–108°, separated. A mixed melting point with the original material was also 107–108°.

Of the various methods for the replacement of the chlorine of the hemi-acetal chloride by methoxyl, the best was that which used methanol and silver carbonate. Koenigs and Knorr[16] discovered that hot methanol converted tetraacetylglucosyl bromide into β-methyl tetraacetylglucoside, but the beneficial effect of silver carbonate was discovered by Fischer and Armstrong.[9] It

(9) Fischer and Armstrong, *Ber.*, **34**, 2985 (1901).

(10) Fischer, *ibid.*, **49**, 584 (1916).

(11) Brauns, THIS JOURNAL, **44**, 401 (1922); **45**, 833 (1923); Freudenberg, *Ber.*, **55**, 929 (1922).

(12) Pacsu, *ibid.*, **61**, 1508 (1928).

(13) Fischer and Armstrong, *Ber.*, **35**, 3153 (1902); Foerg, *Monatsh.*, **23**, 45 (1902); Schliephacke, *Ann.*, **377**, 186 (1910); Brauns, THIS JOURNAL, **51**, 1829 (1929).

(14) Pictet and Sarasin, *Helv. Chim. Acta*, **1**, 87 (1918).

(15) Vongerichten and Müller, *Ber.*, **39**, 245 (1906).

(16) Koenigs and Knorr, *ibid.*, **34**, 965 (1901).

was found that very satisfactory results were obtained in the present work if the reaction mixture was shaken for twelve to fifteen hours.

Alternative procedures for the chloride to acetal conversion were tried and found ineffective. For example, when tetraacetylglucosyl chloride was left for four hours with methanol and pyridine at 0°, the only result was crystallization of the acetochloroglucose. This is in contrast to the known conversion of heptaacetyl-α-maltosyl chloride[17] into β-methyl heptaacetylmaltoside under similar treatment. When the reaction with tetraacetylglucosyl chloride was performed at reflux temperatures, some β-methyl tetraacetylglucoside (21% yield) was formed but secondary reactions also were indicated. For a time, it was thought that sodium methoxide might be effective in introducing the acetal methoxyl and removing the acetyl groups concurrently. However, the method was discarded when it was found that no β-methyl glucoside was obtainable from tetraacetylglucosyl chloride and sodium methoxide. Thus, the silver carbonate method, although time-consuming, seemed to be the only one wherein yield and purity of product were satisfactory.

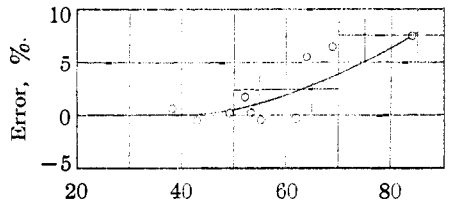


Fig. 1.—Correction curve.

Zemplén's method, namely, the use of sodium methoxide,[18] appeared to be the best means of removing the acetyl groups because of its simplicity and because of the high yields (nearly quantitative) obtained. The necessity of removing the acetyls prior to the final methylation was demonstrated in an attempted methylation of α-methyl tetraacetylglucoside. Only 2.7 g. of a sirup soluble in chloroform was obtained when 20 g. of it was treated with methyl sulfate and 20% aqueous sodium hydroxide solution for four hours at 70° followed by one-half hour at 100°.

For the final step of methylation of the methyl glycosides, Haworth's method was selected, although Muskat's liquid ammonia method might have served equally well. Simpler technique favored Haworth's method. It was recognized that this method would not give complete methylation in one operation, but this did not interfere with the analytical separation.

When glucose and maltose were carried separately through this procedure, the yield of methylated sirup from glucose was 38.3% and that from maltose 37.1%. Thus, no serious preferential action on the two classes of sugars was observed. Neither of the sugars was completely methylated but the sirup from glucose approached tetramethylglucose whereas that from maltose approached heptamethylmaltose.

The procedure was now standardized rigorously, and several duplicate runs were made on mixtures of glucose and maltose over a wide range of composition. The results, collected in Table I, showed that an error in favor of the monosaccharose existed which appeared to increase as the percentage of monosaccharose in the mixture was increased. To compensate for this error, a curve was constructed on the basis of known mixtures, and all unknown mixtures analyzed were corrected from this curve. Later results gave a better observation of the error involved so that the curve shown in Fig. 1 is based upon the average error of fourteen runs.

TABLE I

STANDARDIZATION RUNS ON THE PROCEDURE

Run	Sugars	Composition % by weight, taken	obsd.	Error, %
7A	Monosac.	30.0	29.9	−0.1
	Disacc.	70.0	70.1	.1
8A	Monosac.	30.0	32.2	2.2
	Disacc.	70.0	67.8	−2.2
7B	Monosac.	50.0	49.2	−0.8
	Disacc.	50.0	50.8	.8
8B	Monosac.	50.0	49.6	− .4
	Disacc.	50.0	50.4	.4
7C	Monosac.	70.0	64.4	−5.6
	Disacc.	30.0	35.5	5.6
8C	Monosac.	70.0	64.5	−5.5
	Disacc.	30.0	35.5	5.5

The efficacy of the curve is best demonstrated by the results on two mixtures whose composition was unknown to the observer. A mixture made up to contain 62% glucose and 38% maltose analyzed for a "corrected composition" of 64.3% glucose and 35.7% maltose. A second mixture containing 53.4% glucose and 46.6% lactose gave a "corrected composition" of 55.2% glucose and 44.8% lactose. The absolute error of the method

(17) Freudenberg, *Ber.*, **55**, 929 (1922); Pacsu, THIS JOURNAL, **57**, 587 (1935).

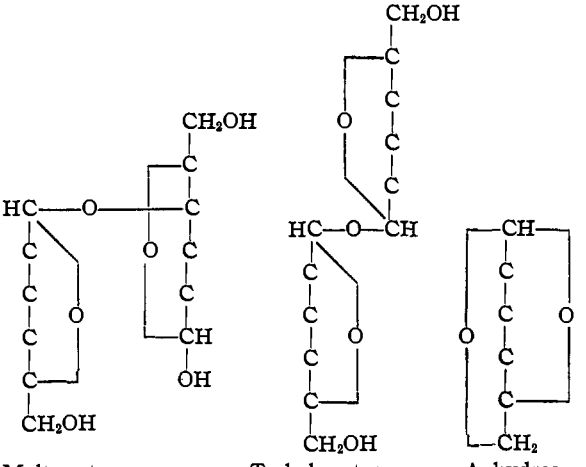
(18) Zemplén, *Ber.*, **56**, 1705 (1923).

varies considerably, but in only one observed case was greater than 3%.

Types of Sugars Studied

Monosaccharoses.—Glucose was chosen as the representative aldohexose. Work with xylose showed that aldopentoses would respond equally well. That methylpentoses were also amenable was shown by results on a mixture of rhamnose (desoxy-*l*-mannose) and maltose. The standardization runs indicated that all such aldohexoses would undergo the procedure successfully. Attempts to apply the technique to mixtures containing fructose, a representative ketohexose, however, were unsuccessful.

Disaccharoses.—Types of disaccharoses studied were necessarily more varied. The application to the maltose or reducing type of disaccharose was shown by the standardization runs. Further results with mixtures containing lactose and gentiobiose showed that such factors as the point of glucosidic attachment, alpha or beta modification of the parent sugar, or the nature of the hexose constituents of the disaccharoses were unimportant in the reactions listed. The chief factor concerned itself with the nature of the hydroxyl group, whether it was alcohol or acetal.



Maltose type
maltose (α at 4)
lactose (β at 4)
gentiobiose (β at 6)

Trehalose type
trehalose ($\alpha\alpha$ at 1)
sucrose (α at 1)

Anhydrose type
levoglucosan

In the trehalose type all eight hydroxyl groups are alcoholic. The trehalose type would, therefore, be expected to undergo acetylation, hydrolysis of acetyl groups and methylation, but no other reactions. Results with sucrose, a trehalose type, indicated that sucrose octaacetate was stable to the action of titanium tetrachloride, but that hydrolysis occurred in the final methylation step, to the extent of 11% by weight. When this correction was applied the analytical data were satisfactory. Trehalose itself, being of a more stable configuration, acted normally and exhibited no hydrolysis.

Applicability to the third or anhydrose type of disaccharose depended upon the ability of the oxygen bridge to withstand the action of titanium tetrachloride. When the triacetate of levoglucosan (1,6-anhydro-*d*-glucose) was treated with the reagent under the conditions of the procedure, no hydrolysis occurred. Other investigators[19] have reported that hydrolysis will result from use of an excess of titanium tetrachloride.

Constants of the Sugars Studied

Monosaccharoses.—Evidence that monosaccharoses were unaltered through the procedure was obtained easily. For example, the methylated glucose fractions showed specific rotations near $-20°$. The specific rotation of pure β-methyl tetramethylgucoside is $-22.4°$. Further evidence was obtained by hydrolyzing a portion of the glucoside to tetramethylglucose and preparing the known aniline condensation product. In general, the evidence obtained from specific rotation, although not indicating absolute purity, was sufficient to demonstrate the nature of the fraction.

Disaccharoses.—Identification in the case of the disaccharoses was less clean cut. Derivatives of the methylated disaccharoses cannot be prepared since hydrolysis of the glucosidic methoxyl group always brings about concurrent hydrolysis of the disaccharose linkage. Thus the disaccharose fractions usually had to be remethylated and distilled in order to obtain a value for the specific rotation which approached the accepted value. The Purdie and Irvine method, involving silver oxide and methyl iodide,[20] was used. With only one remethylation of the maltose fraction a specific rotation of 68° was obtained. That for pure β-methyl heptamethylmaltoside is 79°. Several applications of this technique, accompanied by frequent distillation, would be necessary to obtain samples of optical purity.

Other Applications of the Procedure

Separation of a Pentose from a Hexose.—This separation was of interest to study since the two

(19) Zemplén and Czurös, *Ber.*, **62**, 993 (1929).
(20) Purdie and Irvine, *J. Chem. Soc.*, **83**, 1021 (1903).

sugar types, which differ by only one carbon atom, give methylated derivatives boiling relatively close together. A sample run on a 1:1 mixture of *d*-glucose and *d*-xylose in which volume of distillate was plotted against temperature of distillation (Fig. 2) showed that separation was feasible. An almost quantitative separation of glucose and xylose bore this out. β-Methyl trimethylxyloside, the pentose fraction, was obtained crystalline, indicative of high purity.

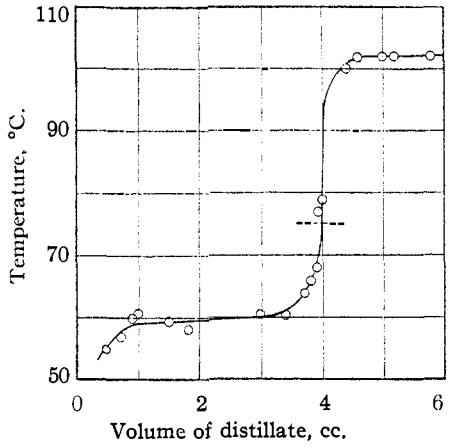


Fig. 2.—Separation of xylose–glucose mixture.

Analysis of Hydrols.—The application of the procedure to "hydrol," the mother liquor obtained after crystallization of glucose from the acid hydrolysate of corn starch, appeared to be an interesting extension of the problem since previous[21] investigators have shown that hydrol contains a mixture of disaccharoses as well as glucose. However, none of these investigators had ever analyzed a complete sample of hydrol, but had attacked the mixture after first fermenting away all fermentable sugars.

Three samples of hydrol were obtained from the Corn Products Refining Company which had various hydrolytic histories, but were alike in that they had all gone through a single crystallization of glucose after the final hydrolytic treatment. Subjection of these samples to the procedure gave the usual mono- and disaccharose fractions, but distillation of the disaccharose fraction after remethylation left a high boiling portion in each case. This material from molecular weight determinations was identified as partially methylated trisaccharoses. The results are shown in Table II.

(21) Berlin, THIS JOURNAL, **48**, 1107, 2627 (1926); Coleman, Buchanan and Paul, *ibid.*, **57**, 1119 (1935).

TABLE II

	Original hydrol	Reconverted hydrol	Kansas City hydrol
Monosaccharoses, %	55.2	55.2	54.8
Disaccharoses	38.4	39.3	37.9
Trisaccharoses	6.4	5.5	7.3

The monosaccharose fraction was identified as glucose. Work is under way at the present time in this Laboratory on the separation and identification of the components of the disaccharose and trisaccharose fractions.

Apparatus for Analytical Vacuum Distillation.—In the standardized procedure (below), one of the steps is that of separation of methylated sugars by vacuum distillation. The details of this apparatus are shown in Fig. 3.

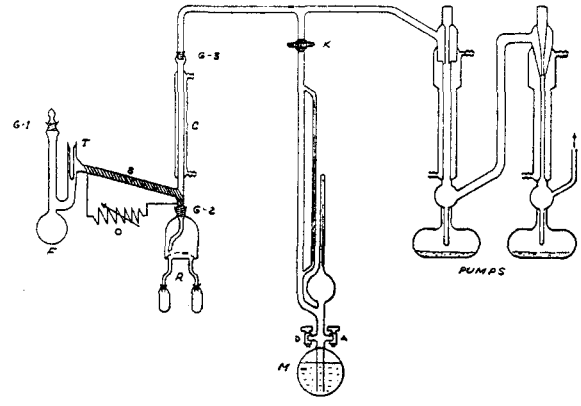


Fig. 3.—Distillation apparatus.

Low pressure was supplied by a set of two mercury vapor pumps connected in series and working against a back pressure of 10–20 mm., supplied by an ordinary water jet.[22] The first mercury pump was a high speed jet and the second was a diffusion pump. These pumps gave a minimum pressure of 1×10^{-5} mm., which was more than sufficient for the purpose.

The distillation flask, F (Fig. 3), was of the conventional Claisen type equipped with a thermometer well, T, and a ground glass stopper, G-1. Three of these flasks of capacities 125, 50 and 20 cc., were used interchangeably depending upon the amount of material to be distilled. The side-arm, S, connecting flask to receiver, was wrapped with nichrome wire surrounded by asbestos rope and was kept electrically heated by the introduction of a current controlled by the variable resistance, O. The temperature in the side-arm was usually kept at 80–90° during a distillation in order to avoid congealing the sirup and to bring to a minimum the holdover in the tubing. The receiver, R, was connected to the bent side-arm by a ground glass

(22) The use of mercury pumps backed by a water pump instead of an oil pump was a decided advantage in this work since it eliminated the necessity for a low temperature trap in the apparatus. Methylated sirups in chloroform solution could be transferred to the distillation flask and the solvent removed through the pumps without danger of corrosion or raising the pressure as is the case with oil pumps.

joint, G-2, and was of the multiple rotary type enabling five fractions to be taken. Two of the small receivers were later connected to the large receiver by ground glass joints which eliminated the necessity of breaking off and sealing on after each distillation. The condenser, C, prevented the entrance of any low boiling sugars into the connection tubing, and the ground glass joint, G-3, allowed removal of the distillation unit for cleaning. Pressures were read by means of the McLeod gage, M, connected to the system by a stopcock, K. The McLeod gage was of a modified variety. The mercury storage bulb was kept evacuated by a water pump at side-arm A. Opening side-arm D to the atmosphere resulted in the mercury rising into the gage from which the pressure was read off. The mercury could then be again lowered by evacuation of the storage chamber.

The Standardized Procedure

The anhydrous sample taken was always 25.0 g., for this quantity was found to give sufficient methylated material for the fractionation. Amounts of reagents were based upon the assumption that all of the material in the mixture was monosaccharose.

Step 1.—The mixture was treated with 120 g. of acetic anhydride and 156 g. of pyridine (dried over lime). This was 100% in excess of that required for complete acetylation if all of the material were monosaccharose. The reaction was allowed to proceed at 0° for forty-eight hours, then for four hours at 70°, which treatment usually completed the acetylation. Two hundred fifty cc. of chloroform was now added and the resulting solution washed twice with 300-cc. portions of 4 *M* hydrochloric acid, twice with 200-cc. portions of a dilute sodium carbonate solution and finally twice with 200-cc. portions of water. The chloroform solution was dried over anhydrous sodium carbonate.

Step 2.—The dried solution from (1) was treated with 28 g. of anhydrous titanium tetrachloride dissolved in 150 cc. of absolute chloroform. The mixture was heated at 70° for two hours on a water-bath, after which it was allowed to stand for five hours. A slight amount of pyridine or an excess of titanium tetrachloride often caused a darkening of this solution, but this effect apparently was not harmful. The resulting homogeneous solution was poured onto cracked ice, and the separated chloroform solution was washed until the washings showed no acid test. The solution was then dried over anhydrous sodium carbonate, and the chloroform removed on the steam-bath.

Step 3.—The sirup from (2) was taken up in 700 cc. of absolute methanol. Then, 25 g. of silver carbonate was added and the mixture shaken for twelve to sixteen hours. Upon completion the silver residues were filtered off.

The type of shaker for this step is sketched in Fig. 4. The box was rotated on its horizontal shaft at approximately 70 r. p. m. With the bottles set at an angle to the direction of rotation, and usually only two-thirds full, the mixing was very effective. The authors are indebted to Mr. Otto Stanger of the Miner Laboratories, Chicago, for suggesting this type of shaker.

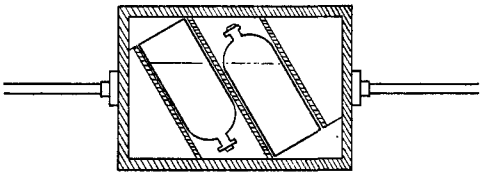


Fig. 4.—Shaking apparatus.

Step 4.—To the clear solution from (3) was added 200 cc. of dry chloroform and a solution made by dissolving 1 g. of sodium in 50 cc. of methanol. The mixture was allowed to stand at 0° for six hours and then diluted with an equal volume of water. The water layer was separated, made neutral to litmus with acetic acid, and reduced in volume to 30 cc. by distillation *in vacuo*. The glycoside sirup obtained at this stage was usually dark brown in color.

Step 5.—The sirup from (4) was transferred to the methylation apparatus, heated to 70° by means of a water-bath, and treated with 150 cc. (200 g.) of methyl sulfate and 140 g. of sodium hydroxide which was dissolved in 200 cc. of water. The reagents were added dropwise over a period of about three hours in an approximate ratio of two drops of methyl sulfate to three drops of sodium hydroxide solution. This kept the base slightly in excess and minimized the danger of hydrolysis. Upon completion of the addition, the mixture was heated with stirring for one-half hour at 100°. The cooled mixture was filtered, and the solid which separated was triturated four times with 25-cc. portions of chloroform. The filtrate was extracted four times with 100-cc. portions of chloroform. The extracts were combined with the washings and dried, without washing, over anhydrous sodium carbonate. Then, the chloroform was removed by distillation on the water-bath and the residual sirup transferred to the distillation apparatus. The chloroform was removed. Bumping under the high vacuum was

TABLE III

ANALYSIS OF MIXTURES OF GLUCOSE AND MALTOSE

Run		Original components (G or M), g.	Methylated derivatives, g.	Equivalent wt. of free sugar, g.	% Components Obsd.	Corr.	Calcd.
7A		G 7.5	3.9	2.89	29.9	29.9	30.0
		M 17.5	8.9	6.80	70.1	70.1	70.0
	Total	25.0	12.8	9.69			
8A		G 7.5	4.5	3.34	32.2	32.2	30.0
		M 17.5	9.2	7.05	67.8	67.8	70.0
	Total	25.0	13.7	10.39			
7B		G 12.5	4.8	3.56	49.2	50.0	50.0
		M 12.5	4.8	3.67	50.8	50.0	50.0
	Total	25.0	9.6	7.23			
8B		G 12.5	6.1	4.52	49.6	50.0	50.0
		M 12.5	6.0	4.59	50.4	49.6	50.0
	Total	25.0	12.1	9.11			
7C		G 17.5	6.1	4.52	64.4	67.0	70.0
		M 7.5	3.3	2.52	35.6	33.0	30.0
	Total	25.0	9.4	7.04			
8C		G 17.5	6.0	4.45	64.5	67.0	70.0
		M 7.5	3.2	2.45	35.5	33.0	30.0
	Total	25.0	9.2	6.90			
12		G 19.0	8.4	6.21	69.3	72.8	76.0
		M 6.0	3.6	2.76	30.7	27.2	24.0
	Total	25.0	12.0	8.97			
14		G 23.0	7.6	5.54	84.8	92.0	92.0
		M 2.0	1.3	1.00	15.2	8.0	8.0
	Total	25.0	8.9	6.54			

relieved by means of small boiling chips. At pressures below 0.02 mm., the limit for removal of the monosaccharose fraction was 110°. If the sample contained only mono- and disaccharoses, the disaccharose fraction was not distilled but the residue was taken as disaccharose. When trisaccharoses were involved, the disaccharose fraction was distilled off and the residue was regarded as the trisaccharose. The fractions were then weighed directly, the weights calculated back to weight of free sugar using the correction curve (Fig. 1).

When glucose and maltose were carried through this procedure separately, the yield of methylated sirup from glucose was 38.3% and that from maltose 37.1%. Thus no serious preferential action on the two classes of sugars was observed. Neither of the sugars was completely methylated. The monosaccharose approached the tetramethyl stage and the disaccharose the heptamethyl. Macdonald[23] has shown that with a chloroform extraction of products obtained in the methyl sulfate methylation of glucose, less than 2% of the methyl dimethylglucoside passes into the chloroform. Hence the extracted material consists almost wholly of the methyl tri- and tetramethylglucosides (A and B). Carrying the analogy to the disaccharoses, one might expect that the chloroform extract would consist mainly of the methyl hexa- and heptamethylglycosides (C and D). From these considerations, it was decided to regard the separated fractions as equimolar mixtures of A and B, or C and D, and to correct the molecular weight accordingly. The molecular weights used for computation of the free sugar weights were thus 243 for the monosaccharose instead of 250, and 447 for the disaccharose instead of 454.

The procedure was now rigorously standardized. Some results on typical analyses of glucose–maltose mixtures are shown in Table III.

The glucose fractions from the first six runs were combined and identified as follows. The specific rotation in chloroform, $[\alpha]^{20}D$, was −20.5°, whereas that for pure β-methyl tetramethylglucoside is −22.4°. Ten grams of the liquid was hydrolyzed by heating for two hours at 100° with 7% hydrochloric acid, then neutralizing with calcium carbonate, evaporating to dryness and extracting with ether. In this way, 5.5 g. of tetramethylglucose was obtained. From this, by heating with aniline,[24] a good yield of tetramethylglucose-aniline, m. p. 135°, was obtained.

The maltose fractions were identified by refluxing 10 g. of the maltose residues (Table III) for six hours with 35 g. of silver oxide and 60 g. of methyl iodide. The silver residues were filtered off and the methyl iodide removed by distillation. The residual sirup was vacuum distilled. A fraction of 3.62 g., b. p. 158° (0.008 mm.), possessed a specific rotation in chloroform, $[\alpha]^{20}D$ of 67.7°. The specific rotation of β-methyl heptamethylmaltoside is accepted as 78.9°.

The method has been tested recently by Mr. P. J. Baker, Jr., of this Laboratory, who reported these data on a glucose–maltose mixture:

	Components, g.	Methylated deriv., g.	Equiv. wt. of free sugar, g.	% Components Obsd.	Corr.	Calcd.
G	17.5	11.75	8.70	67.7	70.7	70.0
M	7.5	5.42	4.15	32.3	29.3	30.0

(23) Macdonald, THIS JOURNAL, 57, 771 (1935).

(24) Greene and Lewis, *ibid.*, 50, 2822 (1928).

Analysis of Other Mixtures

Details for glucose and sucrose are listed in Table IV. If the correction for the 11% hydrolysis of sucrose, noted above, is applied, then the weights of components would be changed from 12.5 g. apiece to 13.9 g. of glucose and 11.1 g. of sucrose. The methylated sucrose was characterized by refluxing it for eight hours with silver oxide and methyl iodide. After the usual procedure, 2.5 g. of liquid was secured: b. p. 130–140° (0.001 mm.), $[\alpha]^{20}D$ in chloroform +63.6°. The rotation of octamethylsucrose[25] is only reported with methanol as solvent, $[\alpha]^{20}D$ + 69.3°.

TABLE IV

ANALYSIS OF MIXTURES OF SUGARS

Components Name	G.	Methylated derivs., g.	Free sugar, g.	% Components Obsd.	Corr.	Calcd.
Glucose	12.5	6.8	5.04	52.4	53.4	56.0
Sucrose	12.5	6.0	4.59	47.6	46.6	44.0
β-Glucose Pentaacetate	35.7	12.6	9.34	63.2	..	62.6
β-Gentiobiose Octaacetate	19.5	6.8	5.20	36.6	..	37.4
Glucose	10.0	4.0	2.96	38.6	39.3	40.0
Trehalose	15.0	6.15	4.70	61.4	60.7	60.0
Glucose	13.3	7.34	5.43	52.5	53.5	53.3
Trehalose	2.77					
Gentiobiose Octaacetate	17.6	6.40	4.90	47.5	46.5	46.7
Rhamnose	11.1	5.54	4.03	43.5	43.9	42.5
Maltose	15.0	7.05	5.22	56.5	56.1	57.5
Glucose[a]	15.35	6.3	4.67	62.8	64.3	62.0
Maltose	9.41	3.6	2.76	37.2	35.7	38.0
Glucose[a]	13.9	6.0	4.45	54.2	55.2	53.4
Lactose	12.1	4.9	3.75	45.8	44.8	46.6

[a] These mixtures were supplied to the analyst as unknowns.

To include gentiobiose in the mixtures it was necessary to start at Step 2, since only gentiobiose octaacetate (Pfanstiehl's), $[\alpha]^{20}D$ in chloroform −5.3°, was available. Therefore, the correction curve was not applied to the data for this run in Table IV. The methylated gentiobiose fraction solidified on standing: $[\alpha]^{20}D$ −7.0° in chloroform. For β-methyl heptamethylgentiobioside, Haworth and Wylam[26] list −29.9° for the specific rotation in alcohol.

Anhydrous trehalose was obtained by heating Pfanstiehl trehalose monohydrate at 105° for three hours. After the analysis (Table IV), 10 g. of the methylated disaccharose fraction was refluxed for eight hours with 37 g. of silver oxide and 70 g. of methyl iodide. When the mixture was worked up, a 5-g. fraction was obtained: b. p. 165–168° (0.01 mm.), $[\alpha]^{20}D$ in chloroform +166.2°. The rotation given for octamethyltrehalose[27] in benzene is +199.8°.

In the mixture of glucose, trehalose and gentiobiose octaacetate, the weight of 17.6 g. of the acetate is equivalent to 8.9 g. of gentiobiose. This represents a total of 11.67 g. of gentiobiose plus trehalose.

In the mixture of rhamnose and maltose, the rhamnose fraction was collected to 110°; $[\alpha]^{20}D$ +12.0° in chloroform. The specific rotation of β-methyl trimethylrhamnoside[28] in water is given as −15°.

The methylated lactose fraction (Table IV) was remethylated with 20 g. of silver oxide and 50 g. of methyl iodide for eight hours. Three grams was obtained: b. p. 170–175° (0.02 mm.), $[\alpha]^{27}D$ +6.19° in chloroform, $[\alpha]^{27}D$ +3.00 in methanol. The specific rotation of β-methyl heptamethyllactoside[29] in methanol is listed at −13.04°.

Separation of a Pentose–Hexose Mixture

Ten grams each of xylose and glucose were mixed and treated by the analysis procedure. The mixture of methylated derivatives obtained in the last step was distilled at a rate of 3–5 drops per minute in the vacuum apparatus. The height of the side-arm on the distillation flask was approximately 10 cm. One of the receivers was previously calibrated in cc., so that temperature of distillation could be plotted against volume of distillate. The curve shown in Fig. 2 was obtained. The pressure was 0.03–0.04 mm.

In a second run 12.5 g. of xylose $[\alpha]^{20}D$ +17.7° and 12.5 g. of glucose were mixed and treated by the standardized procedure. The methylated sirup obtained in the final step was distilled, the cut being made at 75° as indicated by the curve. The pressure was 0.005 mm.

Components	Methylated deriv., g.	Free sugar, g.	% Components Obsd.	Corr.	Calcd.
Pentose	5.63	4.10	49.3	50.1	50.0
Hexose	5.85	4.21	50.7	49.9	50.0

Pentose.—A portion, 3.5 g., of the methylated pentose, crystallized during the distillation. The rotation was determined in chloroform. Sample: 0.1205 g.; ang. rotn., −0.64°; $[\alpha]^{20}D$ −69.9°; volume solution, 25.0 cc.; tube length, 1.89 dm. The melting point of the crystals was 45°. The recorded constants of β-methyl trimethylxyloside are: melting point 51°; $[\alpha]^{20}D$ −69.5° in chloroform.[2]

Hexose.—The specific rotation of the sirupy hexose portion was determined in chloroform. Sample, 0.9276 g.; ang. rotn., −1.75°; $[\alpha]^{20}D$ −33.7°; tube length, 1.89 dm.; volume solution, 25.0 cc. The specific rotation of β-methyl tetramethylglucoside is −22.4° in chloroform.

Analysis of Hydrol

The Drying of Hydrol.—The hydrols used in this work were obtained through the courtesy of Mr. W. B. Newkirk of the Corn Products Refining Company. Hydrol is the term used to represent the mother liquor after glucose has been crystallized from the acid hydrolysis of starch. Ordinarily, starch is hydrolyzed by 0.25 to 0.5% hydrochloric acid under pressure of about 40 pounds (3 atm.) of steam. It is then neutralized, filtered, clarified, concentrated and crystallized. Ordinary hydrol is the residue obtained from one such hydrolytic conversion and crystallization. If the hydrol is again subjected to a second hydrolytic conversion and crystallization, the mother liquors are the so-called Reconverted Hydrol. The material which had undergone two crystallizations for glucose after but one hydrolytic conversion is called Kansas City Hydrol.

(25) Haworth and Law, *J. Chem. Soc.*, **109**, 1314 (1916).
(26) Haworth and Wylam, *ibid.*, **123**, 3120 (1923).
(27) Schlubach and Maurer, *Ber.*, **58**, 1178 (1925).
(28) Purdie and Young, *J. Chem. Soc.*, **89**, 1194 (1906).
(29) Haworth and Leitch, *ibid.*, **113**, 195 (1918).

All of the hydrols used for analysis by the procedure were dried in the following manner. One part of the hydrol was taken up with an equal volume of toluene and distilled at 11–15 mm. from an ordinary Claisen flask surrounded by a water-bath at 95°.

Five such operations usually served to remove almost all of the water, leaving the hydrol as a hard glassy mass. By continued warming on the water-bath the sirup could be softened and poured from the flask. Samples were weighed in this manner. That most of the water was eliminated by this procedure was indicated by a water analysis on dried hydrol. A sample weighing 6.151 g. lost 0.020 g. after four hours of heating at 110–120° in an air-bath; final weight 6.131 g.; % water, 0.32.

Application of the Procedure to Hydrol

The size of the original samples was in all cases 25.0 g. Acetylation with pyridine and acetic anhydride proceeded normally as in other sugar samples. It was found, however, that if the pyridine–hydrol mixture was warmed slightly before adding the acetic anhydride an appreciable amount of the sirup dissolved and hastened the reaction. In this way complete acetylation was usually accomplished in forty-eight hours at 0°.

In the final distillation, after removal of the low boiling fraction, as much as possible of the residual material was distilled by taking the bath temperature to 250°. Conditions for the distillation were substantially the same in all of the runs. The pressure varied from 0.001 to 0.008 mm. All material up to 110° was regarded as monosaccharose; the disaccharose distilled was collected between 160–190° at these pressures.

The weights of the fractions collected in the final distillations of the runs with the hydrols are listed in Table V.

TABLE V

METHYLATED PRODUCTS FROM HYDROLS

	Hydrol, g.				Reconverted hydrol, g.		Kansas City hydrol, g.	
Run	1	2	3	4	1	2	1	2
Fraction 1	5.40	6.44	5.94	4.88	4.94	5.90	5.12	4.86
Fraction 2	3.19	3.50	2.34	3.22	3.29	3.09	2.67	2.58
Residue	1.34	1.66	2.31	0.96	0.83	1.67	1.52	1.45
Calculated to basis of free sugars, g.								
Monosacch.	4.00	4.76	4.40	3.62	3.68	4.37	3.79	3.55
Disacch.	3.02	3.39	2.78	2.88	2.88	3.08	2.70	2.60
Trisacch.	0.45	0.56	0.78	0.32	0.27	0.56	0.51	0.49
Observed percentages of components								
Monosacch.	53.6	54.7	55.3	53.1	53.9	54.6	54.2	53.5
Disacch.	40.4	38.9	36.2	42.2	42.2	38.5	38.6	39.2
Trisacch.	6.0	6.4	8.5	4.7	4.9	6.9	7.2	7.3

Treatment of the Residue: Presence of Trisaccharoses.—To determine the nature of the residue, comparable experiments were performed on the methylated residues from maltose and from hydrol. About 10 g. of undistilled methylated maltose was dissolved in a minimum of chloroform, placed in a 500-cc. bottle, and 250 g. of methyl iodide and 25 g. of silver oxide added. The mixture was heated to boiling, the bottle stoppered and insulated with an asbestos wrapper. The bottle was now shaken mechanically for three days, the shaking being interrupted and heat applied as described about every six hours during the day.

The residues from the four runs of hydrol were treated in exactly the same manner. Upon completion of the shaking, the solutions were filtered, dried and the solvent removed. The sirups obtained were distilled *in vacuo* up to a bath temperature of 250°. Under this condition only would the disaccharose distil; any higher boiling fraction would remain. Upon completion, the distilled portion and the residues were weighed. The results with maltose and hydrol are summarized.

	Maltose	Hydrol
Distilled portion or disaccharose, g.	7.12	3.40
Residue, g.	0.92	2.64
Total, g.	8.04	6.04

The molecular weights of the residues were now determined in camphor:

	Maltose	Hydrol
Wt. camphor	0.0946	0.0907
Wt. sample	.0274	.0397
M. p. camphor, °C.	179.4	179.4
M. p. mixture, °C.	153.0	151.0
Difference, °C.	26.4	30.4
Constant for camphor	407.6	407.6
Molecular weight	429	587

Calculated for fully methylated disaccharose, 454
Calculated for fully methylated trisaccharose, 658

Although the molecular weights did not agree exactly with calculated values, they were different enough to signify that a portion of the original hydrol residues was trisaccharose.

Calculated from the weights obtained by fractionation of the residues, this appeared to be:

Disaccharoses	56.3%
Trisaccharoses	43.7%

The weights of residues obtained in the original fractionation were now corrected using these figures. The weights of methylated sugars were calculated back to the weights of free sugars as was done with the known mixtures, and the percentages of components thus obtained (Table V). The correction from the correction curve was applied only to the mono- and disaccharose fractions.

Molecular Weights Used

Monosaccharose	180	Methylated monosaccharose	243
Disaccharose	342	Methylated disaccharose	447
Trisaccharose	506	Methylated trisaccharose	658

The corrected percentages of components are summarized in Table VI. The whole analysis, including water and ash which were determined in the usual way, for each hydrol is also included.

Only glucose seemed to be present in the monosaccharose portion from hydrol. In two of the runs, the specific rotations taken in chloroform at 20° were −19.5 and −18.1°. This is reasonably near the rotation (−22.4°) for pure β-methyl tetramethylglucoside. Furthermore, glucose crystals separated from hydrol on standing.

Work is being continued on the identification of the sugars in the disaccharose portion. These specific rotations were obtained in chloroform on the methylated disaccharose fractions: hydrol, $[\alpha]^{20}D$ +38.7°; reconverted hydrol, $[\alpha]^{20}D$ +30.8°; Kansas City hydrol, $[\alpha]^{27}D$ +66.7°.

TABLE VI

PERCENTAGE COMPOSITION OF HYDROL

	Monosacch.	Disacch.	Trisacch.	Water	Ash
Hydrol					
Mean (Table V)	54.2	39.4	6.4	..	..
Corrected mean	55.2	38.4	6.4	..	..
Whole analysis	43.2	30.8	5.0	19.8	1.96
Reconverted Hydrol					
Mean	54.2	40.3	5.5	..	..
Corrected mean	55.2	39.3	5.5	..	..
Whole analysis	40.1	28.5	4.0	21.60	5.83
Kansas City Hydrol					
Mean	53.8	38.9	7.3	..	..
Corrected mean	54.8	37.9	7.3	..	..
Whole analysis	40.7	28.2	5.2	20.1	5.60

Summary

A procedure has been developed for the direct analysis of sugar mixtures of these types: mono- and disaccharoses; mono-, di- and trisaccharoses; pentoses and hexoses. The procedure involves indirect methylation, vacuum distillation of the methylated derivatives, and weighing of the fractions thus obtained. An accuracy of 3% was attained. Since the various fractions are separated from each other, they may be used for purposes of identification.

The indirect methylation is necessary to obtain uniformity in yields. Direct methylation of a mixture of mono- and disaccharoses gives results in the fractional distillation which are not indicative of the original percentage composition. The following steps are involved in the standardized procedure: acetylation at 0° by acetic anhydride and pyridine, replacement of the acylal function by chlorine by means of titanium tetrachloride, substitution of the chlorine by methoxyl by prolonged shaking with methanol and silver carbonate, hydrolysis of the remaining acetyl groups by sodium methoxide in methanol, methylation by one treatment with methyl sulfate and sodium hydroxide, and finally fractional distillation *in vacuo* of the methylated sirups.

Monosaccharoses such as xylose, rhamnose, glucose and levoglucosan undergo the procedure successfully. In its present form it does not apply to fructose. The disaccharoses studied included the maltose type (maltose, lactose, gentiobiose) and the trehalose type (trehalose, sucrose).

The procedure has been applied to three types of hydrol and the presence of trisaccharoses in these hydrols has been established.

EVANSTON, ILLINOIS RECEIVED JULY 18, 1938

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA]

The Mechanism of the Aqueous Hydrolysis of β-Butyrolactone

BY A. R. OLSON AND R. J. MILLER

In 1896 Walden[1] showed that *d*-malic acid was obtained when silver oxide was suspended in an aqueous solution of *d*-chlorosuccinic acid. Treatment of the chloro acid with potassium hydroxide yielded an *l*-malic acid. Holmberg[2] explained these results by assuming the primary formation of a lactone from the chloro acid, the lactone then hydrolyzing in several different ways, depending upon the hydrogen ion concentration. He[3] later succeeded in isolating the lactone. The generality of lactone formation from β-halogenated acids has been proved by Johansson's[4] work.

Holmberg[5] and Rørdam[6] studied the hydrolysis of the (+) malolactonic acid which was obtained from the (−) halogenosuccinic acids. They found that in dilute aqueous acids the hydrolysis was first order, and that the product contained a preponderance of (−) malic acid. In stronger acids, up to 2 *N* nitric acid and in basic solutions, the product was largely (+) malic acid. These results were confirmed and extended by Long and Olson,[7] who determined both rates and optical relations using buffered solutions. Their results are summarized in Figs. 1 and 2. The interpretation of these results is complicated by the difficulty of determining the total concentration of lactone and also by the possible existence of an α-lactone as well as a β-lactone. We have therefore turned to β-bromobutyric acid which readily yields a pure β-lactone. We have been able to show that this lactone hydrolyzes in neutral

(1) Walden, *Ber.*, **29**, 133 (1896).
(2) Holmberg, *ibid.*, **45**, 1713 (1922).
(3) Holmberg, *Svensk. Kem. Tids.*, **30**, 190 and 215 (1918), through *Chem. Zentr.*, **90**, I, 223 (1919).
(4) Johansson, *Lunds Universitets Årsskrift*, N. F., Part 2, **12**, No. 8 (1916).
(5) Holmberg, *J. prakt. Chem.*, **88**, 553 (1913).
(6) Rørdam, *J. Chem. Soc.*, **2**, 2931 (1932).
(7) Long and Olson, unpublished work in this Laboratory.