By washing the ether solution of benzaldehyde with 25 cc. of 0.2 N sodium hydroxide solution the quantity of benzoic acid separated is larger and the accuracy of the method increased.

Benzaldehyde.		Benzoic acid.	
Present, gram.	Found, gram.	Present, gram.	Found, gram.
0.4839	0.4835	0.5000	0.4990
0.2419	0.2423	0.2500	0.2496
0.4839	0.4835	0.2500	0.2494

WASHINGTON, D. C.

[CONTRIBUTION FROM THE CARBOHYDRATE LABORATORY, BUREAU OF CHEMISTRY, UNITED STATES DEPARTMENT OF AGRICULTURE.]

THE OCCURRENCE OF MELEZITOSE IN A MANNA FROM THE DOUGLAS FIR.

BY C. S. HUDSON AND S. F. SHERWOOD.

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At the suggestion of Miss Helene M. Boas, of the New York Botanical Garden, Bronx Park, we have recently examined a sample of manna which had been collected by Mr. James A. Teit near Spence's Bridge, British Columbia, from Douglas fir trees (*Pseudotsuga Taxifolia* [(Lamarck) Britton]). This tree is also known as the Douglas spruce and the Oregon pine. It was reported that the manna formed in large quantities on the twigs and needles of the firs during summer droughts, but it was not definitely known whether it was an exudation or was produced by aphids or other insects. The sample of manna that was received from Miss Boas consisted of several small stems and needles of the Douglas fir encrusted with a hard, white, crystalline coating. The stems and needles weighed 4 g., while the manna that surrounded them weighed 42.5 grams. The manna possessed an agreeable, mildly sweet flavor, contained very little moisture, and was not hygroscopic. On drying to constant weight at 100° it lost 2.2% water and became slightly yellow in color. The manna was soluble in cold water and only traces of insoluble matter were left behind.

In order to purify the crystalline substance of which the manna largely consisted, 32 g. of the powdered dry manna was digested with 300 cc. absolute alcohol at 25° during 48 hours. The insoluble residue was dissolved in 25 cc. water, the solution was decolorized with carbon and to it was added an equal volume of absolute alcohol. Crystallization proceeded rapidly and after 12 hours' standing in the ice box the solution yielded 15 g. of pure white crystals of the rare trisaccharide *melezitose*. From the mother liquor 1.5 g. melezitose were also obtained after concentrating to a sirup and adding an equal volume of glacial acetic acid.

The identification of the sugar as melezitose was fully established. Its melting point was 148°, agreeing with that found by Alekhine[1] (147–8°) and by Bourquelot and Herissey[2] (148°). Its specific rotation in water was +88.8° for the anhydrous sugar, in comparison with +88.1° found by Alekhine, +88.5° by Villiers,[3] and +88.8° by Maquenne.[4] The acetyl derivative of the sugar from the manna, prepared by the use of acetic anhydride and sodium acetate, crystallized readily from alcoholic solution on the addition of water. The crystals melted at 117° and showed a specific rotation of +110.8° in benzene solution. These values agree with Alekhine's measurements (117° and +110.4°) on melezitose hendecacetate. We find that the acetate has in chloroform solution the specific dextro-rotation $[\alpha]_D^{20} = +103.6°$ in 1 per cent. solution. Tanret has found that the hydrolysis of melezitose to turanose and glucose

$$\underset{\text{Melezitose.}}{C_{18}H_{32}O_{16}} + H_2O = \underset{\text{Turanose.}}{C_{12}H_{22}O_{11}} + \underset{\text{Glucose.}}{C_6H_{12}O_6}$$

is complete after 2 hours' boiling with 20% acetic acid and that the specific rotation falls during this reaction to +65.8°. The corresponding values which we have found on several occasions for the sugar from the Douglas fir manna are +61.8, 63.4, 63.6 and 63.6°. After hydrolysis the sugar reduced Fehling's solution strongly, but not at all before.

It has been fully established by G. Tanret[5] that turanose yields glucose and fructose on hydrolysis. He has shown that the failure of earlier investigators to obtain any monosaccharide other than glucose is due to the fact that turanose is hydrolyzed with such difficulty that much of the liberated fructose is destroyed by the strong mineral acid solutions that must be used. Tanret mentions that melezitose gives the Seliwanoff reaction for fructose. We have found that it also gives the color reaction with α-naphthol which Pinoff[6] has shown to be characteristic of ketoses in distinction from aldoses. In addition to the ketoses, fructose and sorbose, that Pinoff examined, we have tested manno-keto-heptose[7] and sedoheptose[8] and find that both these ketoses give Pinoff's α-naphthol test.

The portion of the dried manna (32 g.) that dissolved in absolute alcohol at 25° weighed 4.2 g. and thus constituted 13% of the dry manna. It consisted principally of reducing sugars but also contained some compound sugar that is hydrolyzed by invertase, probably sucrose, because a Clerget estimation with top yeast invertase as the hydrolyzing agent

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[1] *Ann. chim. phys.*, [6] **18,** 532 (1889).

[2] *J. Pharm. chim.*, [6] **4,** 385 (1896).

[3] *Compt. rend.*, **84,** 35 (1877).

[4] *Ibid.*, **117,** 127 (1893).

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

[6] *Ber.*, **38,** 3308 (1905).

[7] LaForge, *J. Biol. Chem.*, **28,** 511 (1917).

[8] LaForge and Hudson, *Ibid.*. **30,** 61 (1917).

showed 0.94 g. sucrose in the 4.2 g. residue, which corresponds to 2.9% of the manna. The invertase solution was without any hydrolyzing action upon a solution of pure melezitose. The dry manna reduces Fehling's solution to an extent that corresponds to 11.5% glucose. The sum of this reducing sugar and the sucrose is 14.4%, which checks fairly well with the 13.0% of alcohol-soluble material in the manna. An aqueous solution of the 4.2 g. residue, made up to 50 cc., rotated to the right 17.75° Ventzke in a 200 mm. tube, and after hydrolysis with invertase rotated +8.40° at the same concentration. As mentioned, this change in rotation is interpreted as indicating the presence of 0.94 g. sucrose in the residue, or 2.9% in the manna. Since a solution of 0.94 g. sucrose in 50 cc. shows a reading of +7.23°, the difference 17.75° — 7.23° = 10.52°, may be ascribed to the 4.2 — 0.94 = 3.3 g. portion of the residue. This reading corresponds to a specific rotation of about +26° for the 3.3 g. substance. Probably the material is a mixture of considerable glucose with some fructose.

The proportion of melezitose in the manna is a large one. Over 50% was obtained as purified crystals. The following observations and calculations indicate that the percentage in the manna is about 75 to 83%. An aqueous solution of the manna, on the basis of 26 g. dry manna in 100 cc. solution rotated in a 2 dcm. tube +107.9° Ventzke, and after hydrolysis by the Clerget acid method rotated +72.8, all readings at 22°. Since the alcohol-soluble material of the manna amounts to 13% and has the rotation that has been recorded (+17.75°), one calculates that +7.2° of the reading 107.9° is due to the glucose, fructose and sucrose that make up the alcohol-soluble material of the manna. If it is assumed that the large difference, 107.9 — 7.2 = 100.7°, is due entirely to melezitose the percentage of this sugar in the manna is 75%. This calculation can be controlled by the data on the change in rotation after acid hydrolysis. It has been mentioned that this change is 107.9 — 72.8 = 35.1°. Since it has been shown by the invertase hydrolysis that the manna contains 2.9% sucrose, the change in reading that is due to the hydrolysis of this sugar may be calculated to be 3.5°. If the difference, 35.1 — 3.5 = 31.6°, is attributed to the hydrolysis of melezitose, the specific rotation of which is known to change by the Clerget acid method from +88.8 to about +63.5, the percentage of melezitose in the manna is 82%.[1] An estimation of the proportion of melezitose in the manna can also be obtained from reducing sugar determinations. Before hy-

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[1] The method of calculation is as follows: If the manna were 100% melezitose, the Ventzke readings before and after acid hydrolysis of the sugar would be 134° and 95.5°, respectively, for 26 g. substance in 100 cc. solution, read in a 2 dcm. tube. These values are obtained from the recorded specific rotations 88.8° and 63.5°. Since the difference, 134 — 95.5 = 38.5°, would indicate 100% melezitose, the observed corrected value 31.6° corresponding to (31.6/38.5)100 = 82% melezitose in the manna.

drolysis the manna showed 11.5% reducing sugars by Fehling's solution, calculated as glucose. After acid hydrolysis by the Clerget method at 68–9° the reducing sugars were 71.9%, calculated as glucose. Since the manna is known to contain 2.9% sucrose, which is equivalent after inversion to 2.9% glucose, the increase in reducing sugars that is not due to sucrose is $(71.9 - 11.5) - 2.9 = 57.5\%$. Assuming that this quantity is due to the hydrolysis of melezitose, 100 g. of which we find to be equivalent in reducing power to 69 g. of glucose, the percentage of melezitose is $(57.5 \div 69)100 = 83\%$. The two determinations which depend upon the hydrolysis of the melezitose and the measurements of the resulting change in rotation and the increase in reducing power, respectively, agree closely (82 and 83%); from theoretical grounds they appear more reliable than the method of estimation by which the value 75% was found.

Summarizing, the sample of Douglas fir manna yielded about 50% of pure crystalline melezitose, and there is evidence that the manna contains sucrose and some reducing sugar, probably a mixture of glucose with a smaller quantity of fructose. The percentage composition of the sample of dry manna that we examined was approximately:

Melezitose	75 to 83%
Sucrose	2.9%
Reducing sugars	11.5%

Isolation of Sucrose, Melezitose and Mannite from Turkestan Manna (Tarandjabine).—A sample of tarandjabine, which is a manna that forms on a shrub or small tree (Alhagi camelorum) in Palestine, Arabia, Persia and Turkestan,[1] was obtained through the aid of the U. S. Consul General at Teheran, Persia. It has been recorded[2] that sucrose can be crystallized from tarandjabine and the occurrence of melezitose in it has been shown by Alekhine, who recommends it as the best source for this trisaccharide. We have examined tarandjabine in order to compare its value as a source for melezitose with the manna from the Douglas fir. Plant stems, sand and other insoluble material made up 27% of the tarandjabine. Ten g. of the manna were extracted with water, the extract evaporated to dryness, the residue (7.3 g.) dissolved in a few drops of water to a thick sirup, an equal volume of glacial acetic acid stirred in, and the solution seeded with sucrose and kept two weeks during a slow crystallization. The crystals were purified by two recrystallizations. They weighed 0.69 g. and were pure sucrose, of specific rotation + 65°. But we are unable to state that sucrose is a natural ingredient of the tarandjabine manna, as it may have been added to it.

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[1] Alekhine (*Ann. chim. phys.*, **6**, 18, 532 (1889)) has recorded the best description of this manna and its mode of occurrence.

[2] Villiers, *Compt. rend.*, **84**, 35 (1877).

Melezitose was crystallized from tarandjabine after removing the sucrose by fermentation. An aqueous extract of 50 g. of the manna was fermented with baker's yeast, the solution was then clarified with basic lead acetate and the excess of lead was removed as sulfide. Most of the acetic acid was removed by 5 extractions with ether and the solution was then boiled *in vacuo* to a sirup. On seeding it with crystals of melezitose it rapidly formed a solid mass, and from it 3.1 g. of pure melezitose, of specific rotation +89°, were obtained. It is possible that the small yield may have been due to a partial fermentation of the melezitose.

Mannitol was isolated from 10 g. of the tarandjabine, after some sucrose had been crystallized from it, by very thoroughly fermenting the mother liquor with baker's yeast. Under the conditions employed the melezitose seems also to have been fermented. The concentrated liquid yielded 0.91 g. crystalline mannitol. It melted at 165–7° and this value was not changed by the addition of an equal quantity of pure mannitol. The crystals did not reduce Fehling's solution. On acetylation they yielded mannitol hexacetate of m. p. 120° and $[\alpha]_D^{20} = +26°$ in chloroform solution. For mannitol hexacetate prepared from *d*-mannite we found m. p. 120° and $[\alpha]_D^{20} = +26°$.

At the present time only two natural sources of melezitose yield enough of this sugar and occur in large enough quantities to be of importance as raw materials for its preparation, namely, tarandjabine and the Douglas fir manna. Alekhine obtained as high as 38% melezitose from tarandjabine and G. Tanret obtained 20 to 25%; these quantities are considerably lower than the 50% yield that we have crystallized from the Douglas fir manna. If the latter can be obtained in large quantity, which appears to be the case, it will indeed furnish an excellent source for melezitose.

In relation to the occurrence of this manna on the Douglas fir it seems reasonable to suppose that bees may collect it and store it in the form of honey-dew honey. We are not aware that it is known whether bees hydrolyze melezitose, but in case they do not it is to be expected that melezitose may occur in some honey-dew honeys. We have recently had this expectation strikingly verified. Four samples of comb honey, which were made by the bees during July, 1917, in Central Pennsylvania, were almost solid from the crystallization of melezitose. The bee-keepers reported that the bees collected this honey from the pine trees along the mountain sides and they considered the honey to be of honey-dew honey type. We expect to report further upon this honey in a subsequent article.

WASHINGTON, D. C.