THE WATER-SOLUBLE CARBOHYDRATES OF *PAPAVER SOMNIFERUM* L.

BY ELI OTTESTAD, EINAR BROCHMANN-HANSSEN*, DAGRUN ÖISETH AND ARNOLD NORDAL

From the Department of Pharmacognosy, Institute of Pharmacy, University of Oslo, Norway

Received June 29, 1959

From an aqueous extract of opium poppy capsules of Yugoslavian origin, the following carbohydrates were isolated: glucose, fructose, sucrose, sedoheptulose, mannoheptulose, plus a complex polysaccharide. The polysaccharide was isolated in two different ways, by means of an ion exchange resin and by precipitation with ethanol. The first-mentioned procedure appeared to give the most genuine product, as the substance thus obtained formed a gel when sulphuric acid was added to an aqueous solution. Enzymatic hydrolysis with a pectase preparation liberated arabinose. The product isolated by ethanol precipitation gave no arabinose upon treatment with the enzyme preparation. By hydrolysis with acid, both products yielded the same monosaccharides, arabinose, xylose, rhamnose, glucose, galactose, uronic acid, and an unidentified component.

DURING the last two to three decades, increasing amounts of morphine have been manufactured directly from opium poppy capsules[1]. As the chemical composition of the poppy capsules is different from that of opium, the morphine manufacturers have to face numerous new problems when changing from opium to opium poppy capsules as their starting material.

In the manufacture of morphine and other opium alkaloids from the capsules, the starting material is usually extracted with water or with various aqueous solutions. The main difficulties in working with such extracts are caused by the huge amount of pectic substances present, but practically nothing has been done to investigate the physical properties and the chemical composition of these substances. It, therefore, seemed important from a scientific, as well as a practical point of view, to undertake a study of the water-soluble carbohydrates of the opium poppy capsules. Dried poppy capsules without seeds were used for this work. The material was obtained from Yugoslavia from a white-seeded poppy variety†.

EXPERIMENTAL

Free Sugars

Eighty g. of poppy capsules was ground to a coarse powder and macerated for 16 hours with 750 ml. of 80 per cent ethanol. The extract was filtered and the ethanol removed under reduced pressure (25 mm. Hg).

* While on leave from the University of California School of Pharmacy, San Francisco, California, U.S.A.

† The authors are indebted to Weiders Farmasöytiske A/S, Oslo, for supplying the necessary material.

The residue, amounting to 75 ml. of a brown, turbid mixture, was centrifuged and purified by precipitation with 10 ml. of basic lead acetate solution, followed by filtration and de-ionisation of the filtrate with Amberlite IR-120 and Amberlite IR-4B.

The partly purified solution was evaporated under reduced pressure to a syrupy liquid from which the free sugars were extracted with 1 to 2 times its volume of hot ethanol. The filtered solution gave positive tests for free sugars[2]. Also, reactions indicating the presence of pentoses and heptoses, such as Rosenthalers'[3], Bial's[4] and Thomas'[5] reactions, were positive.

The sugar solution was chromatographed by the descending method on Whatman No. 1 filter paper using pure sugars as reference substances. In this way, glucose, fructose, sucrose, mannoheptulose and sedoheptulose were detected. A solvent mixture consisting of ethyl acetate, acetic acid and water (3:1:3) was used for separation of the three ketoses, and the spots were developed with a ketoheptulose reagent[6,7].

Separation and identification of glucose was accomplished by means of an ethyl acetate-pyridine-water mixture (2:1:2), using aniline-phthalate[8] as a spray reagent. No galactose could be detected in the mixture.

To confirm the identification of the heptoses, sucrose and free hexoses were removed by fermentation as follows. The alcoholic solution of the sugar mixture (25 ml.) was evaporated to dryness on a steam bath and the residue dissolved in 10 ml. of water. Bakers' yeast was added, and the mixture allowed to ferment for 6 hours at 35°, after which it was filtered and the filtrate evaporated to dryness. The residue was extracted with 50 per cent ethanol, the extract filtered and chromatographed on filter paper as described above. Treatment with the ketoheptulose reagent[6,7] produced two distinct spots, corresponding to mannoheptulose and sedoheptulose, plus one very faint spot of sucrose.

Water-soluble Polysaccharides

Isolation by adsorption on an ion exchange resin. In the course of a project aimed at developing a satisfactory assay procedure for morphine in poppy capsules, the results of which will be published elsewhere, the powdered capsule material was shaken with an aqueous suspension of a cationic exchange resin of the sulphonated polystyrene type (Dowex 50-X_2, (H^+)). It was found that not only the alkaloids, but also the pectic polysaccharides were adsorbed on the resin. Because these polysaccharides caused difficulties during the elution and purification of morphine, they were removed by selective elution with a dilute aqueous pyridine solution. This eluate, which was light yellow in colour and rather viscous, was used as a source of polysaccharides. The solution was concentrated under reduced pressure to a small volume and precipitated with ethanol. The polysaccharides were filtered off and purified by repeated solution in water and precipitation with ethanol.

Larger amounts of the polysaccharides were isolated in the following way. A mixture of 400 g. of Dowex 50-X_2 (H^+), 100 g. of poppy capsules

(coarse powder) and 3 l. of water was shaken mechanically for 3 hours. It was then set aside for a short while to allow the heavy ion exchange resin to settle to the bottom, whereupon the supernatant aqueous suspension of the extracted capsule powder was poured off. By means of a few washings with water followed by careful decantation, most of the capsule powder could be removed. Finally, the ion exchange resin was transferred to a large chromatographic column fitted with a stopcock, and the polysaccharides were eluted with a 0·5 per cent solution of pyridine in water. The eluate was concentrated under reduced pressure and the polysaccharides precipitated with ethanol. The precipitate was purified as described above and dried in a vacuum desiccator. Yield: 0·70 g. of a yellowish-brown, gum-like product.

An aqueous solution of the polysaccharides (1 : 35) was brown, viscous and turbid. It had a pH of 3·1. The equivalent weight, as determined by titration with alkali, was 501. Qualitative tests for sugars and uronic acids were positive. A gel was formed when sulphuric acid was added to the aqueous solution.

The polysaccharides were hydrolysed by heating 0·70 g. of the substance with 70 ml. of N sulphuric acid in a closed container at 100° for 7 hours. During hydrolysis the solution lost its viscosity, and a grayish-brown precipitate separated out. As this precipitate gave negative tests for sugars, it was filtered off and the filtrate neutralised with 45 g. of barium carbonate. The precipitate of barium sulphate was removed by filtration and washed with water, and the combined filtrate and washings evaporated to 20 ml. under reduced pressure. Addition of ethanol produced a precipitate, separating the hydrolysate into an ethanol-soluble and an ethanol-insoluble part.

The ethanol-soluble part of the hydrolysate was concentrated and analysed by means of paper chromatography as described above. The following monosaccharides were identified: arabinose, xylose, rhamnose, glucose, galactose, traces of a uronic acid, plus an unidentified sugar component with a high R_F value.

The carbohydrates of the sugar mixture were also separated and isolated on a column of Whatman cellulose powder[8]. The isolated sugars were converted into osazones and identified by means of their micro melting points (Kofler). Authentic samples of the corresponding osazones were used as reference substances. The results are shown in Table I.

TABLE I

MELTING POINTS OF OSAZONES OF ISOLATED AND OF AUTHENTIC CARBOHYDRATES

Name of compound	Melting point of component from hydrolysis °C	Melting point of authentic sample °C
Arabinosazone	155	155
Xylosazone	167	166·5
Rhamnosazone	185–187	187
Glucosazone	202	205·5–207
Galactosazone	170–187	190–193

The column chromatography technique also revealed a compound which gave the reactions of a uronic acid. The compound is probably galacturonic acid, as no lactone could be detected by means of paper chromatography, and the oxidation product yielded a substance that melted at 215 to 217° (Kofler). Under corresponding conditions an authentic sample of mucic acid melted at 215 to 217°.

The ethanol-insoluble part of the hydrolysate gave a strong test for uronic acids. It was purified by repeated solution in water and precipitated with ethanol. It was then dissolved in 2N sulphuric acid and heated at 100° for 16 hours. The reaction mixture was filtered, neutralised with barium carbonate and filtered again. The Ba^{++} ions were removed from the filtrate by means of a cationic exchange resin (Amberlite IR-120). The solution obtained in this way was evaporated to dryness, the residue was treated with ethanol and the ethanol-soluble part analysed by means of paper chromatography, which revealed the presence of a uronic acid.

Enzymatic degradation of the polysaccharides was as follows. One hundred mg. of the polysaccharide was dissolved in 10 ml. of MacIlvaine's buffer[9] of pH 5·0. Two mg. of pectase was added and the mixture was placed in a thermostat at 35°. An additional 2 mg. of the enzyme preparation was added after 4½ hours and again after 9 hours. Samples were taken for paper chromatography after 1, 4½ and 19 hours. The last sample was found to contain free arabinose. Prolonged treatment with pectase did not split off other monosaccharides.

Isolation of polysaccharides by direct precipitation with ethanol. Four hundred g. of the coarse capsule powder was extracted twice by maceration at 30 to 40°, first with 8 l. then with 4 l. of water for 2½ hours each time. The combined aqueous extracts were filtered and the filtrate concentrated to about 30 ml. under reduced pressure (25 mm. Hg). The polysaccharides were precipitated with ethanol and collected on a sintered glass filter.

The brown, granular substance was purified by dissolving it in water and precipitating with ethanol. This was repeated and the product dissolved in 1 per cent acetic acid and again precipitated with ethanol. As the precipitate was still dark brown in colour, it was dissolved in water and the solution macerated with charcoal at 30 to 40° for 2 hours. After filtration, precipitation with ethanol and drying, the polysaccharides appeared as a grayish powder. Yield: 5·3 g.

When dissolved in water in the proportion 1:20, the product gave a brown, turbid but not viscous solution with a pH of 6·0. The product did not reduce Fehling's solution, but gave positive test with Molisch's reagent. The naphthoresorcinol-hydrochloric acid test for uronic acids[10] was also positive. The equivalent weight, as determined by titration with alkali was near 1000. Hydrolysis with acid produced a mixture which showed the same qualitative composition with respect to carbohydrates as the hydrolysate described under water-soluble polysaccharides. However, no arabinose could be detected after treatment with pectase.

REFERENCES

1. Permanent Central Opium Board, United Nations, Geneva. Report to the Economic and Social Council on Statistics of Narcotics for 1953 and the Work of the Board in 1954. United Nations Publications, Sales Number, 1954, **XI**, 4.
2. Tollens-Elsner, *Kurzes Handbuch der Kohlenhydrate*, Leipzig, 1935, p. 87.
3. Tollens-Elsner, *ibid.*, p. 158.
4. Tollens-Elsner, *ibid.*, p. 112.
5. Tollens-Elsner, *ibid.*, p. 113.
6. Klevstrand and Nordal, *Acta chem. scand.*, 1950, **4**, 1320.
7. Bevenue and Williams, *Arch. Biochem. Biophys.*, 1951, **34**, 225.
8. Hough, Jones and Wadman, *J. chem. Soc.*, 1949, 2511.
9. MacIlvaine, *J. biol. Chem.*, 1921, **49**, 183.
10. Patridge, *Nature, Lond.*, 1949, **164**, 443.