Isolation of D-*erythro*-L-*gluco*-Nonulose from the Avocado[1]

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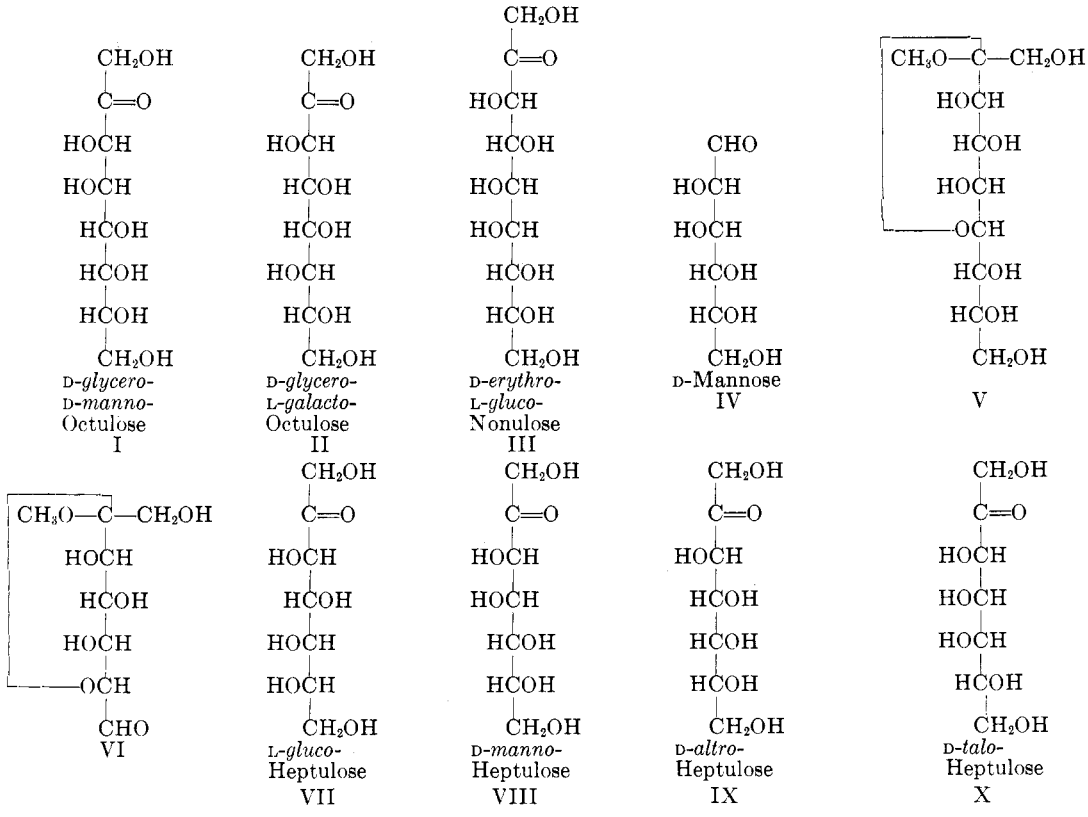
The first nonulose to be found in nature has been isolated from the avocado. Its structure has been established as D-*erythro*-L-*gluco*-nonulose by degradation to D-mannose (asymmetric carbon atoms 5–8 of the nonulose) and to L-*gluco*-heptulose (asymmetric carbon atoms 3–6 of the nonulose), as well as by comparison with the synthetic product (Wolfrom and Wood, 1955).

The first naturally occurring octulose to be isolated and identified was D-*glycero*-D-*manno*-octulose (I), and it was found both in the avocado (Calavo, Fuerte variety) and in *Sedum* species.[3] Continuing these studies we described in the preceding paper[4] the isolation also of a second octulose, namely, D-*glycero*-L-*galacto*-octulose (II), from the avocado (Calavo, Hass variety). In addition to these two octuloses, paper chromatography indicated the presence of two still slower moving components that we believed to be nonuloses. The isolation and identification of one of these, D-*erythro*-L-*gluco*-nonulose (III), is forthwith described.



The structure of the sirupy nonulose was proved as follows. Upon degradation with two molecular equivalents of lead tetraacetate according to the method of Perlin and Brice,[5] it yielded mainly D-mannose (IV), which was identified through its crystalline phenylhydrazone; this established the configuration of asymmetric carbon atoms 5, 6, 7, and 8. To establish the configuration of asymmetric carbon atoms 3, 4, 5, and 6 we employed periodate oxidation as used by Jones and Sephton.[6] In this sequence of reactions, the nonulose was converted to a methyl nonulopyranoside (or a mixture of the anomeric nonulopyranosides of which V would be one isomer). Upon treatment of this product with two molecular equivalents of sodium metaperiodate, oxidation occurred preferentially with the *exo*-cyclic glycol groups (C-7–C-9) rather than with those having the all-*trans* configuration inside the glycoside ring (C-3–C-5).[7] Besides formaldehyde and formic acid, a methyl heptosuloside (such as VI) was formed, and subsequent reduction of the aldehyde group with sodium borohydride yielded a methyl heptuloside. Acid hydrolysis then liberated L-*gluco*-heptulose (VII), which was obtained in crystalline form and identified by comparison with an authentic sample.[8]

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(3) (a) A. J. Charlson and N. K. Richtmyer, *J. Am. Chem. Soc.*, **81**, 1512 (1959); (b) **82**, 3428 (1960).

(4) H. H. Sephton and N. K. Richtmyer, *J. Org. Chem.*, **28**, 1691 (1963).

(5) A. S. Perlin and C. Brice, *Can. J. Chem.*, **34**, 541 (1956).

(6) J. K. N. Jones and H. H. Sephton, *ibid.*, **38**, 753 (1960); see also N. J. Antia and M. B. Perry, *ibid.*, **38**, 1917 (1960).

(7) *Cf.* O. Kjølberg, *Acta Chem. Scand.*, **14**, 1118 (1960).

(8) W. D. Maclay, R. M. Hann, and C. S. Hudson, *J. Am. Chem. Soc.*, **64**, 1606 (1942).

Finally, the identity of the first avocado nonulose with synthetic D-*erythro*-L-*gluco*-nonulose, prepared according to Wolfrom and Wood,[9] was confirmed by paper chromatography, by infrared spectra, and by melting points and infrared spectra of the 2,5-dichlorophenylosazones.

In regard to the biosynthesis of the six higher carbon ketoses so far identified in nature, we know only that the three heptuloses [D-*manno*-heptulose (VIII), D-*altro*-heptulose (sedoheptulose, IX), and the recently discovered D-*talo*-heptulose (X)[3b]], the two octuloses (I and II), and the first avocado nonulose (III) all belong to the D series and all have the hydroxyl group at C-3 on the left when written in the Fischer projection formula. On the other hand, extensive studies, especially by Jones and his collaborators,[6,10] have shown that the aldolase-catalyzed synthesis of sugars from an aldehyde and dihydroxyacetone phosphate (1,3-dihydroxy-2-propanone phosphate) leads to the production of ketoses with the D-*threo* configuration at C-3 and C-4; that is, not only do they all have the hydroxyl group at C-3 on the left, but they also have the hydroxyl group at C-4 on the right.[11] Since the latter situation exists for only three of the six ketoses, it would appear that another enzyme or other enzymes also are involved in the production of the sugars that do not possess the D-*threo* configuration.

It may be mentioned that, from paper chromatographic evidence alone, *Sedum* appears to contain the same two octuloses and the same two nonuloses as the avocado. The separation and identification of the *Sedum* octuloses and nonuloses are in progress.

Experimental

Paper chromatography was carried out 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, 1-butanol–ethanol–water (40:11:19); C, 1-butanol–pyridine–water (6:4:3); and D, ethyl acetate–pyridine–water saturated with boric acid (12:5:4). Spray reagents used were aniline hydrogen phthalate for aldoses, orcinol–hydrochloric acid for ketoses, and silver nitrate (ammoniacal, or in conjunction with sodium hydroxide in ethanol) for alditols, sugars, and other polyhydroxy substances in general. With the orcinol–hydrochloric acid spray and heating at 100–110°, heptuloses give a pinkish orange color changing to blue (or greenish blue with *manno*-heptulose), octuloses give a pink to red color changing to a brownish gray, and nonuloses give the same pink to red changing to a greenish gray. The octulose and nonulose spots fluoresce bluish white under the ultraviolet light. All concentrations were carried out *in vacuo* at temperatures not over 50°; the final drying of sirups was completed in evacuated desiccators over granular calcium chloride. Melting points were determined on a Kofler micro hot stage.

Isolation of D-*erythro*-L-*gluco*-Nonulose (III) from the Avocado.—Sirupy fraction E (0.35 g.), containing principally the first nonulose isolated from the avocado as described in the preceding paper,[4] was dissolved in a small volume of water and the turbidity removed by filtration through a decolorizing carbon (Darco X). The filtrate was concentrated to a dry sirup that showed $[\alpha]^{20}D$ −30.5° in water (*c* 1.9). This material was rechromatographed on a cellulose column (100 cm. × 1 cm.) by elution with aqueous 1-butanol and the best fraction was considered to be the one with $[\alpha]^{20}D$ −40.0° in water (*c* 0.6). Paper chromatography of the nonulose was carried out with solvents A, B, and C; the chromatograms, sprayed with orcinol–hydrochloric acid, showed the presence of only one ketose, but when solvent D was used small quantities of a heptose (probably D-*glycero*-D-*galacto*-heptose[4]) and of a third, nonreducing compound were detected with the aniline hydrogen phthalate and silver nitrate reagents, respectively.

Degradation of D-*erythro*-L-*gluco*-Nonulose (III) to D-Mannose (IV) with Lead Tetraacetate.—A 38-mg. portion of the nonulose in 40 ml. of glacial acetic acid was treated with 7.8 ml. of 0.04 *M* lead tetraacetate in glacial acetic acid (2.2 molecular equivalents) for 15 min. at room temperature. The lead ions were precipitated by adding a slight excess of a 10% solution of oxalic acid in acetic acid, and the lead oxalate was removed by filtration. The filtrate was concentrated to a sirup that was heated with 0.1 *N* sulfuric acid for 5 hr. on the steam bath to hydrolyze formyl and glycolyl esters. The hydrolysate was deacidified with Duolite A-4 ion-exchange resin and concentrated to a sirup. The sirup was dissolved in methanol, clarified by filtration through Darco X, and concentrated to 30 mg. of dry sirup. Paper chromatograms showed principally mannose, with smaller proportions of a heptose, arabinose, and xylose (derived presumably from some unknown impurity). The sirup was fractionated on Whatman no. 3 MM paper in solvent A, and the area corresponding to the mannose cut out and extracted with methanol. The extract, after filtration through Darco X and concentration, yielded 10 mg. of sirup with $[\alpha]^{20}D$ +16° in water (*c* 1; $[\alpha]^{20}D$ +14° for known D-mannose).

For further identification, 8 mg. of the sirupy D-mannose was dissolved in 0.16 ml. of water and to it was added a mixture containing 0.12 ml. of phenylhydrazine, 0.12 ml. of glacial acetic acid, and 0.36 ml. of water. D-Mannose phenylhydrazone crystallized on standing overnight in the refrigerator and, after being filtered, washed successively with water, ethanol, and ether, and dried, it weighed 12 mg. (quantitative) and had m.p. and m.m.p. 196–198°; melting point was depressed 6° when the product was mixed with the L-isomer. The infrared spectrum (Nujol mull) of the phenylhydrazone derived from the nonulose degradation product had absorption peaks of the same wave lengths and of similar intensities as that of authentic D-mannose phenylhydrazone.

The Preparation of Methyl D-*erythro*-L-*gluco*-Nonuloside (V) and Its Degradation to L-*gluco*-Heptulose (VII).—A solution of 19.7 mg. of sirupy nonulose (III) in 5 ml. of 5% methanolic hydrogen chloride was refluxed for 5 hr., cooled, and adjusted to pH 5 by the addition of methanolic potassium hydroxide. The precipitated potassium chloride was removed by filtration and the filtrate concentrated at room temperature. The sirupy residue was dissolved in water and to it was added a solution of 40 mg. of sodium metaperiodate (2.5 molecular equivalents) in 5 ml. of water. After 15 min. at room temperature, 100 mg. of sodium borohydride was added and the solution left for 4.5 hr. to effect reduction of the aldehydo groups formed in the periodate oxidation. Cations were removed with Amberlite IR-120 ion-exchange resin and the acidic solution was heated on the steam bath for 2.5 hr. to hydrolyze methyl glycosides. The hydrolysate was concentrated to a sirup that was freed of boric acid by repeatedly dissolving it in methanol and concentrating. The final residue was deionized completely with Amberlite IR-120 and Duolite A-4 resins and concentrated to a sirup. Paper chromatography carried out with solvents A, B, and C indicated that the product consisted mainly of a heptulose indistinguishable from *gluco*-heptulose; there was also a small amount of nonulose. The heptulose was purified by paper chromatography on Whatman no. 1 paper in solvent A and extraction of the appropriate area with methanol. The extract was filtered through Darco X and concentrated to 5 mg. of sirup. This was dissolved in a few drops of aqueous ethanol and nucleated with L-*gluco*-heptulose. When the crystalline product was separated from its mother liquor, washed, and dried, it melted at 166–169° alone, and at 167–170° when mixed with authentic L-*gluco*-heptulose.[8]

Synthetic D-*erythro*-L-*gluco*-Nonulose (III).—A sample of 1-deoxy-1-diazo-*keto*-D-*erythro*-L-*gluco*-nonulose heptaacetate, kindly supplied by Dr. Harry B. Wood, Jr., was recrystallized twice from benzene–petroleum ether (90–100°); m.p. 75–78°. In a

(9) M. L. Wolfrom and H. B. Wood, Jr., *J. Am. Chem. Soc.*, **77**, 3096 (1955).

(10) For a review, see L. Hough and J. K. N. Jones, *Advan. Carbohydrate Chem.*, **11**, 185 (1956); also, J. K. N. Jones and N. K. Matheson, *Can. J. Chem.*, **37**, 1754 (1959), and earlier papers.

(11) V. V. Rendig and E. A. McComb [*Arch. Biochem. Biophys.*, **97**, 562 (1962); **99**, 409 (1962)] have shown recently that the feeding of L-arabinose to plants results in the synthesis and accumulation of L-*gluco*-heptulose (VII) in the plant leaf tissue. Similarly, D-ribose produces D-*altro*-heptulose (IX), D-xylose produces D-*ido*-heptulose, and L-lyxose produces L-*galacto*-heptulose. All four heptuloses have the D-*threo* configuration at C-3 and C-4.

simplified variation of the procedure used by Wolfrom and Wood,[9] 5.5 g. of the recrystallized material was dissolved in 65 ml. of glacial acetic acid containing a trace each of copper powder and cupric acetate, as catalysts. As the solution was heated to just below its boiling point, a sudden and copious evolution of nitrogen occurred; after this had subsided, the solution was refluxed for 5 min., cooled, and concentrated to a sirup that contained, presumably, principally *keto*-D-*erythro*-L-*gluco*-nonulose heptaacetate. The sirupy product was cooled to 0° and deacetylated by adding a solution of 18.5 g. of barium hydroxide octahydrate in 110 ml. of water also at 0°, and stirring for 0.5 hr. to dissolve the sirup and then 3 hr. more at 0°. Barium ions were precipitated by the addition of oxalic acid, the barium oxalate was removed by filtration through Celite, and the filtrate was deionized completely by passing it through columns of Dowex 50 and Duolite A-4 ion-exchange resins. The eluate was concentrated to a sirup that was dissolved in 10 ml. of methanol and the nonulose precipitated by the addition of ethanol. After the precipitate had been freed from solvents *in vacuo* the nonulose remained as a dry, white, amorphous, hygroscopic powder weighing 2.17 g. and showing $[\alpha]^{20}D$ −47.2° in water (*c* 18); Wolfrom and Wood[9] reported $[\alpha]^{23}D$ also −47.2° in water (*c* 1.24). Paper chromatography carried out with solvents A, B, C, and D for periods of 4, 6, 5, and 1 days, respectively, failed to show any difference between the mobilities of the synthetic and the first avocado nonulose. The infrared spectra of the two nonuloses, as films from methanol on sodium chloride plates, had absorption bands at the same wave length and gave a further indication of the identity of the two sugars.

Preparation and Comparison of the D-*erythro*-L-*gluco*-Nonose 2,5-Dichlorophenylosazones from the First Avocado Nonulose and from the Synthetic Nonulose.—A 60-mg. sample of the avocado nonulose was refluxed with 250 mg. of 2,5-dichlorophenylhydrazine in 5 ml. of absolute ethanol containing 0.5 ml. of glacial acetic acid for 15 hr. on the steam bath. The osazone crystallized in clusters of fine, yellow needles from the boiling solution and, after being cooled, filtered, washed with ethanol and methanol, and dried, it weighed 58 mg.; m.p. 244–246° dec. The product appeared to be only sparingly soluble in the usual organic solvents and so was not recrystallized.

The 2,5-dichlorophenylosazone of the synthetic nonulose was obtained in the same manner; m.p. 248–250° dec. A mixture of the two osazones melted at 244–248° dec. The infrared spectra of the two osazones in Nujol mull also confirmed their identity.

Anal. Calcd. for $C_{21}H_{24}Cl_4N_4O_7$: C, 43.02; H, 4.13; Cl, 24.19; N, 9.56. Found (found avocado nonulose): C, 43.41; H, 4.40; Cl, 24.80; N, 9.50. Found (from synthetic nonulose): C, 43.29; H, 4.88; Cl, 23.57; N, 9.36.

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Terpenoids. LIII.[1a] Demonstration of Ring Conformational Changes in Triterpenes of the β-Amyrin Class Isolated from *Stryphnodendron coriaceum*

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Acid hydrolysis of the saponins from *S. coriaceum* leads to a series of novel triterpenoid sapogenins, two of them—named *Stryphnodendron* sapogenins B and F—having been obtained in pure form. Mass spectrometry pointed towards an amyrin skeleton and chemical conversion demonstrated that B is the lactone of the cactus triterpene machaerinic acid, F being 2α-hydroxy-B. In contrast to all of the other known members of the α- or β-amyrin series, sapogenins B and F are readily hydrogenated, this unprecedented behavior being rationalized by the conformational alteration of rings D and E associated with lactone formation. In agreement with this conclusion is the observation that opening of the lactone ring restores the "nonreducibility" of the 12–13 double bond. Caution has to be exercised, therefore, in utilizing this criterion for excluding membership of an unknown triterpene in the α- or β-amyrin class.

Considerable mortality of cattle is registered during droughts in some parts of Brazil's Northeast, following ingestion of the bean pods of the plant *Stryphnodendron coriaceum* Benth. (family *Leguminosae Mimosacaea*). The hepatic and renal parenchyma of the animals are attacked and severe photosensitized lesions of the skin appear.[1b] These facts prompted chemical investigation of the plant at the Instituto de Quimica Agricola in Rio de Janeiro.[1b]

Extraction of the dried bean pods of *Stryphnodendron coriaceum* with ethanol afforded a considerable quantity of saponins. In this work no attempt was made towards their separation. The crude product was hydrolyzed and afforded a complex mixture of polyoxygenated triterpenic sapogenins. Separation of the individual sapogenins was effected by column chromatography and the compounds named A, B, C, etc.,—in the order of their elution.

Sapogenin B, m.p. 240–243°, $[\alpha]D$ −16°, had the empirical formula $C_{30}H_{46}O_3$, confirmed by mass spectrometry. The infrared spectrum showed a hydroxyl band at 3400 cm.$^{-1}$ and a band at 1776 cm.$^{-1}$, attributable to a γ-lactone. The nature of the three oxygen atoms was thus accounted for. The 60-Mc. n.m.r. spectrum of B showed signals corresponding to one vinyl proton at 5.55 δ (complex structure), one proton at 4.12 δ appearing as a neat doublet with J = 6 c.p.s. (attributable to the CH–O–CO lactonic proton), 9 protons at 1.02 δ (3 methyl groups), and 12 protons at 1.10, 0.90, 0.82 and 0.75 δ (4 methyl groups appearing as singlets). The mass spectrum, besides the molecular ion, showed prominent peaks at m/e 201 and 246.

The secondary nature of the hydroxyl group was readily confirmed by oxidation with Jones' reagent[2] to B ketone (VII), $C_{30}H_{44}O_3$, while the presence of the lactone was established by hydrolysis of B to B acid (III), $C_{30}H_{48}O_4$. The acid III was esterified with diazomethane, giving B methyl ester (IV), $C_{31}H_{50}O_4$, which in turn was diacetylated to diacetoxy B methyl ester (V), $C_{35}H_{54}O_6$; furthermore, IV also could be

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(2) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).