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THE ISOLATION OF SOME HEPTOSES, HEPTULOSES, OCTULOSES, AND NONULOSES FROM *PRIMULA OFFICINALIS* JACQ*

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When Nordal and Öiseth[1] examined an aqueous extract of the dried roots of *Primula elatior* (L.) Hill, they established the presence of sedoheptulose (I; D-*altro*-heptulose) by isolating the crystalline di-*O*-benzylidene acetal of sedoheptulosan (2,7-anhydro-β-D-*altro*-heptulopyranose). This was the first reported occurrence of sedoheptulose in a plant outside the family Crassulaceae (except for some concurrent studies on photosynthesis by Benson *et al.*[2]) since the discovery of that sugar in *Sedum spectabile* Bor. by LaForge and Hudson[3] in 1917. Nordal and Öiseth obtained paper-chromatographic evidence for the presence of a *manno*-heptulose also. This was the first reported occurrence of that sugar since the discovery by LaForge[4] of D-*manno*-heptulose (II) in the fruit of the avocado (*Persea gratissima* Gaertn.) in 1917; it was isolated in 1954 by Nordal and Benson[5] from avocado leaves.

Although sedoheptulose is now believed to occur extensively in nature (although usually in relatively small amounts), the report of the finding of *two* heptuloses in a single plant seemed novel enough to warrant the effort to obtain a definitive proof of the configuration of the second heptulose. Therefore, with the encouragement of Professor Nordal, we undertook a large-scale study of the higher-carbon sugars in *Primula officinalis* Jacq. Before the completion of this study, however, reports from some other workers were published. Nordal and co-workers[6] detected, by paper chromatography, both sedoheptulose and *manno*-heptulose in the capsules of *Papaver somniferum* L. (opium poppy). Next, Rendig and McComb[7] stated that *manno*-heptulose occurs in alfalfa (*Medicago sativa* L.), and Rendig, McComb, and Hu[8] identified both sedoheptulose and *manno*-heptulose in the leaf-petiole fraction of alfalfa by paper chromatography; the *manno*-heptulose was also identified by its X-ray powder diffraction pattern. Bevenue *et al.*[9] obtained evidence, by paper chromatography, of the presence of sedoheptulose and *manno*-heptulose in both the fruit and the leaf of the fig tree (*Ficus carica* L.), and confirmed this finding through the microscopic identification of a crystalline osazone from each of the isolated sugars[10]. Esau and Amerine[11] examined the residual sugars in a grape wine and, after a paper-

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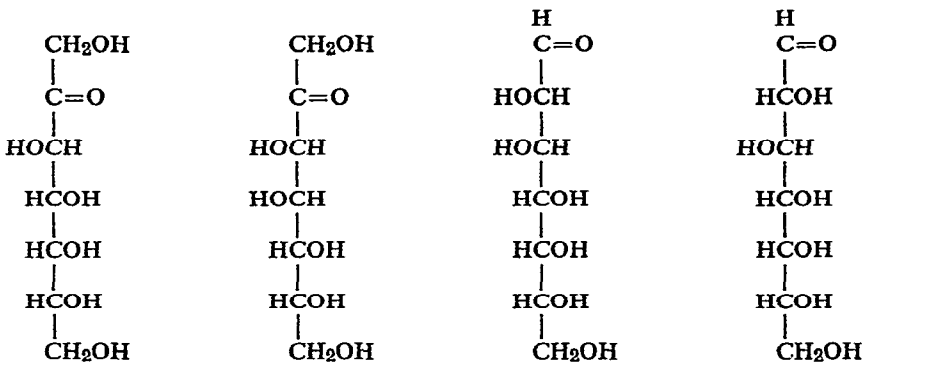
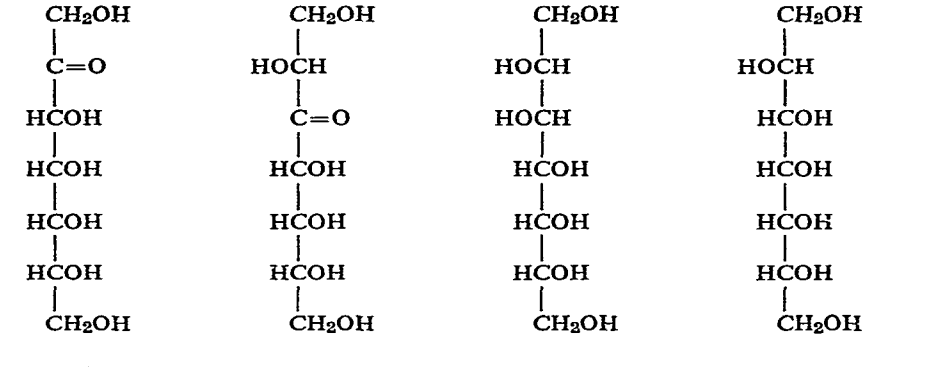
chromatographic study, assumed that the two heptuloses present were *altro*-heptulose and *manno*-heptulose.

Although it seems highly probable that the sugars identified above by paper chromatography and other methods actually are D-*altro*-heptulose and D-*manno*-



I II III IV



V VI VII VIII

heptulose, there is no positive evidence, either from optical rotations or from mixed melting points, that the heptuloses belong to the D series. We have now remedied this lack of evidence, in one case at least, by isolating two heptuloses from the roots of *Primula officinalis* Jacq. and proving, by optical rotations and by mixed melting points with authentic samples, that they are, indeed, D-*altro*-heptulose (sedoheptulose) and D-*manno*-heptulose.

In addition to these two heptuloses, other C_7 sugars have been isolated from natural products in recent years. Several aldoheptoses, for example, have been found

as components of various bacterial polysaccharides[12], and D-*glycero*-D-*galacto*-heptose has been reported as the first aldoheptose found in higher plants[13]. Now, by fractionation of an aqueous extract of *P. officinalis* Jacq., first on a column of cellulose and then on a column of Dowex 50W-X8 (Ba^{2+}), we have isolated sirupy D-*glycero*-D-*manno*-heptose (III) and crystalline D-*glycero*-D-*gluco*-heptose (IV) as the β anomer [m.p. 156–157°; $[\alpha]_D^{20}$ +17 → +46.2° (in water)*]. Identification of these two heptoses was accomplished by conversion into the known[14] D-*glycero*-α-D-*manno*- and D-*glycero*-α-D-*gluco*-heptose hexaacetates. Low-temperature acetylation of the new crystalline heptose has furnished the previously unknown D-*glycero*-β-D-*gluco*-heptose hexaacetate in crystalline form.

The isolation of these two heptoses that are so closely related to D-*altro*-heptulose recalls the isolation, by Ginsburg, O'Brien, and Hall[15], of D-*glycero*-D-*manno*-heptose from a guanosine pyrophosphate nucleotide found in bakers' yeast; they suggested that there may be a parallelism between the heptoses and hexoses with regard to their biosynthesis and incorporation into polysaccharides.

In addition to the long-known D-*manno*- and D-*altro*-heptuloses, one other has recently been reported to occur naturally**, namely, D-*talo*-heptulose from the avocado[19]. Although crystalline D-*talo*-heptulose[20] was not isolated, considerable evidence was obtained to establish the identity of the sugar.

We have now isolated crystalline D-*allo*-heptulose (V) from *P. officinalis* extract, and have identified it conclusively by direct comparison with a sample of the synthetic sugar[20]. Another heptulose that we have obtained in crystalline form from the fractionation of the *P. officinalis* extract has been identified as D-*altro*-3-heptulose (VI) through direct comparison with a product obtained from the rearrangement of sedoheptulose (I) in boiling pyridine; the structure of that product was based upon its reduction with borohydride to D-*glycero*-D-*manno*-heptitol (VII) and D-*glycero*-D-*altro*-heptitol (VIII). This is the first reported isolation of a 3-heptulose from a plant source.

Although compounds III to VI have, at C-4 to C-7, the same configuration as sedoheptulose (I), and although it is conceivable that they were formed by isomerizations occurring at C-1 to C-3 during the course of their isolation, we do not believe them to be artifacts but, rather, that their formation was caused by the action of enzymes within the plant itself.

Two octuloses have recently been discovered in nature, namely, D-*glycero*-

*In a personal communication, Professor Donald L. MacDonald, of Oregon State University, has informed us that he and Mr. Roger Wong have crystallized this sugar independently; they found m.p. 150–151° (dec.) and $[\alpha]_D^{20}$ +18 (5 min)→+47° (in water).

**Still other heptuloses have been obtained from plants by deliberately feeding them with precursors. McComb and Rendig (Ref. 16) fed L-sorbose to alfalfa shoots, and isolated L-*galacto*-heptulose. When Rendig and McComb (Ref. 17) fed D-ribose, they found that D-*altro*-heptulose accumulated; L-arabinose similarly gave L-*gluco*-heptulose, D-xylose gave D-*ido*-heptulose, and L-lyxose gave L-*galacto*-heptulose. The same authors (Ref. 18) later showed that each of the four tetroses induces an accumulation of a specific heptulose that could be identified through paper-chromatographic examination of the extract of the alfalfa leaves to which the tetroses had been fed.

D-*manno*-octulose in the avocado[13,19,21], in *Sedum*[19], and, probably, in a red wine[11]; and D-*glycero*-L-*galacto*-octulose in the avocado[13] and, probably, in *Sedum*[22]. We have now isolated the same two octuloses from the *P. officinalis* extract, and have identified them by direct comparisons of paper chromatograms, opticai rotations, infrared spectra, and crystalline derivatives with those of the octuloses from the avocado and with those of the same octuloses previously synthesized in this laboratory[13,19]. Degradations modeled on those employed in their original characterization by Charlson and Richtmyer[19,21] and by Sephton and Richtmyer[13] have furnished confirmatory evidence for these structures.

Two nonuloses also have been reported to occur naturally, namely, D-*erythro*-L-*gluco*-nonulose[22] and D-*erythro*-L-*galacto*-nonulose[23], both in the avocado, and both, probably, in *Sedum*[22]. We have now isolated the same two nonuloses from *P. officinalis*, and identified them by direct comparisons and by degradations modeled on those employed in their original characterization by Sephton and Richtmyer[22,23].

In addition to volemitol (D-*glycero*-D-*manno*-heptitol, VII), which was first isolated from the roots of *Primula* species (including *P. grandiflora* Lam., *P. elatior* Jacq., and *P. officinalis* Jacq.) by Bougault and Allard[24], following its earlier discovery in the mushroom *Lactarius volemus* Fr. by Bourquelot[25], we have isolated β-sedoheptitol (D-*glycero*-D-*gluco*-heptitol) from the *P. officinalis* extract. Although the latter heptitol had previously been reported as accompanying sedoheptulose in *Sedum* species[19], this appears to be the first example of two heptitols occurring in the same plant.

Primeverose (6-*O*-β-D-xylopyranosyl-D-glucose) is the carbohydrate constituent of a number of naturally occurring phenolic glycosides, including primeverin and primulaverin from the fresh roots of *Primula officinalis* Jacq.[26]. We were not surprised, therefore, when we found the free sugar in the aqueous extract of the dried roots of that plant, even though primeverose has been isolated directly only once previously: Wallenfels and Lehmann[27] obtained it from ripe carob beans (St. John's bread; *Ceratonia siliqua* L.). A portion of the primeverose was reduced with borohydride, to yield the new, crystalline primeveritol, which was characterized further as its crystalline octaacetate.

The other substances that we isolated from the *P. officinalis* extract and then identified were glycerol, erythritol, D-xylose, xylitol, and *myo*-inositol. Although xylitol had been detected previously[28] in the edible mushroom *Psalliota campestris* by means of a specific enzyme called TPN-xylitol (L-xylulose) dehydrogenase[29], we believe that this is the first time that xylitol has been isolated in crystalline form from a plant source.

EXPERIMENTAL

General methods

Paper chromatography was carried out with 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, ethyl acetate–pyridine–water (10:4:3); C, the same as A, except that it contained 0.5% of benzeneboronic acid[30]; and D, butyl alcohol–pyridine–water (6:4:3). Spray reagents used were aniline hydrogen phthalate for aldoses, orcinol–hydrochloric acid for ketoses, alkaline hydroxylamine–ferric chloride for lactones, and silver nitrate (ammoniacal, or followed by sodium hydroxide in ethanol) or 0.25% sodium periodate in 5% aqueous acetic acid followed by 2% *p*-anisidine in 5% acetic acid for alditols, sugars, and other polyhydroxy compounds. The values for R_{Rha}, R_{Sed}, and R_{Fru} refer to the rate of movement of the compounds on paper chromatograms (developed in solvent A, unless otherwise noted) relative to that of rhamnose, sedoheptulosan, and fructose. All concentrations were carried out at temperatures below 50°, and final drying of sirups was effected over granular calcium chloride *in vacuo* or by lyophilization. Unless otherwise specified, melting points were determined on a Kofler micro hot-stage, and infrared spectra were measured in Nujol mulls.

Preparation of the extract

Twenty kg of the dried roots of *Primula officinalis* Jacq.* was ground to a fine powder and extracted in 500-g batches. Each 500 g was suspended in 8 l of distilled water, 50 g of calcium carbonate was added to neutralize any acidity that might develop, and the mixture was digested for 8 h on a steam bath. About 150 g of a filter aid (Johns–Manville Celite 535) was stirred into the mixture, which was then filtered through a large Büchner funnel precoated with about 100 g of the same filter aid. The filtration was very slow. The residue on the funnel was extracted a second time with 8 l of water. The combined, dark-colored extracts were deproteinized by dissolving 200 g of zinc sulfate heptahydrate in the solution and adding slowly, with mechanical stirring, a saturated aqueous solution of barium hydroxide until the main solution was nearly alkaline to phenolphthalein paper. About 150 g of decolorizing carbon (Darco G-60) was stirred into the mixture, and the mixture, after being kept for about 2 h to let the precipitated material coagulate and settle, was filtered through a large Büchner funnel. The clear, almost colorless filtrate was deionized by successive passage through columns of Amberlite IR-120(H^+) and Duolite A-4 (OH^-) ion-exchange resins. Unless the aqueous solution was quite dilute (volume at least 30 l), a fine, white precipitate formed in the Amberlite column, and retarded or even stopped the flow of solution through the resin; the precipitate was suspected to be a rather insoluble, acidic substance, but no attempt was made to establish its identity.

When the deproteinized, deionized extracts from ten batches of *P. officinalis* were combined, and concentrated *in vacuo* to a thick sirup, the volemitol (D-*glycero*-D-*manno*-heptitol ≡ D-*glycero*-D-*talo*-heptitol, VII) crystallized spontaneously. It was isolated by redissolving the mixture in hot water, diluting with methanol, cooling, and filtering off the prismatic needles. Identification was made through its m.p. of

*This material was purchased from S.B. Penick & Company, 100 Church Street, New York, N.Y., through the special cooperation of Mr. Hans R. Schmidt.

154–155° (alone, and when mixed with authentic material[31]), by comparison of infrared spectra, and through the preparation of its heptaacetate[31], having m.p. 62–63° and $[\alpha]_D^{20}$ +36.0° (*c* 1.3, dichloromethane). The total yield of 344 g from 20 kg (1.7%) is comparable to the 1.5% recorded by Bougault and Allard[24].

Concentration *in vacuo* of the volemitol filtrates yielded 152 g of a sirup whose paper-chromatographic examination in solvents A and B indicated the presence of at least twelve components, including relatively large proportions of D-glucose, D-fructose, and sucrose. These last three were removed by dissolving the sirup in 3.2 l of water, adding 1.25 cakes of bakers' yeast and 75 g of D-glucose as a primer, and allowing the mixture to ferment for 3 days at 37°. Successive deproteinization, deionization, and concentration *in vacuo* yielded 84 g of sirup in which xylose, *manno*-heptulose, sedoheptulose, and primeverose could be tentatively identified through their mobilities and color reactions on paper chromatograms. In a separate experiment, a mixture of D-glucose, D-fructose, D-xylose, D-arabinose, and D-ribose was fermented with bakers' yeast; no higher-carbon aldose or ketose could be detected. Bevenue *et al.*[32] had similarly fermented pure sucrose, but could detect no formation of heptulose.

Fractionation of the extract on a cellulose column

A glass tube (100 × 10 cm) was packed with 2 kg of Whatman standard-grade, cellulose powder (effective height, 68 cm) and the 84 g of sirupy extract, made into a smooth slurry with 150 g of cellulose powder and 750 ml of quarter-saturated aqueous butyl alcohol, was put on top of the cellulose column, by the procedure described earlier[13]. Elution was begun with quarter-saturated aqueous butyl alcohol, continued in a stepwise manner with half- and three-quarter-saturated aqueous butyl alcohol, and concluded with fully saturated aqueous butyl alcohol. With the aid of an automatic fraction-collector, the eluate was distributed among 14,200 tubes, each containing 21 ml; the contents of these tubes were combined on the basis of their paper-chromatographic assays, and concentrated to give 66 fractions. The constituents of these fractions, insofar as they have been identified, are reported below.

Glycerol

Fractions 9–11 (tubes 331–550; 4.68 g) appeared to contain glycerol (R_{Rha} 1.20) as the principal constituent. Accordingly, a small portion of the thick, mobile liquid of fraction 10 was heated with *p*-nitrobenzoyl chloride and pyridine for 2 h at 80–90°, and the product isolated; it was identified as glycerol tris-*p*-nitrobenzoate, m.p. 195–196° (capillary tube) both alone and when mixed with authentic material[33]. The infrared spectra of the two substances were identical.

Erythritol

Fraction 12 (tubes 551–700; 1.04 g) crystallized in part, and furnished 0.78 g of stout prisms (R_{Rha} 0.96). After one recrystallization from aqueous ethanol, the erythritol melted at 120–122°, both alone and when mixed with authentic material; a comparison of the infrared spectra confirmed the identification.

*2,7-Anhydro-β-*D-altro*-heptulofuranose*

Fraction 13 (tubes 701–885; 0.52 g) yielded 24 mg of stout prisms (R_{Sed} 1.37 and R_{Fru} 1.23 in solvent D) that melted at 195–196°, alone, and at 195–197° when mixed with authentic anhydro compound[34] of m.p. 198-200° (all in capillary tubes). Identification was confirmed through paper-chromatographic and infrared-spectral comparisons.

*α-*D*-Xylose*

Fractions 15 and 16 (tubes 1011–1130; 1.02 g) showed strong evidence for xylose (R_{Rha} 0.74) as the only carbohydrate constituent, and prisms of α-D-xylose were separated from the sirup and also from fraction 14. After recrystallization from aqueous methanol, the compound had m.p. 151–152° and $[\alpha]_D^{20}$ +95° (3 min)→ +20.5° (*c* 1.5, water), in good agreement with the recorded values[35] of m.p. 153° and $[\alpha]_D^{25}$ +96° → +20°. A mixture of the compound with authentic α-D-xylose melted at 151–153°, and the infrared spectra of the two samples were identical.

Subfractionation on Dowex 50W-X8 (Ba^{2+}) resin

After the initial separation of the *Primula officinalis* constituents on a cellulose column, some of the fractions were resolved further on a column of Dowex 50W-X8 (Ba^{2+}) ion-exchange resin[36]. To this end, the resin (200–400 mesh) was converted into its barium form by treatment with several portions of aqueous barium chloride (barium acetate would probably be better, to avoid possible release of hydrogen chloride that might occur later because of incomplete washing), and washed thoroughly with water. The resin was poured, as a slurry, into a glass tube (2.5 cm, inside diameter) equipped with a needle-valve stopcock and with a plug of polyurethane foam in the bottom to retain the resin; after the resin had settled, the effective height of the column was 116 cm. The fraction to be investigated was dissolved in the minimum amount of water, and the solution was added to the top of the column; after the solution had passed into the resin at the top of the column, elution was effected with water at a flow rate of 4–8 ml/h, and the eluate was collected automatically in 1- to 1.5-ml portions. In many cases, these fractions were chromatographically pure. Tubes were combined according to their chromatographic behavior, and the solutions were treated with small amounts of Amberlite IR-120 and Duolite A-4 ion-exchange resins to insure that they would be free from ionic material before being concentrated.

Xylitol

Fractions 17–19 (tubes 1131–1370; 3.09 g) showed the presence of three orcinol-positive compounds having R_{Fru} 0.88, 0.99, and 1.09 on paper chromatograms. The first and third spots corresponded to sedoheptulose and sedoheptulosan, but for the complete identification of the intermediate spot we resorted to subfractionation on Dowex 50W-X8 (Ba^{2+}) as described above. During that procedure, an intermediate fraction (420 mg) crystallized very slowly from its solution in methanol. The m.p. of 92–93°, and the chromatographic mobility, suggested xylitol, and the infrared spec-

trum was indistinguishable from that of an authentic sample of xylitol. Upon acetylation with acetic anhydride and fused sodium acetate, the compound afforded xylitol pentaacetate as hexagonal plates (from aqueous ethanol); the m.p. was 62–63° alone (as well as when mixed with authentic material), and the infrared spectra of the two specimens of pentaacetate were identical.

D-altro-*3-Heptulose* (VI)

After the xylitol had been eluted in the subfractionation of fractions 17–19 on Dowex 50W-X8 (Ba^{2+}) resin, the next tubes yielded D-*altro*-3-heptulose (VI; m.p. *ca.* 165°, $[\alpha]_D^{20}$ *ca.* +20°). On paper chromatograms sprayed with orcinol–hydrochloric acid, it appeared as an orange-brown spot that sometimes faded to a grayish-brown color; the isomeric D-*manno*-3-heptulose has been described by Schaffer[37] as forming a gray-brown spot with the orcinol–trichloroacetic acid spray reagent. The infrared spectrum of the compound isolated from fractions 17–19 was indistinguishable from that of a compound isolated following the rearrangement of sedoheptulose (D-*altro*-heptulose) in boiling pyridine; their chromatographic mobilities were the same, and the m.p. of a mixture of the two substances was not depressed. The sodium borohydride reduction of the sample isolated from the latter source yielded D-*glycero*-D-*manno*-heptitol (VII) and D-*glycero*-D-*altro*-heptitol, which were identified by comparison of melting points and infrared spectra with those of known specimens of these heptitols. Further details of the synthesis and properties of D-*altro*-3-heptulose will be included in a later publication.

D-allo-*Heptulose* (V)

The final tubes from the subfractionation of fractions 17–19 on Dowex 50W-X8 (Ba^{2+}) resin contained a heptulose that was readily separated from the other constituents through its relatively high retention-volume. Crystals were obtained by the slow evaporation of a solution of the sirupy sugar in methanol. Recrystallization from 95% ethyl alcohol yielded long needles whose melting-point behavior (75–80° on rapid heating, 90–97° on slow heating, and 128—130° after being dried at 75° *in vacuo* for 24 h) was characteristic of an *allo*-heptulose hydrate[38,39]. The *allo*-heptulose structure received confirmation through paper chromatography and by comparison of an infrared spectrum of the hydrate with that of a sample prepared by crystallization of authentic, anhydrous D-*allo*-heptulose* from 95% ethyl alcohol. A mixture of the two anhydrous forms showed no depression of the melting point. Finally, a phenylosazone was prepared by heating 35 mg of the sirupy heptulose (obtained by concentrating the mother liquor from which the crystalline D-*allo*-heptulose had separated) with 0.08 ml of phenylhydrazine and 0.04 ml of glacial acetic acid in 1.3 ml of 2-methoxyethanol[40] for 3 h at 100°. The reaction mixture was poured onto ice, and the yellow precipitate was collected on a sintered-glass funnel and washed successively with 10% acetic acid and water. After one recrystal-

*Kindly supplied by Dr. Robert Schaffer.

lization from ethyl alcohol, the yellow needles (24 mg) of D-*allo*-heptosulose bis(phenylhydrazone) melted at 164–167°, a value that was not significantly different from that of a mixture of this compound and the phenylosazone prepared similarly from authentic* D-*glycero*-D-*allo*-heptose[38]. The infrared spectra of the two phenylosazones were identical.

Sedoheptulose (D-altro-*heptulose*, I)

Paper chromatograms indicated that fractions 18–27 contained both sedoheptulose and sedoheptulosan (2,7-anhydro-β-D-*altro*-heptulopyranose). To verify this conclusion, fraction 23 (tubes 1731–1850; 0.89 g) was dissolved in 25 ml of water containing 0.4 ml of concentrated sulfuric acid, and the mixture was heated for 6 h on the steam bath, cooled, neutralized with barium carbonate, and filtered, and the filtrate deionized by successive treatments with Amberlite IR-120(H^+) and IR-45(OH^-) resins, and concentrated to a sirup. When the sirup was dissolved in a small amount of hot methanol, and the solution cooled and inoculated with a seed crystal, 0.62 g of the anhydrous modification of sedoheptulosan was obtained. After three recrystallizations from methanol, the clear, chunky prisms melted at 155–156° both alone and when mixed with authentic sedoheptulosan; $[\alpha]_D^{20}$ −145° (*c* 1, water); and the infrared spectrum was indistinguishable from that of authentic material. For further identification, a portion of the crystalline product obtained from fraction 23 was benzoylated, to give a substance having m.p. 164–165° and $[\alpha]_D^{20}$ −185° (*c* 0.5, dichloromethane), in good agreement with the reported values[41] of 165–166° and −188°, respectively. A mixture with authentic sedoheptulosan tetrabenzoate melted at 164–165°.

D-manno-*Heptulose* (II)

Fraction 28 (tubes 2331–2570; 0.72 g) deposited chunky prisms of D-*manno*-heptulose from its concentrated solution in methanol. The sugar had m.p. 152–153° alone, as well as when mixed with authentic material; its rotation, $[\alpha]_D^{20}$ +29° (*c* 1, water), agreed with the reported rotation; and infrared spectra of the two specimens were identical. The identity was confirmed by treating the mother liquor (from which the crystals had been separated) with a mixture of phenylhydrazine and 1-benzyl-1-phenylhydrazine according to the directions of White and Secor[42]. The resulting D-*manno*-heptosulose 1-(2-benzyl-2-phenylhydrazone) 2-phenylhydrazone, after two recrystallizations from absolute ethyl alcohol, melted at 199–200° when heated at a rate of 1.5°/min up to 180° and then at 1°/min. A mixture with authentic material showed no significant depression of the melting point, and the identity of the infrared spectra of the two samples confirmed their identity. Analyses for C, H, and N were also confirmatory.

*Dr. J.W. Pratt had prepared the same phenylosazone from D-*glycero*-D-*allo*-heptose, but its description had been omitted from the publication (Ref. 38).

Anal. Calc. for $C_{19}H_{24}N_4O_5$: C, 58.75; H, 6.23; N, 14.43. Found: C, 59.00; H, 6.15; N, 14.27.

The infrared spectrum of the phenylosazone prepared by Dr. Pratt was indistinguishable from the other two spectra. (N.K.R.)

Volemitol (VII)

In addition to the 344 g of volemitol that had crystallized before the *Primula officinalis* extract was fractionated on cellulose, 8 g crystallized later from fractions 32–38 (tubes 3171–6300; 9.6 g).

D-glycero-D-manno-*Heptose* (III)

The subfractionation, on Dowex 50W-X8 (Ba^{2+}) resin, of 1.31 g of fractions 29–33 (tubes 2571–4100; 4.04 g, from which 3.40 g of crystalline volemitol had been removed) yielded a middle fraction of 363 mg of sirup that appeared, from paper-chromatographic evidence, to contain a heptose, but no heptulose or other ketose. Since the product did not crystallize, an 86-mg portion of it was heated with 43 mg of fused sodium acetate in 5 ml of acetic anhydride for 5 h at 110°, and 114 mg of crude, sirupy acetate was isolated in the usual way. After decolorization with Darco X, and reconcentration, a solution of the sirup in a small amount of 60% aqueous methanol began to deposit crystals after standing at 0° for 2 weeks. After one recrystallization from aqueous methanol, the prisms were identified as D-*glycero*-α-D-*manno*-heptose hexaacetate through a melting point of 137–138° alone, and 137–139° when mixed with authentic material[14] of m.p. 138–139°. The identity of the two hexaacetates was confirmed by comparison of their infrared spectra.

Crystalline D-glycero-β-D-gluco-*heptose*

Fractions 34–36 (tubes 4101–5810; 5.12 g, from which 4.08 g of crystalline volemitol had been removed) appeared to contain an octulose. To isolate it, the remaining 916 mg of these fractions was chromatographed on a Dowex 50W-X8 (Ba^{2+}) resin column, as described earlier. Of the 8 subfractions thus obtained, subfractions 2–4 were richest in octulose; these were combined (530 mg), and rechromatographed on a cellulose column (90 × 2.2 cm) with 92.5% aqueous acetone as eluent. Of the 12 new fractions thus obtained, fractions 7 and 8 deposited 43 mg of crystals when their concentrated solutions in methanol were kept in a refrigerator for about 10 days. The compound, after recrystallization from methanol, melted at 156–157° (unchanged after a second recrystallization) and showed $[\alpha]_D^{20}$ +17° (extrapolated)→ +46.2° (6 h, constant; *c* 2.4, water). Its eventual identification as D-*glycero*-β-D-*gluco*-heptose was based on the evidence that follows.

Anal. Calc. for $C_7H_{14}O_7$: C, 40.00; H, 6.71. Found: C, 40.15; H, 6.87.

On a paper chromatogram sprayed with aniline hydrogen phthalate, the compound gave the brown color that is characteristic of hexoses and higher aldoses; its mobility was less than that of any of the hexoses. When oxidized with 1.25 molecular equivalents of lead tetraacetate*, a paper chromatogram showed ribose as the only

*Perlin and Brice (Ref. 43) used 2 molecular equivalents of oxidant. We used less than that, in an attempt to identify, also, the hexose that should be an intermediate in the degradation. Failure to obtain any chromatographic evidence for such an intermediate here, and in other oxidations to be described later, may be the result of a very rapid oxidation of the intermediate, together with the use of too small an amount of substrate.

degradation product. For acetylation, 25 mg of sirupy heptose (recovered from measurements of optical rotation) was dissolved in 5 ml of acetic anhydride containing 7 mg of freshly fused zinc chloride, and the mixture was kept for 6 h at room temperature. The product, isolated in the usual way, was identified as D-*glycero*-α-D-*gluco*-heptose hexaacetate through (*a*) its m.p. 180–182°, alone as well as when mixed with authentic material[14], (*b*) its rotation $[\alpha]_D^{20}$ +105° (*c* 1, dichloromethane), and (*c*) a comparison of infrared spectra. Finally, deacetylation of a sample of authentic α-hexaacetate[14] with methanolic sodium methoxide yielded crystalline D-*glycero*-β-D-*gluco*-heptose, indistinguishable in m.p. and infrared spectrum from those of the substance isolated from the subfractionations of fractions 34–36 as described above.

D-glycero-β-D-gluco-*Heptose hexaacetate*

The acetylation of 200 mg of D-*glycero*-β-D-*gluco*-heptose at 0° with a mixture of 1 ml of acetic anhydride and 1.5 ml of pyridine yielded 405 mg of the corresponding β-hexaacetate. After recrystallization from aqueous ethyl alcohol, and then several times from dichloromethane–pentane, the clusters of small prisms of the new hexaacetate melted at 133–134° and showed $[\alpha]_D^{20}$ +19.6° (*c* 1, chloroform).

Anal. Calc. for $C_{19}H_{26}O_{13}$: C, 49.35; H, 5.67; CH_3CO, 55.9. Found: C, 49.45; H, 5.39; CH_3CO, 56.3.

β-Sedoheptitol (D-glycero-D-gluco-*heptitol*; L-glycero-D-talo-*heptitol*)

As described above, the further separation of fractions 34–36 on Dowex 50W-X8 (Ba^{2+}) yielded 8 subfractions. Of these, subfractions 5–7 deposited, on standing, 38 mg of a crystalline substance that was identified as β-sedoheptitol through direct comparison (m.p., mixed m.p., paper chromatography, and infrared spectra) with an authentic sample of m.p. 129–130°, prepared by the reduction of sedoheptulose.

D-glycero-D-manno-*Octulose*

As described above, fractions 34–36 were fractionated on Dowex 50W-X8 (Ba^{2+}) resin, and the octulose-rich subfractions 2–4 were rechromatographed on a cellulose column. The new fractions 7–8 (from which 43 mg of crystalline D-*glycero*-D-*gluco*-heptose had been removed) and 9–11 were combined (288 mg of sirup) and dissolved in 14 ml of water, and the remaining aldoheptose was oxidized in the dark with 0.3 ml of bromine in the presence of an excess of barium carbonate. After 18 h, the excess of bromine was removed by aeration, and the solution was filtered and then deionized with a mixture of Amberlite IR-120 (H^+) and IR-45 (OH^-) ion-exchange resins. Concentration of the solution gave 98 mg of a yellowish sirup that contained an octulose, contaminated (as determined by paper chromatography) with lactones and a small proportion of a hexulose-containing disaccharide. These impurities were removed by chromatography on sheets of Whatman 3MM filter paper developed in solvent A. In this manner, there was obtained 40 mg of chromatographically pure, sirupy D-*glycero*-D-*manno*-octulose having R_{Sed} 0.46 and $[\alpha]_D^{20}$ +25.2° (*c* 2, 90% aqueous methanol); the latter value is comparable to the value of +26.5° (*c* 5,

methanol) reported previously[13] for the same octulose isolated from the avocado, and to the value of +27° (*c* 1, methanol) measured for the same octulose synthesized by the diazomethane method[44].

The infrared spectrum of the D-*glycero*-D-*manno*-octulose from *Primula officinalis* roots, obtained for a dried film from methanol, was indistinguishable from those of the same octulose isolated from two avocado varieties and from *Sedum* species, as well as from the spectrum of the synthetic specimen[44]. The (2,5-dichlorophenyl)hydrazone prepared from this *Primula* octulose, after recrystallization from aqueous methanol as yellowish needles, melted at 171–172°; a mixed melting point with the product prepared from the same octulose from the avocado[19,21] was undepressed, and the infrared spectra of the two compounds were identical. Degradation of this *Primula* octulose with two molecular equivalents of lead tetraacetate, as described previously[19,21], yielded, as reported, a single pentose whose mobility on paper chromatograms (developed in solvents A and B) was the same as that of ribose.

Finally, by the procedures described previously[22,45], 4.7 mg of the sirupy D-*glycero*-D-*manno*-octulose from *Primula officinalis* roots was converted into a mixture of the corresponding methyl octulosides; oxidation with one molecular equivalent of sodium metaperiodate, followed by reduction of the aldehyde group with sodium borohydride and hydrolysis of the methyl glycosides, yielded 2.5 mg of a reducing sugar. Its chromatographic mobility in solvents A, B, and C was, as previously reported, identical with that of *manno*-heptulose; and, on a paper chromatogram sprayed with orcinol–hydrochloric acid, it gave the characteristic greenish-blue color reaction of *manno*-heptulose instead of the blue color that is given by the other heptuloses.

D-glycero-L-galacto-*Octulose*

The original fractions 34–36 had been fractionated further on Dowex 50W-X8 (Ba^{2+}) resin as described above, and the subfractions 5–7 (after removal of 38 mg of crystalline β-sedoheptitol) were then found to contain a second octulose. This sirup (140 mg) was fractionated further, on sheets of Whatman 3MM filter paper developed in solvent A, and 13.5 mg of a (chromatographically) practically pure octulose (R_{Sed} 0.42) was thus obtained. Its mobility on paper chromatograms developed in solvents A, B, and C was the same as that of the known D-*glycero*-L-*galacto*-octulose[13,45], and its infrared spectrum, obtained for a dried film from methanol, was very closely similar to that of the previously described octulose[13]. Degradation of 1.8 mg of this second octulose from *Primula* roots, with somewhat more than one molecular equivalent of lead tetraacetate in glacial acetic acid, furnished xylose as the only pentose detectable on a paper chromatogram (solvents A and B). This second octulose yielded a (2,5-dichlorophenyl)hydrazone whose melting point of 176–178° (unrecrystallized) was not depressed significantly (175–179°) when it was mixed with the corresponding derivative of D-*glycero*-L-*galacto*-octulose from the avocado[13].

D-erythro-L-gluco-*Nonulose*

From their behavior on paper chromatograms, fractions 37–41 (tubes 5811–7130; 2.39 g) appeared to contain two nonuloses; these were separated relatively easily on a column of Dowex 50W-X8 (Ba^{2+}) resin. The first nonulose was purified further by chromatography on a cellulose column, with elution by 92.5% aqueous acetone (which removed much non-nonulosic material) followed by methanol. Final purification of the 148 mg thus obtained was effected on Whatman 3MM filter paper developed with solvent A. The middle zone from this chromatogram yielded 59 mg of chromatographically pure D-*erythro*-L-*gluco*-nonulose. Its rotation of $[\alpha]_D^{20}$ −42.8° (*c* 1.3, water) is intermediate between the values of −40.0° for the same nonulose isolated from the avocado[22] and −47.2° for the synthetic nonulose[22,46]. The infrared spectrum of this nonulose, obtained for a dried film from methanol, was indistinguishable from that of the synthetic D-*erythro*-L-*gluco*-nonulose[22]. In confirmation of earlier findings[22], degradation of 13.9 mg of this nonulose with 2 molecular equivalents of lead tetraacetate in 98% acetic acid, by the procedure described earlier, yielded 5.7 mg of a sirup identified by chromatography in solvents A and B, and by its rotation of $[\alpha]_D^{20}$ +10° (*c* 0.57, water), as D-mannose; neither a pentose nor a heptose was detectable. For confirmation, 3.7 mg of the sirup containing D-mannose was dissolved in 0.075 ml of water; to the solution was added 0.055 ml of redistilled phenylhydrazine, 0.055 ml of glacial acetic acid, and 0.105 ml of water and the mixture was kept overnight at 0°; 4 mg of white needles of D-mannose phenylhydrazone was deposited. The m.p. was 198–199° (dec.), both alone and when mixed with authentic material, and the infrared spectra of the two phenylhydrazones were identical.

Another portion (16.5 mg) of this *Primula* root nonulose was converted into a mixture of methyl glycosides, and this mixture was oxidized with 2 molecular equivalents of sodium metaperiodate for 30 min at 0°. After reduction of the product with sodium borohydride, followed by acid hydrolysis, the 5.8 mg of sirup that was obtained was identified as a *gluco*-heptulose by paper chromatography in solvents A, B, and C, and as belonging to the L series because of its rotation of $[\alpha]_D^{20}$ −41° (*c* 0.3, water). This result is in agreement with the earlier degradation studies on the same nonulose isolated from the avocado[22].

Finally, 14.6 mg of this nonulose was refluxed for 14.5 h with 63.5 mg of (2,5-dichlorophenyl)hydrazine in 1.25 ml of ethyl alcohol containing 0.125 ml of glacial acetic acid. The quite insoluble, yellow needles obtained weighed 7.1 mg and melted at 242–245° (dec.); this value was not depressed when the compound was mixed with a sample of the (2,5-dichlorophenyl)osazone derived from synthetic D-*erythro*-L-*gluco*-nonulose[22]. The infrared spectra confirmed the identity of the two samples.

D-erythro-L-galacto-*Nonulose*

The second nonulose from *Primula* roots had a relatively high retention-time on the Dowex 50W-X8 (Ba^{2+}) column, and the material thus obtained from fractions 37–41 weighed 203 mg. A final purification on Whatman 3MM filter paper, developed in

solvent A, gave 100 mg of chromatographically pure D-*erythro*-L-*galacto*-nonulose as a sirup having $[\alpha]_D^{20}$ −37° (*c* 3.7, water). This value is very close to that of −36.2° (*c* 5.2, 90% methanol) for the synthetic nonulose[23]. Its identity was confirmed by repeating the two degradation procedures by which it was originally characterized. Thus, treatment of 8.3 mg of this second nonulose with 2 molecular equivalents of lead tetraacetate in 15 ml of glacial acetic acid for 20 min at room temperature afforded 3.5 mg of a sirup that contained a hexose having the same mobility as D-glucose on paper chromatograms developed in solvents A, B, and C. A trace of a pentose having a mobility corresponding to that of an arabinose was also observed. That the hexose was D-glucose was shown by digesting a solution of 1.5 mg of the sirupy oxidation product in 1 ml of water with 0.1 ml of a 4% solution of D-glucose oxidase*; subsequent paper chromatography showed that the hexose had been completely destroyed.

Secondly, 3 mg of this second nonulose from *Primula* roots was converted into a mixture of methyl nonulosides that was oxidized with 2 molecular equivalents of sodium metaperiodate for 30 min in an ice bath; borohydride reduction and acid hydrolysis, as described previously[23], yielded 2 mg of a sirup that contained a heptulose having the mobility of a *galacto*-heptulose on paper chromatograms developed in solvents A, B, and C.

α-Primeverose (*6*-O-*β*-D-*xylopyranosyl*-*β*-D-*glucopyranose*)

Fractions 47–50 (tubes 8106–9200; 8.24 g) yielded 5.76 g of crystalline primeverose. When recrystallized from methanol, it separated as clusters of flat, wedge-shaped prisms that melted at 191–192° with browning when heated slowly, and at about 210° when heated rapidly. It showed mutarotation $[\alpha]_D^{20}$ +24 → −3.5° (*c* 2.5, water). All these values are in accord with those recorded for this compound in the literature[26]. Analyses for C and H were also confirmatory.

In addition, a sample of the sugar was acetylated with acetic anhydride and fused sodium acetate; the *β*-primeverose heptaacetate melted at 215–216° and showed $[\alpha]_D^{20}$ −20.1° (*c* 1, chloroform). These values are comparable to the m.p. 216° and $[\alpha]_D^{20}$ −23.4° reported by Helferich and Rauch[47] for the *β*-heptaacetate of synthetic primeverose. The infrared spectrum of our primeverose heptaacetate and that of a sample derived from *Ceratonia siliqua* L.** were identical when obtained in chloroform solutions; and a mixture of our heptaacetate with a synthetic sample*** showed no depression of melting point (capillary tube).

Primeveritol (*6*-O-*β*-D-*xylopyranosyl*-D-*glucitol*)

A 310-mg portion of primeverose was reduced with 125 mg of sodium borohydride in the usual way. Upon crystallization and recrystallization from aqueous

*Worthington Biochemical Corporation's "Glucostat", in a phosphate buffer.
**Kindly supplied by Dr. Jochen Lehmann (Ref. 27).
***Kindly supplied by Dr. George H. Coleman (Ref. 48).

ethyl alcohol, the small needles of primeveritol melted at 141–142° and showed $[\alpha]_D^{20}$ —34.0° (*c* 1, water).

Anal. Calc. for $C_{11}H_{22}O_{10}$: C, 42.04; H, 7.06. Found: 42.10; H, 7.08.

Primeveritol octaacetate (*6-O-β-D-xylopyranosyl-D-glucitol octaacetate*)

The acetylation of a sample of primeveritol with acetic anhydride and fused sodium acetate, at 110° overnight, yielded a sirup that crystallized when its solution in aqueous ethyl alcohol was kept at 0° for 2 weeks. The primeveritol octaacetate was recrystallized from aqueous methanol, and then from dichloromethane by the addition of pentane; the fine needles melted at 119–120° and showed $[\alpha]_D^{20}$ —25.8° (*c* 1, chloroform).

Anal. Calcd. for $C_{27}H_{38}O_{18}$: C, 49.84; H, 5.89. Found: C, 50.18; H, 6.07.

myo-*Inositol*

Fractions 51 and 52 (tubes 9201–10,055; 2.84 g) yielded the rather insoluble *myo*-inositol in the first crop of crystals. After recrystallization from aqueous methanol, identification was effected by paper chromatography, by a melting point and mixed melting point of 222–224°, and finally by a comparison of infrared spectra.

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SUMMARY

An aqueous extract of 20 kg of the dried roots of *Primula officinalis* Jacq. has been deproteinized, deionized, fermented with bakers' yeast, and fractionated by chromatography on cellulose columns, Dowex 50W-X8 (Ba^{2+}) resin columns, and sheets of filter paper. The following higher-carbon sugars were isolated: D-*altro*-heptulose (sedoheptulose), D-*manno*-heptulose, D-*allo*-heptulose, D-*altro*-3-heptulose, D-*glycero*-D-*manno*-heptose, D-*glycero*-D-*gluco*-heptose, D-*glycero*-D-*manno*-octulose, D-*glycero*-L-*galacto*-octulose, D-*erythro*-L-*gluco*-nonulose, and D-*erythro*-L-*galacto*-nonulose. Two higher-carbon polyhydric alcohols — volemitol (D-*glycero*-D-*manno*-heptitol) and β-sedoheptitol (D-*glycero*-D-*gluco*-heptitol) — were also isolated, as well as glycerol, erythritol, xylitol, *myo*-inositol, D-xylose, and primeverose. Of all these substances, only volemitol had previously been isolated from *P. officinalis*, although primeverose was known to occur there as a constituent of the glycosides primeverin

and primulaverin. In addition, primeverose has been reduced to primeveritol(6-*O*-β-D-xylopyranosyl-D-glucitol), which was characterized further as the crystalline octaacetate; and the crystalline D-*glycero*-β-D-*gluco*-heptose has been converted into the new, crystalline D-*glycero*-β-D-*gluco*-heptose hexaacetate.

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