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Note

The isolation of volemitol and other polyhydric alcohols from avocado seeds

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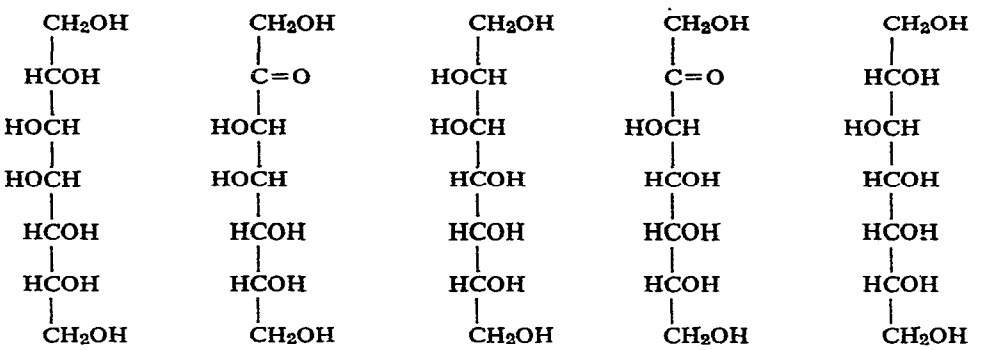
Perseitol (D-*glycero*-D-*galacto*-heptitol, **1**) was first isolated from the avocado by Avequin[1] in 1831. The closely related D-*manno*-heptulose (**2**) was discovered in the avocado by LaForge[2] in 1917. Sedoheptulose (D-*altro*-heptulose, **4**) was first isolated from *Sedum spectabile* Bor. by LaForge and Hudson[3] in 1917, and the closely related *β*-sedoheptitol (D-*glycero*-D-*gluco*-heptitol, **5**) was found in *Sedum* species by Charlson and Richtmyer[4] in 1960. Compounds **1** and **2** are related by oxidation



Perseitol | D-*manno*-Heptulose | Volemitol | Sedoheptulose | *β*-Sedoheptitol

1 | **2** | **3** | **4** | **5**

and reduction, as are compounds **5** and **4** also. The fifth compound whose formula is shown is volemitol (D-*glycero*-D-*manno*-heptitol, **3**); it was discovered in the mushroom *Lactarius volemus* Fr. by Bourquelot[5] in 1889. It is related, similarly, to both D-*manno*-heptulose (**2**) and sedoheptulose (**4**). Nordal and Öiseth[6] isolated both volemitol (**3**) and sedoheptulose (**4**) from *Primula elatior* (L.) Hill, and obtained paper-chromatographic evidence for the probable presence of D-*manno*-heptulose (**2**) also. Begbie and Richtmyer[7], in 1966, described a large-scale study of *Primula officinalis* Jacq. in which they isolated not only D-*manno*-heptulose (**2**) and sedoheptulose (**4**) but also volemitol (**3**) and *β*-sedoheptitol (**5**).

The present paper reports the isolation of volemitol (3) from the avocado, where it occurs together with perseitol (1) and D-*manno*-heptulose (2). Glycerol, D-arabinitol, galactitol, *myo*-inositol, and D-*erythro*-D-*galacto*-octitol[4] were also isolated during the course of the investigation. According to two recent reviews[8], D-arabinitol is known to occur in fungi and lichens, but this seems to be the first time that it has been isolated from a higher plant.

EXPERIMENTAL

General. — Paper chromatography was performed on Whatman No. 1 filter paper by the descending method at room temperature. The following solvent systems were used: A, ethyl acetate–acetic acid–formic acid–water (18:3:1:4); B, butyl alcohol–ethyl alcohol–water (40:11:19); and C, butyl alcohol–pyridine–water (6:4:3). The spray reagents used were aniline hydrogen phthalate for aldoses, orcinol–hydrochloric acid for ketoses, and ammoniacal silver nitrate for alditols and other polyhydroxy compounds in general.

Isolation of polyhydric alcohols from avocado seeds. — The seeds from four hundred ripe avocados (Californian Calavo, Hass variety[9]) were crushed in a meat grinder and stored for several months at −5°. The meal (22,650 g) was then extracted twice with hot water, and the extract was deproteinized by the addition of aqueous zinc sulfate solution followed by saturated aqueous barium hydroxide solution until the solution was neutral to phenolphthalein. The precipitate was removed, and the filtrate was deionized by passage through columns of Amberlite IR-120 and Duolite A-4 ion-exchange resins. The eluate was concentrated *in vacuo* until considerable crystallization occurred, and the product was then filtered off and washed, first with 1:1 water–methanol and then with methanol. The air-dried perseitol (1) weighed 217 g; it melted at 187–188°, in agreement with the value reported by Hann and Hudson[10] for pure perseitol. Concentration of the mother liquor yielded 80 g of perseitol, m.p. 186–188°. Paper-chromatographic examination of the resulting mother liquor indicated the probable presence of D-fructose, D-glucose, and D-*manno*-heptulose, together with very small proportions of an aldopentose, a second aldohexose, and a second heptulose, besides several polyhydric alcohols. After concentration of the solution to remove methanol, the reducing sugars were decomposed by heating the aqueous solution in an open, stainless-steel container with an excess of barium hydroxide for about 20 h. The excess of alkali was then neutralized with carbon dioxide, and the mixture was filtered, deionized with Amberlite IR-120 and Duolite A-4 ion-exchange resins, and concentrated *in vacuo*; 14 g of perseitol, m.p. 185–187°, was obtained by crystallization. The mother liquor, when concentrated and examined by paper chromatography, appeared to contain lactones (revealed through the use of alkaline hydroxylamine followed by acid ferric chloride spray reagents). The solution was, therefore, passed slowly through a column of the strongly basic resin Amberlite IRA-400. Paper-chromatographic examination of the concentrated eluate showed that it contained a large proportion of perseitol and at least five other components having greater mobilities.

Separation of the polyhydric alcohols on cellulose columns. — One third (8.4 g) of the mixture of polyhydric alcohols thus obtained was mixed with powdered cellulose, and transferred to the top of a column (34 × 3.7 cm) containing washed cellulose powder (Whatman). Elution was begun with quarter-saturated aqueous butyl alcohol and, with the aid of an automatic fraction-collector, 14-ml fractions were collected as soon as a sample of the eluate left a residue when the solvent was evaporated. Thus, fractions 1–50 yielded 2.7 g of a syrup having the same mobility as glycerol on a paper chromatogram. Complete identification as **glycerol** was established by converting a portion of it into its tris-*p*-nitrobenzoate, m.p. 192–193° alone and when mixed with authentic ester[11].

Fractions 126–175 (0.168 g) yielded crystals (29 mg) having m.p. 99–102°, raised to 101–102° by recrystallization from absolute ethyl alcohol. The position of this compound on paper chromatograms suggested that it was a pentitol; in solvents A and B, it was indistinguishable from ribitol and D- and L-arabinitol (all of which melt at 102°); however, in solvent C, it migrated at the same rate as the arabinitols but more slowly than ribitol. Its i.r. spectrum (Nujol mull) agreed with that of the arabinitols. It was identified as **D-arabinitol** through mixed melting points: undepressed when the compound was mixed with authentic material, but depressed when it was mixed with either ribitol or L-arabinitol.

Fractions 301–625 (0.231 g) yielded 75 mg of crystalline material, m.p. 125–130°. Upon recrystallization from 30 ml of methanol, clusters of small, heavy prisms separated, together with a few needle-like crystals that were removed by decantation. The prisms melted at 180–188°; a second recrystallization from methanol gave prisms (22 mg) of a compound identified as **galactitol** because it had m.p. and mixed m.p. of 187–188°, and i.r. spectrum (Nujol mull) and paper-chromatographic behavior (solvents A and B) identical with those of authentic material.

The rest of the mixture of polyhydric alcohols (16.8 g) was fractionated similarly, and some of the resulting fractions, together with the corresponding ones from the first fractionation, were combined into (*a*) a "hexitol–volemitol" fraction and (*b*) a "perseitol–octitol" fraction for further examination.

The "hexitol–volemitol" fraction (0.35 g) was transferred to a column of cellulose, and at first eluted with eighth-saturated aqueous butyl alcohol, and later with quarter-saturated aqueous butyl alcohol, 16-ml fractions being collected. The first portions of eluate (fractions 301–750) contained galactitol and a small amount of a second substance (m.p. 137–138°) that was not identified. Fractions 1201–1825 (67 mg of syrup) were, from paper-chromatographic comparisons, believed to contain **volemitol** (3) and, on inoculation with that compound, yielded a product that, after recrystallization from methanol, melted at 153–154°, alone, and when mixed with authentic volemitol. Its identity was verified by its i.r. spectrum (Nujol mull).

The "perseitol–octitol" fraction (5.9 g) was refractionated on a larger cellulose column (83 × 5 cm) by elution with half-saturated aqueous butyl alcohol, the eluate being collected in 16-ml fractions. The first fractions, which appeared to contain perseitol only, were discarded; the middle fractions (1251–2375) contained perseitol

and an octitol; and the final fractions, obtained by elution with methanol, yielded 0.4 g of the readily identified *myo*-inositol, having m.p. and mixed m.p. 224–225°.

Fractions 1251–2375 (1.7 g) were refractionated on the larger cellulose column in the same way. The new fractions 1251–1500 (0.68 g) were combined, treated with Darco X decolorizing carbon, and evaporated to give 0.68 g of solid residue. Recrystallization from aqueous methanol afforded 0.49 g of clusters of small, prismatic needles having m.p. 167–168°; the mother liquor was combined with fractions 1501–1850 (0.12 g) to give an additional 0.15 g of a compound having m.p. 166–168°. This compound was identified as the anhydrous modification of **D-*erythro*-D-*galacto*-octitol**[4] by mixed m.p., i.r. spectrum, and the fact that it lost no weight when heated at 100° overnight in a high vacuum.

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REFERENCES

1 J. B. AVEQUIN, *J. Chim. Méd. Pharm. Toxicol.*, [1] 7 (1831) 467; see also, R. M. HANN AND C. S. HUDSON, *J. Amer. Chem. Soc.*, 61 (1939) 336.
2 F. B. LAFORGE, *J. Biol. Chem.*, 28 (1917) 511.
3 F. B. LAFORGE AND C. S. HUDSON, *J. Biol. Chem.*, 30 (1917) 61.
4 A. J. CHARLSON AND N. K. RICHTMYER, *J. Amer. Chem. Soc.*, 82 (1960) 3428.
5 E. BOURQUELOT, *Bull. Soc. Mycol. Fr.*, 5 (1889) 132.
6 A. NORDAL AND D. ÖISETH, *Acta Chem. Scand.*, 5 (1951) 1289.
7 R. BEGBIE AND N. K. RICHTMYER, *Carbohyd. Res.*, 2 (1966) 272.
8 V. PLOUVIER, *Bull. Soc. Chim. Biol.*, 45 (1963) 1079; D. H. LEWIS AND D. C. SMITH, *New Phytologist*, 66 (1967) 143.
9 H. H. SEPHTON AND N. K. RICHTMYER, *J. Org. Chem.*, 28 (1963) 1691.
10 R. M. HANN AND C. S. HUDSON, *J. Amer. Chem. Soc.*, 61 (1939) 336.
11 J. U. NEF, *Ann.*, 335 (1904) 284.