[CONTRIBUTION FROM THE HYGIENIC LABORATORY, U. S. PUBLIC HEALTH SERVICE]

RELATIONS BETWEEN ROTATORY POWER AND STRUCTURE IN THE SUGAR GROUP. XXIX. THE PREPARATION OF CRYSTALLINE TURANOSE[1]

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Alekhine[2] found that when the trisaccharide melezitose is gently hydrolyzed by weak acid solutions there results as one of the products a disaccharide to which he gave the name turanose. He observed that turanose is dextrorotatory and he states that it melts at 65–70° and is very hygroscopic; this description indicates in the light of our present results that his preparation must have been an amorphous substance. Alekhine obtained only glucose by the complete hydrolysis of melezitose with strong acids and he concluded that turanose is a di-glucose. This inference was later revised by G. Tanret,[3] who obtained both glucose and fructose from the hydrolysis of turanose. Tanret describes turanose as a white powder consisting of extremely hygroscopic round grains containing one-half molecule of alcohol of crystallization, melting at 60–65° and showing $[\alpha]_D$ +71.8, without mutarotation. His description indicates in the light of our results that the substance was not obtained in crystalline condition. Alekhine observed that turanose is a reducing sugar; decision as to whether it is a glucosido-fructose or a fructosido-glucose was made by Kuhn and Von Grundherr,[4] who found that it is not oxidized by alkaline hypoiodite solution and must consequently be a glucosido-fructose.

This partial determination of the structure of turanose leads at once to some very interesting speculations concerning the structure of melezitose. Since melezitose is a non-reducing sugar, its structure must be indicated as glucose $<>$ fructose $>$ glucose, where the disaccharide $>$ fructose $>$ glucose is turanose. What is the identity of its other potential disaccharide, which must be a non-reducing sugar, glucose $<>$ fructose? Obviously this sugar may be sucrose. If such is not the case it must be an isomer closely related to sucrose. Kuhn and Von Grundherr have found that certain enzyme preparations which hydrolyze sucrose also split melezitose into glucose and

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[1] Publication authorized by the Surgeon General of the U. S. Public Health Service. The crystallization of turanose was reported by the authors at the April, 1929, meeting of the National Academy of Sciences and a short article was published in *Science*, **69**, 278 (1929). No. XXVIII of this series was published in THIS JOURNAL, **52**, 2106 (1930).

[2] Alekhine, *Ann. phys. chim.*, **18**, 532 (1889).

[3] Tanret, *Bull. soc. chim.*, **35**, 816 (1906).

[4] Kuhn and Von Grundherr, *Ber.*, **59**, 1655 (1926); Bougault, *J. pharm. chim.*, **16**, 97 (1917); Votoček and Nemeček, *Z. Zuckerind. Böhmen*, **34**, 399 (1910); Nef, *Ann.*, **403**, 204 (1914).

turanose and they conclude that melezitose is probably a derivative of sucrose. When these authors published their observations, one of us (C. S. H.) made the following calculations from the application of the isorotation rules to the rotations of the substances in question. It has long been known[5] that the mixture of the forms of glucose and fructose which are initially liberated through the hydrolysis of sucrose by invertase possesses the same rotation as sucrose, that the glucose form is ordinary α-glucose ($[\alpha]_D$ 113) and that consequently the fructose form is one having an $[\alpha]_D$ value of about +17. This form must be a β-fructose; if it were an α-fructose the mixture immediately resulting from the hydrolysis would show a much lower dextrorotation than sucrose, which is not the fact. Assume now with Kuhn and Von Grundherr that melezitose is a derivative of sucrose and apply similar considerations. The initial products of its hydrolysis must be α-glucose (113) and a β-form of turanose; this mixture must show the same rotation as melezitose ($[\alpha]_D$ 88.6). The $[\alpha]_D$ value of this hypothetical β-turanose is obtained from these data, when the molecular weights are introduced, as $((88.6)(504) - (113)(180))/342 = 71$. This calculated value is quite near the rotation of turanose which Tanret has measured (71.8). Obviously the measurement applies to stable solutions of turanose. It seemed therefore from the calculations that melezitose might indeed be a derivative of sucrose and that if such is the case turanose might be found to be a ketose which, like sorbose, tagatose and mannoketoheptose, would not exhibit mutarotation. Obviously the existence or absence of mutarotation could only be determined from the study of truly crystalline turanose, but since Tanret believed that he had the crystalline sugar in hand and found no mutarotation, the calculations appeared to be substantiated throughout. However, it was not considered wise to publish these considerations at the time because of the following uncertainty: the statement by Tanret that his turanose preparation melted at as low a temperature as 60–65° and was exceedingly hygroscopic and the fact that accurately discernible crystals were not reported suggested that the substance was really amorphous because the reported properties would be most unusual for a pure crystalline

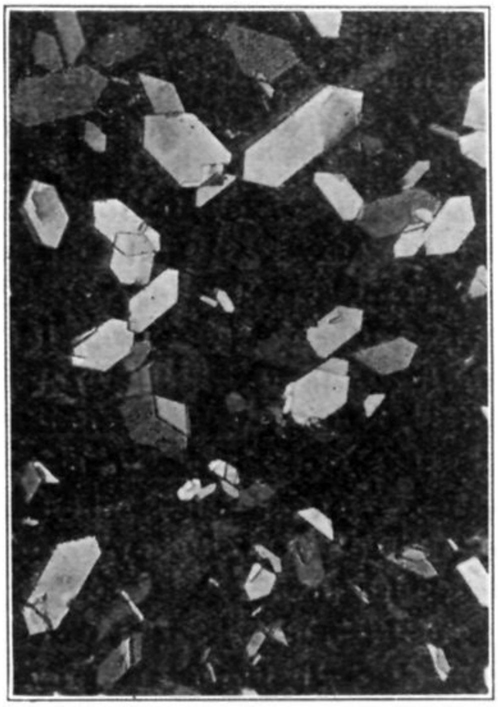

Fig. 1.—Crystals of turanose in polarized light.

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[5] Hudson, THIS JOURNAL, **31**, 655 (1909); see also *ibid.*, **52**, 1715 (1930).

disaccharide. Indeed, Bridel and Aagaard[6] have expressed the conviction that Tanret did not crystallize turanose. It was decided, therefore to await the production of further experimental evidence. This has fortunately been obtained lately in an unexpected way, as will now be described. In 1918 one of us found an abundant supply of the rare melezitose in a certain kind of honey-dew honey[7] and from it he prepared a small quantity of sirupy turanose in the hope of crystallizing it. Other samples of turanose sirup were prepared subsequently from this stock of melezitose by other workers in the same laboratory. Recently it was observed by D. H. Brauns that one of these sirups, the exact history of which is not now known, had crystallized after standing many years. By the use of these crystals to nucleate turanose sirups which we have lately prepared from melezitose, it has been possible to obtain a rapid crystallization of the sugar in abundant quantities.[8]

The physical properties of crystalline turanose differ entirely from those reported by G. Tanret and there can no longer be any doubt that his preparation was an amorphous material. The sugar is readily purified by recrystallization from hot methyl alcohol, in which it is moderately soluble. Its melting point is 157°, it crystallizes in well-formed prisms with many faces developed (see photograph), its composition is $C_{12}H_{22}O_{11}$ and it is not hygroscopic. We were surprised to find that it exhibits a large and rapid

TABLE I

MUTAROTATION OF TURANOSE AT 22° IN AQUEOUS SOLUTION (CONCENTRATION, 3.928 G./100 CC.)

Time, minutes	$[\alpha]_D^{22}$	$k = \frac{1}{t} \log \frac{R_\infty - R_0}{R_\infty - R}$
0	(+22.0)	..
4	+53.5	..
5	+58.1	0.103
6	+61.4	.098
7	+64.5	.101
8	+66.2	.095
9	+68.2	.091
10	+69.7	.098
11	+70.6	.095
12	+71.7	.098
13	+72.4	.097
14	+73.0	.098
25	+75.3	
60	+75.3	Average .097

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[6] Bridel and Aagaard, *Bull. soc. chim. biol.*, **9**, 884 (1927).

[7] Hudson and Sherwood, THIS JOURNAL, **42**, 116 (1920).

[8] During the hot dry season in early July, 1928, an unusually large flow of honey-dew honey occurred in apiaries around Washington, D. C., and we were able to prepare five kilograms of melezitose from such honey kindly supplied by the Office of Bee Culture Investigations, U. S. Department of Agriculture, from their apiary at Somerset, Maryland.

mutarotation, from the direction of which it is inferred that the sugar is a beta modification. The designation is provisional, however, because it is not excluded that a ring shift may occur during the mutarotation. The course of the change is shown in the table; it follows closely the unimolecular law and consequently the initial rotation can be obtained with fair accuracy by extrapolation. The value of the final rotation (75.3) shows that the previous estimates by Tanret (71.8) and by Bridel and Aagaard (72–73) from the study of amorphous materials were fair approximations.

Let us return now to the theoretical considerations. Observation shows that the form in which turanose has crystallized is not the hypothetical β-turanose of initial $[\alpha]_D$ approximately 71, predicted by theory. Nevertheless, the theory may be correct; if such is the case the hypothetical sugar must have a different ring structure from that of the known form. Such a result would not be surprising because by analogy with the structure of sucrose, the fructose constituent of which apparently possesses a 2,4-ring that shifts to the 2,5-position after the hydrolysis,[9] the fructose constituent of melezitose would be expected to possess the same 2,4-labile ring which could conceivably change to another type in crystalline turanose. It will apparently require new experimental evidence before this phase of the considerations can be further advanced and it is mentioned that the studies of Bridel and Aagaard on the hydrolysis of melezitose and turanose by various enzyme preparations lead them to doubt that sucrose is a constituent of the melezitose molecule. The writers are continuing the investigation of turanose; one (E. P.) will publish soon the description of several acyl derivatives of it which he has prepared and the other (C. S. H.) is studying its behavior in the Lobry de Bruyn alkaline rearrangement in the hope of converting it to a disaccharide aldose.

The Preparation of Crystalline Turanose from Melezitose.—The one-stage acid hydrolysis of melezitose must be carefully controlled for a good yield of turanose. Bridel and Aagaard recommend heating a solution containing 10% melezitose and 0.6% sulfuric acid for one and three-quarters hours on the water-bath but our results indicate that the hydrolysis proceeds under these conditions much beyond the first stage. The rotations of test samples of such a solution which were kept definitely at 100° for different lengths of time and then cooled, indicated that the first stage of hydrolysis is complete at this temperature in fifteen minutes. Three hundred grams of pure melezitose was dissolved in 2800 cc. of boiling water and a hot mixture of 19.2 g. of concentrated sulfuric acid (95%) and 150 cc. of water was added in one portion. The solution was kept boiling gently for fifteen minutes and was then cooled rapidly to room temperature and neutralized with an excess (22 g.) of calcium carbonate. About 10 g. of baker's yeast was then added to the unfiltered solution and fermentation was allowed to proceed at 40° for thirty-six to forty-eight hours, when it appeared to have nearly ceased. The solution was filtered after adding decolorizing carbon and the filtrate was concentrated under reduced pressure to a thick sirup, which was taken up with 500 cc. of

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[9] Hudson, THIS JOURNAL, **52**, 1717 (1930).

absolute methyl alcohol and filtered from the insoluble calcium salts, using decolorizing carbon again. On nucleation of the solution with crystals of turanose it crystallized rapidly and became a solid mass after twenty-four hours at room temperature. The crystals were washed with absolute methyl alcohol and dried in a desiccator; yield, 110–125 g., and a further crop of 35–40 g. was obtainable from the mother liquor by reconcentration to a sirup and taking this up as before. Even the second mother liquor yielded a crop of 8–12 g. by removal of the methyl alcohol, solution of the residue in water, fermentation and retreatment by the usual procedure. The highest yield from a batch of 300 g. of melezitose was 178 g. or 87% of the theoretical, the lowest 80%. The sugar was recrystallized from boiling methyl alcohol and refluxing is advisable for obtaining a concentrated solution.

The separation of glucose from turanose, which is accomplished in the preceding directions through fermentation, may also be made by oxidizing the hexose with bromine water, which leaves the turanose largely unchanged, and precipitating calcium gluconate by the addition of alcohol.[10] One hundred grams of melezitose was hydrolyzed as directed and the cooled solution was neutralized with 11 g. of barium carbonate. Without filtration there was added a solution of 78 g. of barium benzoate in 1800 cc. of water followed by 10.2 cc. of bromine, which rapidly dissolved on stirring. After twelve hours the excess bromine was blown out, the barium was removed as sulfate and the main portion of the hydrobromic acid as lead bromide (using litharge to neutrality for congo red), and the final part as silver bromide. Excess heavy metals were then precipitated as sulfides. Dissolved benzoic acid was largely removed by several extractions with chloroform. The resulting colorless solution contained little besides turanose and gluconic acid. It was kept slightly alkaline to phenolphthalein with milk of lime for one hour at room temperature and then neutralized with sulfuric acid, filtered and concentrated to 500 cc. under reduced pressure. This solution was made slightly alkaline with lime and 450 cc. of 95% alcohol was added, followed by filtration and nucleation with calcium gluconate crystals. After standing overnight in the ice chest the salt was filtered off, 300 cc. of 95% alcohol was added and the filtration repeated. The filtrate was concentrated under reduced pressure to a thick sirup, which was dissolved in 400 cc. of cold methyl alcohol. A slight quantity of calcium gluconate soon separated but the filtrate gave no further precipitate on adding methyl alcohol. The solution was then concentrated to a thin sirup, which was seeded with turanose crystals; after a day it had become solid and yielded 44 g. of turanose. A second crop of 7.5 g. was obtained from the mother liquor, bringing the yield to 80% of the theoretical. The fermentation procedure is less laborious than the oxidation method but the latter may be used if difficulties arise in connection with the control of the growth of the yeast, as is sometimes the case.

Turanose crystallizes from methyl alcohol without solvent of crystallization and its analysis corresponds to the formula $C_{12}H_{22}O_{11}$. After drying at room temperature over calcium chloride in a vacuum desiccator to constant weight it lost no further weight when heated for two-hour intervals at 77, 98 and 110°, respectively, under reduced pressure. Micro combustions of samples weighing 2–3 milligrams showed C, 42.09, 42.17, 42.10; H, 6.38, 6.58, 6.56%; calculated for $C_{12}H_{22}O_{11}$: C, 42.08; H, 6.48. The combustions were made by Dr. R. T. K. Cornwell, to whom our thanks are expressed.

One of the authors (E. P.) expresses his thanks to the International Education Board for the grant of a fellowship which made possible his participation in this research.

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[10] Montgomery and Hudson, THIS JOURNAL, **52**, 2101 (1930).

Summary

After several years' standing a sirupy preparation of turanose crystallized spontaneously and by nucleation with these crystals it has been possible to obtain rapid crystallization of large quantities of turanose. Directions for the preparation of the sugar in good yield from melezitose are given and some of its properties are recorded. Turanose shows a large and rapid mutarotation. A discussion of the structures of turanose and melezitose, in so far as present evidence permits, is presented.

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[CONTRIBUTION FROM THE KENT CHEMICAL LABORATORY OF THE UNIVERSITY OF CHICAGO]

SALTS OF NITRILES. IV. SODIUM ALPHA-PHENYLBUTYRONITRILE[1,2]

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Previous reports[3] have described the preparation of the sodium and potassium salts of certain nitriles, and presented evidence for the nitride–carbide tautomerism of the anions of the salts, *e. g.*,

$$[C_6H_5C(R){=}C{=}N^-]Me^+ \rightleftharpoons [C_6H_5C^-(R)CN]Me^+ \qquad (1)$$

Rising and Braun[3] made a quantitative study of the course of the reaction of phenylacetonitrile with sodium, and of the chemical behavior of the salt sodium phenylacetonitrile, $[C_6H_5CHCN]Na$. The present authors have studied quantitatively the analogous reaction of α-phenylbutyronitrile with sodium. They have found that the course of the reaction may be summarized in the equation

$$2C_6H_5C(C_2H_5)HCN + 2Na \longrightarrow [C_6H_5C(C_2H_5){=}C{=}N]Na + NaCN + C_6H_5C_3H_7 \qquad (2)$$

The hydrogen formed in the reaction of sodium with the nitrile reduces part of the salt to toluene and sodium cyanide

$$2C_6H_5C(C_2H_5)HCN + 2Na \longrightarrow 2[C_6H_5C(C_2H_5){=}C{=}N]Na + 2H \qquad (3)$$

$$[C_6H_5C(C_2H_5){=}C{=}N]Na + 2H \longrightarrow C_6H_5C_3H_7 + NaCN \qquad (4)$$

This reaction corresponds to that observed by Rising and Braun with sodium phenylacetonitrile, which is reduced by hydrogen to toluene and sodium cyanide. There are described also herein the conditions used for the preparation of the salts sodium and potassium α-phenylbutyronitrile. A study of the behavior of sodium α-phenylbutyronitrile has

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[1] The work here reported forms part of the dissertation of E. W. Lowe, to be presented in partial fulfilment of requirements for the doctorate degree at the University of Chicago.

[2] Original manuscript received August 31, 1929.

[3] Rising and Zee, THIS JOURNAL, (a) **49**, 541 (1927) and (b) **50**, 1699 (1928); Rising, Muskat and Lowe, *ibid.*, **51**, 262 (1929); Rising and Braun, *ibid.*, **52**, 1069 (1930).