Isolation of Trehalose from Desert Manna

By J. LEIBOWITZ, *Cancer Research Laboratories, Department of Chemistry,
The Hebrew University, Jerusalem*

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The manna found on the leaves of *Tamarix* in the Sinai Desert has been described in detail by Bodenheimer & Theodor (1929), and has been identified as the excretion of the scale insects *Trabutina mannipara* and *Najacoccus serpentinus*. Its chemical analysis by Fodor & Cohn (1929) established the presence of sucrose and invert sugar as the principal or sole sugar constituents.

Some specimens of another manna collected in the North Iraqi Desert (Suleimanya District) were kindly supplied to me by Prof. Bodenheimer, who believes that this manna, too, is the excretion of some scale insect. It is gathered by the Bedouin from the leaves of trees and bushes and is used as a source of sweetening substance.

EXPERIMENTAL

The specimens examined included two batches of the raw material, and one of the syrup which is produced from the manna. The method of preparation of the syrup from its natural source is unknown to me. It is water-clear, with a slightly yellowish colour, and is of a thick consistency; it has a pronounced sweet taste. The substance consists mainly of carbohydrate and gives only a very slight precipitate with trichloroacetic acid and other protein precipitants. The aqueous solution shows a surprisingly high dextrorotation, corresponding to a $[\alpha]_D$ of over $+100^\circ$, which rules out the possibility that sucrose or invert sugar or any of the common hexoses is the principal constituent. The solution was analyzed by differential hydrolysis, the changes in optical activity and reducing power to Bertrand reagent and Willstätter-Schudel reagent being followed simultaneously.

The stock solution consisted of 5 g. of syrup (dried in a desiccator, but still containing some water) dissolved in 50 ml. of water. Hydrolysis was carried out on samples of this solution by heating with measured amounts of H_2SO_4 of varying concentrations, or by incubating with a solution of yeast invertase in phosphate buffer of pH 4.5. All reducing values refer to 1 ml. of the original solution or to equivalent amounts of the hydrolysates. Total reducing sugar was determined by the method of Bertrand. Aldoses were determined by the method of Willstätter & Schudel as modified by Auerbach & Bodländer (1923). Ketose was calculated as the difference between the Bertrand and the Willstätter-Schudel reducing values. All polarimetric data refer to readings in a 1 dm. tube. In the case of the hydro-

lysates the values are corrected for the changes in volume induced in the original solution by the treatment. The data are summarized in Table 1.

Table 1. *Changes in optical rotation and reducing power produced by hydrolysis of manna*

	Treatment of original solution	$[\alpha]_D$	Total hexose (mg.)	Aldose (mg.)	Ketose (mg.)
(1)	None	$+10.3^\circ$	22.1	15.1	7.0
(2)	0.01 N- H_2SO_4 , 30 min. at 100°	$+9.2^\circ$	34.4	21.0	13.4
(3)	Invertase, 24 hr. at 37°	$+9.0^\circ$	34.0	22.2	11.8
(4)	0.5 N- H_2SO_4 , 60 min. at 100°	*	57.5	44.0	13.5
(5)	N- H_2SO_4 , 60 min. at 100°	*	76.6	68.3	(8.3)
(6)	N- H_2SO_4 , 75 min. at 100°	*	78.0	70.3	(7.7)

* Owing to a dark coloration of the solution no observation was possible.

It follows from (1) and (6) of Table 1 that about one-quarter of the carbohydrate consists of reducing sugar, apparently invert sugar with an excess of glucose. The transition from (1) to (2) marks the inversion of sucrose, identified by its susceptibility to mild hydrolysis and its conversion into an approximately equimolecular mixture of aldose and ketose. This identification is corroborated by the agreement between the data of the mild acid hydrolysis (2) and of the enzymatic hydrolysis (3), as well as by the amount of decrease in rotational power: observed for (2) $10.3 - 9.2 = 1.1^\circ$, observed for (3) $10.3 - 9.0 = 1.3^\circ$. Calculated for the inversion of 1.17% sucrose, corresponding to 1.23% invert sugar,

$$\left[66 \times \frac{1.17}{100} \right] - \left[-20 \times \frac{1.23}{100} \right] = 1.02^\circ.$$

The bulk of the substance, however, is some non-reducing carbohydrate different from sucrose. It is remarkably resistant to acid hydrolysis and yields on ultimate hydrolysis only aldose. The apparent drop in the ketose values from (4) through (5) to (6) is caused by the prolonged action of the hot strong acid on fructose. Control experiments with invert sugar showed that under the same conditions fructose is partially destroyed, causing a dark coloration

of the solution, while the glucose component remains unaffected. By comparing (6) with (2) or (3) of Table 1, the amount of this fraction can be calculated as 60 % of the total carbohydrate, and it is obvious that its rotation must be about $[\alpha]_D = +200^\circ$. These results suggest the identity of this fraction with trehalose. The data would have been consistent also with the presence of glycogen or starch, but this is definitely excluded by the failure of the solution to give a colour with iodine.

On the basis of this assumption it should be possible to compute the optical rotation of the original solution as follows:

$$\begin{aligned} 1.51\% \text{ glucose (1) } \dots \dots [\alpha]_D &= 52 \times \frac{1.51}{100} = +0.78^\circ \\ 0.70\% \text{ fructose (1) } \dots \dots [\alpha]_D &= (-92) \times \frac{0.7}{100} = -0.64^\circ \\ 1.17\% \text{ sucrose (2) - (1) } \dots [\alpha]_D &= 66 \times \frac{1.17}{100} = +0.77^\circ \\ (\equiv 1.23\% \text{ invert sugar}) \dots \dots & \\ 4.68\% \text{ trehalose (6) - (2) } \dots [\alpha]_D &= 197 \times \frac{4.68}{100} = +9.22^\circ \\ (\equiv 4.93\% \text{ glucose}) \dots \dots & \\ \text{Total } [\alpha]_D &= +10.13^\circ \end{aligned}$$

The excellent agreement between this calculated value and that observed ($+10.3^\circ$) strongly supports the assumptions employed in making this calculation.

To confirm the nature of the unknown sugar the following experiment was undertaken with a specimen of the natural manna. The raw material was a dark grey sticky mass containing some granular material together with leaf and insect residues. The material (30 g.) was fermented with baker's yeast until all reducing sugar (and presumably also all the sucrose) had been removed. After centrifugation, the supernatant fluid was deproteinized with basic lead acetate; the excess of lead was precipitated with H_2S and the H_2S removed by aeration. The solution was neutralized, filtered and concentrated *in vacuo*, at 40° , to 50 ml. The clear solution had $[\alpha]_D = +8.6^\circ$, and was almost non-reducing. Incubation with invertase and treatment with hot 0.01 N-acid failed to liberate reducing sugar or to bring about any change in optical rotation. After total hydrolysis with hot N-acid, the solution reduced Bertrand (1 ml. solution gave 85.5 mg. Cu = 44.5 mg. 'glucose') and Auerbach-Bodländer (1 ml. solution required 5.1 ml. 0.1 N- $Na_2S_2O_3$ = 45.9 mg.

aldose) reagents to the same extent. The observed rotation was $[\alpha]_D = 2.4^\circ$; hence $[\alpha]_D = \frac{2.4 \times 100}{4.5} = +53.3^\circ$.

The hydrolysate presumably contained only glucose. On this basis the amount of the parent oligosaccharide may be calculated as $45 \times 342/360 = 42.7$ mg. in 1 ml., and its specific rotation as

$$[\alpha]_D = \frac{8.6 \times 100}{42.7} = +201^\circ,$$

in good agreement with the specific rotation ($[\alpha]_D = +197^\circ$) of trehalose. The disaccharide present amounts to 7 % of the raw material.

In a further experiment, a second sample of raw material (14 g.) was treated according to the method of Harding (1924) for the preparation of trehalose from *Selaginella*. The substance was thrice extracted with 50–75 ml. boiling ethanol; the extract was then evaporated and the residue taken up in water. After elimination of the fermentable sugar with yeast, deproteinization, as previously described, and decolorization with charcoal, the solution was concentrated to a thin syrup and diluted with hot ethanol. The aqueous ethanolic solution was seeded with a few trehalose crystals. On slow concentration in a desiccator over P_2O_5 with repeated additions of ethanol, a crystalline deposit was formed. The substance was filtered off and washed with cold ethanol and ether. Yield 0.41 g., m.p. 96° . The hydrated form of trehalose melts at 97° . On drying at 78° over P_2O_5 *in vacuo*, 0.362 g. of the material lost 0.035 g. = 9.7 %. Calc. for $C_{12}H_{22}O_{11} \cdot 2H_2O$: H_2O , 9.5 %. A solution of the anhydrous sugar in water was non-reducing; $[\alpha]_D = +198.5$ ($c = 0.327$). The recorded value for trehalose is $+197^\circ$ (Schukoff, 1900).

It is already recorded that trehalose may be obtained from the so-called *Trehala* manna, described as an excretion of a larva or beetle (Meyer & Jacobsen, 1913), or as the product of the plant *Echinops persicus* on which the beetle lives (Wehmer, Thies & Hadders, 1932):

SUMMARY

1. A manna found in the North Iraqi Desert and said to be the excretion of a scale insect contains about 7 % of trehalose.

2. A sugar syrup prepared from this manna contains as much as 60 % trehalose.

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