Higher-carbon Sugars of Opium Poppy (*Papaver somniferum* L.)

GURI HAUSTVEIT and JENS K. WOLD

Institute of Pharmacy, Department of Pharmacognosy, University of Oslo, Oslo 3, Norway

The capsule of the opium poppy contains a vast number of different substances, of which only the alkaloid fraction has been the subject of extensive investigation.

In a preliminary study of the carbohydrates of the poppy capsule Ottestad *et al.*[1] obtained chromatographic evidence for the presence of *altro*-heptulose (sedoheptulose) and of *manno*-heptulose. The present report confirms this finding and furthermore describes the characterization of D-*glycero*-D-*manno*-octulose, also found to occur in the poppy capsule.

In view of its role as an intermediate in photosynthesis sedoheptulose is probably uniformly distributed throughout the plant kingdom. In recent years a number of other higher-carbon sugars has been isolated from members of several plant families.[2] The possible role of these sugars in the carbohydrate metabolism of the plants is obscure, and the pathways for their biosynthesis have not yet been elucidated. It has been suggested[3] that the higher-carbon ketoses with a D-*threo* configuration at C_3 and C_4 may be synthesized by a mechanism involving the enzymes aldolase, transaldolase or transketolase, and experimental support for this has been obtained.[4,5] The mode of biosynthesis of the higher-carbon ketoses with an L-*erythro* configuration at C_3 and C_4 is not understood at present. Both D-*manno*-heptulose and D-*glycero*-D-*manno*-octulose possess this configuration.

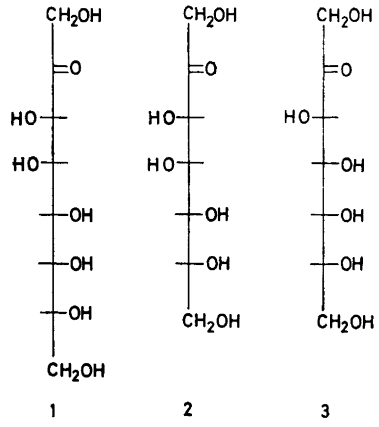

Fig. 1. Open chain forms of D-*glycero*-D-*manno*-octulose (1), D-*manno*-heptulose (2) and D-*altro*-heptulose (3).

Extraction of fresh or dried poppy capsules with aqueous ethanol furnished a mixture containing several sugars and related compounds, *e.g.* sedoheptulose, mannoheptulose, arabinose, xylose, erythritol, and *myo*-inositol; however, glucose, fructose, and sucrose constituted by far the major part of the extract. It was found necessary to remove the latter sugars prior to the isolation of the substances of interest, and this was most effectively done by fermentation with bakers yeast. It should be noted that the yeast proved to contain low-molecular compounds of carbohydrate nature; chromatographic evidence was obtained for the presence of glycerol, erythritol, *myo*-inositol, and a few other unidentified polyol-type compounds in an aqueous extract of the yeast. After treatment of a mixture of glucose, fructose, and sucrose with the yeast the same alcohols were detected, but there was no sign of any higher-carbon sugars, which made it reasonable to conclude that these sugars originated entirely from the plant material. In a similar experiment Begbie and Richtmyer[2] were also unable to detect any higher-carbon aldose or ketose in the fermentation digest. After removal of the yeast and purification of the extract, paper chromatography indicated the presence of an octulose in addition to the sugars detected previously. The compound gave the same greyish colour with the orcinol spray reagent[6] for ketoses, and it had the same chromatographic and electrophoretic mobility as D-*glycero*-D-*manno*-octulose, previously isolated from *Persea gratissima*[7] (avocado) and from *Primula officinalis*.[2]

The two heptuloses and the octulose were isolated by preparative filter sheet chromatography and subjected to paper chromatography, gas-liquid chromatography, and paper electrophoresis for comparison with authentic specimens.

The two heptuloses on oxidation with two molar equivalents of lead tetraacetate[7] gave erythrose as the major product. This result confirming the *erythro*-configuration at positions 5 and 6 in the two sugars, is in

agreement with the mechanism suggested [8] for the oxidation of ketoses with lead tetraacetate. Oxidation of the octulose under the same conditions furnished mainly ribose, confirming the configuration at positions 5, 6, and 7 in the molecule. Furthermore, oxidation of the octulose methylpyranoside with one molar equivalent of sodium metaperiodate at 0° followed by reduction with sodium hydridoborate and acid hydrolysis gave *manno*-heptulose. As expected, the three ketoses occur in the D-form judging from their optical rotations.

Experimental. Paper chromatograms were run on Whatman No. 1 and for preparative purposes on No. 3 MM filter paper in the solvent systems (v/v): A. Ethyl acetate, acetic acid, formic acid, water, 18:3:1:4. B. Ethyl acetate, pyridine, water, 8:2:1. C. Ethyl acetate, pyridine, acetic acid, water, 5:5:1:3. Zone electrophoresis was performed on Munktell No. 302 filter paper at *ca.* 40 V/cm in 0.05 M sodium tetraborate, pH 9.2. Sugars were located on chromatograms and electropherograms with the following reagents: a. Aniline oxalate, saturated aqueous solution. b. Silver nitrate-sodium hydroxide.[9] c. Trichloroacetic acid-orcinol.[6] Gas-liquid chromatography of trimethylsilylated sugars [10] was carried out on a column 600 × 0.9 cm using 3 % w/w of SE 30 on Chromosorb W at 200°, gas rate 55 ml/min, and on a column 300 × 0.9 cm using 15 % w/w of EGS on Chromosorb W at 185–200°, gas rate 115–125 ml/min. Unless otherwise stated, optical rotations were measured at 20° in water at a Perkin Elmer Model 141 photo-electric polarimeter.

Isolation and purification of the sugar fraction. Powdered capsules (850 g) were subjected to Soxhlet-extraction with, successively, light petroleum (b.p. 60–80°), benzene, and chloroform to remove non-polar substances. The dried, pre-treated material was then extracted twice with 80 % ethanol (5 l) at 70–75° for 8 h. The combined filtrates were deionized and concentrated to a syrup (32 g), which contained glucose, fructose, and sucrose as the major constituents. The mixture was subjected to fermentation with bakers yeast (25 g) at 37° for two days under toluene. After centrifugation and removal of residual protein in the supernatant by precipitation with lead acetate, the mixture was again deionized and concentrated to a syrup (12.5 g). Paper chromatography revealed spots corresponding to arabinose, xylose, sedoheptulose, *manno*-heptulose, *glycero-manno*-octulose, *myo*-inositol, erythritol, glycerol, and a few other unidentified spots (reagents a, b, and c).

Isolation and characterization of the higher-carbon sugars. In a typical experiment a portion of the syrupy mixture (0.75 g) was subjected to chromatography on 3 MM filter paper in solvent B. The fractions isolated proved heterogeneous, but after rechromatography in solvent A, pure samples were obtained of the three higher-carbon ketoses. On paper chromatography in solvents A, B and C, on gas-liquid chromatography on the two columns, and on zone electrophoresis in borate buffer the three sugars had the same mobilities and gave the same colour reactions as the corresponding authentic substances.

D-glycero-D-manno-*Octulose*, (6 mg), $[\alpha]_D$ +23.5° (*c* 0.55, methanol).

Degradation to ribose with lead tetraacetate.[7] To a solution of the octulose (9.6 mg, 40 μmoles) in glacial acetic acid (5 ml) was added 1.60 ml of a 0.05 M solution of lead tetraacetate in glacial acetic acid. After 15 min at room temperature the reaction was terminated by the addition of 0.05 M oxalic acid in glacial acetic acid (3 ml). The precipitated lead oxalate was removed by filtration and the filtrate concentrated to a syrup which was heated with 0.1 N sulphuric acid (5 ml) at 100° for 5 h to hydrolyse formyl and glycolyl groups. After neutralization with barium carbonate and subsequent deionization the main product proved indistinguishable from ribose in its behaviour on chromatography and electrophoresis.

Degradation to manno-*heptulose with sodium metaperiodate.*[11] The octulose (4.8 mg) was converted to the methyl glycoside by reflux with Dowex-50 (H^+) in methanol. The product was dissolved in water (5 ml) and the solution cooled to 0° followed by the addition of a cooled solution of 0.01 M sodium metaperiodate (2.00 ml). After 15 min at 0° sodium hydridoborate (5 mg) was added and the mixture was left at room temperature for 4 h, followed by addition of N sulphuric acid (7 ml) and heating of the solution at 100° for 2 h. After neutralization and deionization, chromatographic and electrophoretic examination showed that the main product formed corresponded to *manno*-heptulose.

D-altro-*Heptulose* (sedoheptulose), (2 mg), $[\alpha]_D$ −9.5° (*c* 0.45). Oxydative degradation of the sugar (40 μmoles) with two molar equivalents of lead tetraacetate as described for D-*glycero*-D-*manno*-octulose yielded mainly erythrose, verified by paper chromatography and electrophoresis. The low specific rotation compared with that reported in the literature, $[\alpha]_D$ +8°,[12] is ascribed to the presence of a small proportion of 2,7-anhydro-β-D-*altro*-heptulopyranose (sedoheptulosan), $[\alpha]_D$ −146°, easily

formed from sedoheptulose.[13] Gas-liquid chromatography revealed peaks corresponding to sedoheptulosan as well as to sedoheptulose.

D-manno-*Heptulose*, (10 mg), $[\alpha]_D$ +17° (*c* 1.75). Lead tetraacetate oxidation of the sugar (40 μmoles) under the conditions stated above gave erythrose as the main product, as confirmed by paper chromatography and electrophoresis. D-*manno*-heptulose 1-(*N'*-benzyl-*N'*-phenyl)-2-(*N'*-phenyl)-osazone was prepared according to White and Secor,[14] m.p. and mixed m.p. 192–196°.

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Biosynthesis of Spermidine and Spermine in Regenerating Rat Liver: Some Properties of the Enzyme Systems Involved

AARNE RAINA and PEKKA HANNONEN

Department of Medical Chemistry, University of Helsinki, Helsinki, Finland

In previous reports from this laboratory it was shown that the enzyme activities catalysing the synthesis of spermidine and spermine are increased during liver regeneration.[1,2] In these studies the enzymatic synthesis of spermine was demonstrated for the first time with liver preparations. Putrescine was also shown to influence the rate of spermine synthesis, stimulating it at low concentrations, whereas higher concentrations were inhibitory.[2] A report has lately appeared, showing that putrescine is a competitive inhibitor of spermine synthesis by prostatic enzyme.[3] Evidence has also been presented which suggests that a single prostatic enzyme may catalyse the production of both spermidine and spermine.[3] Therefore, our observation on the stimulation of spermine synthesis by low concentrations of putrescine seemed to be of considerable interest for understanding the mechanisms possibly regulating liver polyamine synthesis and prompted a closer study with partially purified enzyme preparations from regenerating liver. In the present study some properties of the crude enzyme preparations are described.

Material and methods. Putrescine-1,4-^{14}C dihydrochloride (specific activity 11.27 mC/mmole), DL-methionine-2-^{14}C (sp. act. 4.08 mC/mmole) and DL-methionine-1-^{14}C (sp. act. 3.54 mC/mmole) were purchased from the New England Nuclear Corporation. Unlabelled putrescine, spermidine, and spermine were supplied by Calbiochem. Before use, the radioactive putrescine and unlabelled spermidine were purified by chromatography with Dowex 50-H^+. Yeast inorganic pyrophosphatase was obtained from Sigma.

Labelled *S*-adenosylmethionine (SAM) was synthesized from ATP and either DL-methionine-2-^{14}C or DL-methionine-1-^{14}C, as described earlier,[2] and purified by chromatography with Dowex 50-H^+. Labelled 5'-deoxyadenosyl-(5'),3-aminopropyl-(1), methyl-