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SOME CARBOHYDRATES OF LOW MOLECULAR WEIGHT PRESENT IN *Cannabis sativa* L.*

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ABSTRACT

The following sugars and sugar alcohols have been isolated from *Cannabis sativa* L. and identified: arabinose, D-*manno*-heptulose, *altro*-heptulose (sedoheptulose), D-*glycero*-D-*manno*-octulose, *myo*-inositol, 1L-2-*O*-methyl-*chiro*-inositol (quebrachitol), glycerol, erythritol, arabinitol, and xylitol. The sugars were isolated from an extract after fermentation with yeast, and the alcohols were isolated from an unfermented extract.

INTRODUCTION

Except for the reported occurrence[1,2] of quebrachitol as a constituent of *Cannabis sativa* L., this plant has not previously been thoroughly analyzed for carbohydrates of low molecular weight. A comprehensive study of the constituents of *Cannabis sativa* L. has been initiated by the Division of Narcotic Drugs, United Nations, Geneva. The present investigation represents a part of that research programme.

RESULTS AND DISCUSSION

Our interest was focused on the non-fermentable monosaccharides and the acyclic and cyclic polyhydric alcohols. Small amounts of several oligosaccharides were also present in the plant, but these were not investigated.

The following compounds were isolated and fully or partly characterized: arabinose, D-*manno*-heptulose, *altro*-heptulose, D-*glycero*-D-*manno*-octulose, *myo*-inositol, 1L-2-*O*-methyl-*chiro*-inositol, glycerol, erythritol, arabinitol, and xylitol. As expected, an unfermented extract contained substantial quantities of glucose, fructose, sucrose, and also some raffinose, but these sugars were not investigated further.

The aqueous, ethanolic extract of the plant material constituted a complex mixture which required extensive purification before isolation of the substances of

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interest. The bulk of the carbohydrate material (glucose, fructose, sucrose) was removed by fermentation with bakers' yeast. When a mixture of glucose, fructose, and sucrose was fermented under identical conditions, no artefacts were detected. This result accords with those obtained by Begbie and Richtmyer[3] and by Bevenue *et al.*[4], and indicates that the subsequently investigated sugars originated from the plant material.

A partial fractionation of the sugars on a carbon–Celite column was followed by further purification of the appropriate fractions by preparative paper chromatography (p.p.c.).

The ketoses isolated were subjected to p.c., g.l.c., and paper electrophoresis. D-*manno*-Heptulose and D-*glycero*-D-*manno*-octulose were obtained pure, but p.c. of *altro*-heptulose showed it to be contaminated with 2,7-anhydro-*altro*-heptulose (sedoheptulosan), which is easily formed from *altro*-heptulose[5].

On oxidation with two molar equivalents of lead tetra-acetate, erythrose was formed from both heptuloses, and ribose from the octulose. This confirms the *erythro* configuration at positions 5 and 6 in the heptuloses, and the *ribo* configuration at positions 5–7 in the octulose. The results are in agreement with the mechanism suggested for the oxidation of ketoses with lead treta-acetate[6]. 2,7-Anhydro-*altro*-heptulose was not degraded by lead tetra-acetate under the conditions used, and the presence of this compound in the sedoheptulose fraction would therefore not influence the result. Oxidation of the octulose methyl pyranoside with one molar equivalent of sodium metaperiodate in the cold, followed by reduction with sodium borohydride and subsequent acid hydrolysis of the glycoside, gave *manno*-heptulose as the major product, thus confirming the configuration at C-3–C-6 of the octulose.

Although fermentation did not yield higher-carbon ketoses or arabinose, it has been shown to give rise to several polyhydric compounds[7]. Therefore, the fermentation step was avoided for the investigation of the alcohols. After acid hydrolysis, the reducing compounds were removed by adsorption[8] on Dowex-1(HO^-) resin, and the residual mixture of alcohols was fractionated by p.p.c. Xylitol and arabinitol were subsequently separated as their borate complexes on a column of Biorad AG-1 resin in the borate form[9]. The amount of arabinitol obtained was too small to allow determination of the optical rotation, and thus it could not be established whether the arabinitol belonged to the D or L series. As far as the authors are aware, arabinitol (D form) has previously been isolated only twice[10,11] and xylitol once[3] from higher plants.

EXPERIMENTAL

General methods. — Paper chromatography (p.c.) was effected on Whatman No. 1 paper, and preparative p.c. on Whatman No. 3MM paper washed with water, using 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*) 1-butanol–pyridine–water (6:4:3); (*D*) same as (*C*), but with molybdate impregnation of the paper before the

run[12]; (*E*) ethyl acetate–pyridine–acetic acid–water (5:5:1:3); (*F*) ethyl acetate–pyridine–water saturated with boric acid[13] (60:25:20). Zone electrophoresis was performed on Munktell No. 302 filter paper at ~40 volts/cm in 50mM sodium tetraborate (pH 9.2).

Staining reagents were: (*a*) saturated, aqueous aniline oxalate to which 10% glacial acetic acid was added for the staining of borate complexes (reducing sugars); (*b*) silver nitrate–sodium hydroxide (polyols)[14]; (*c*) trichloroacetic acid–orcinol (ketoses)[15]; (*d*) periodate–benzidine (polyols)[16]. When using solvent systems *D* and *F*, staining was done with reagents *b* and *d*, respectively.

G.l.c. was carried out in the following systems, using helium as the carrier gas in system 3, and nitrogen in systems 1, 2, and 4. 1, 3% SE 52 on Gas-Chrom *O*, for *O*-trimethylsilyl derivatives of sugars and alcohols[17] (150 × 0.31 cm). 2, 13.1% EGS on Varaport 30, for *O*-trimethylsilylated sugars and alcohols[17] (150 × 0.31 cm). 3, 3% XE 60 on Chromosorb W 80/100, high performance, for trifluoroacetates[18] of alcohols (170 × 0.31 cm). 4, Apiezon L 9.5% on Varaport 30 for *O*-trimethylsilyl derivatives of alcohols[19] (180 × 0.31 cm).

Unless otherwise stated, optical rotations were measured in water.

Isolation and partial purification of the sugar–polyhydric alcohol fraction. — The carbohydrates were extracted from leaves and attached stems from *Cannabis sativa* L. (grown in the Botanical Garden of the University of Oslo from Swiss seeds) by boiling the plant material with 65% ethanol for 20 min. Batches (100 g) of the extract obtained after filtration and evaporation were treated with lead acetate, deionized, and concentrated to a thick syrup (73 g) which contained (p.c.) large proportions of glucose, sucrose, and fructose. In a typical experiment, the syrup (45 g) was subjected to fermentation with bakers' yeast, as previously described[7]. After centrifugation and removal of residual protein with lead acetate, the mixture was deionized and concentrated to a thick syrup (15 g). P.c. then revealed reducing compounds corresponding to arabinose, *manno*-heptulose, *altro*-heptulose, *glycero*-D-*manno*-octulose, and several as yet unidentified spots in the oligosaccharide region.

A column (2.5 × 40 cm) of carbon–Celite[20] was loaded with 2.2 g of the fermented mixture. Arabinose and the heptuloses were eluted with water, and the octulose with 3% ethanol in water. The fractions containing the respective ketoses were combined and further purified by p.p.c. (systems *A* and *B*). The mixture resulting from fermentation and purification (15 g) yielded, after column chromatography and p.p.c., D-*glycero*-D-*manno*-octulose (15 mg), D-*manno*-heptulose (8 mg), and *altro*-heptulose (3.8 mg, contaminated with 2,7-anhydro-*altro*-heptulose).

The fractions containing arabinose from the carbon column were not purified further, and the compound was identified by p.c. (systems *A*, *B*, and *C*), g.l.c. (systems 1 and 2), and paper electrophoresis in comparison with an authentic sample.

The higher-carbon sugars. — The three ketoses were identified by p.c. and paper electrophoresis in the same systems as for arabinose, in comparison with authentic samples. Identification was further established by oxidative degradation to the respective, lower-carbon sugars.

D-*glycero*-D-*manno*-Octulose, $[\alpha]_D$ +25.6° (*c* 0.5, methanol). The octulose (10 μmoles) was oxidized with lead tetra-acetate by the method of Sephton and Richtmyer[21], and the product was hydrolyzed as previously described[22]. Neutralization was effected with 5% dioctylamine in chloroform. This process was repeated until the aqueous layer was negative for sulphate. P.c. (systems *A*, *B*, and *C*) of the deionized solution revealed the main oxidation product to be ribose.

The octulose (12 μmoles) was transformed into the methyl pyranosides according to Bollenback[23]. The product was oxidized at −2° with an equimolar amount of aqueous sodium metaperiodate and then was reduced and hydrolyzed as previously described[22]. Sulphuric acid was removed with 5% dioctylamine in chloroform, and the deionized product was shown to be *manno*-heptulose by p.c. (systems *A*, *B*, and *C*).

D-*manno*-Heptulose, $[\alpha]_D$ +29.3° (*c* 0.8, methanol). The heptulose was oxidized with lead tetra-acetate as described above, and the product was subjected in sequence to hydrolysis, neutralization, and reduction with sodium borohydride. After removal of boric acid by successive evaporations with methanol, and deionization, the main degradation product was erythritol (p.c.; systems *A*, *B*, and *C*).

altro-Heptulose, when treated similarly, also gave erythritol (p.c.).

Purification of the alcohol fraction. — Hydrolysis of oligosaccharides and removal of reducing compounds. The unfermented mixture (20 g) resulting from the isolation and partial purification of the sugar–alcohol fraction was boiled for 4 h with 2M hydrochloric acid (200 ml). The filtrate was diluted with water (1 litre) and treated on a boiling water-bath with Dowex-1(HO^-) resin (450 ml) for 6 h. The resin was filtered off and washed with distilled water. The filtrate and washings, which were shown not to contain reducing sugars by the phenol–sulphuric acid test[24], were combined and concentrated to a syrup (1.5 g). P.c. revealed at least five non-reducing polyhydric alcohols with mobilities corresponding to glycerol, erythritol, *myo*-inositol, 1L-2-*O*-methyl-*chiro*-inositol, and arabinitol and/or xylitol.

Glycerol had the same chromatographic mobility as the authentic substance when subjected to p.c. (systems *A*, *B*, and *C*) and to g.l.c. (systems 1 and 2).

The mixture of the other alcohols was partly resolved by p.p.c. (system *B*), the various alcohols being eluted with water.

The erythritol fraction (28 mg) was impure, but erythritol was the main component, as indicated by p.c. (systems *A*, *B*, and *D*), g.l.c. (systems 1 and 2), and paper electrophoresis.

myo-Inositol crystallized from aqueous ethanol; yield 30 mg, m.p. 223.5–225° and mixed m.p. 222–224°. The alcohol had the same chromatographic mobility by p.c. and g.l.c. (systems *A*, *B*, and *D*; 1 and 2) as an authentic sample.

1L-2-*O*-Methyl-*chiro*-inositol (quebrachitol) (40 mg) crystallized from water and had m.p. and mixed m.p. 192–193°, $[\alpha]_D$ −75.3° (*c* 1.18). Chromatographic mobilities (systems *A*, *B*, and *D*; 2 and 3) were identical to those of authentic substance.

Column chromatography of the xylitol–arabinitol fraction. — Xylitol and arabinitol were not resolved by p.c. The pentitol fraction (7 mg) was dissolved in

50mM sodium tetraborate (0.2 ml), and applied to a column (11 × 52 cm) of Biorad AG–I x4 resin (400 mesh). Prior to use, the resin was fractionated by settling, according to the method of Walborg and Lantz[25]. Removal of contaminants and equilibration were performed as described by Ohms *et al.*[26], with the exception that BRIJ-35 was omitted, and the packing of the column was done at room temperature. The pentitol mixture was eluted at 12 ml/h with the borate–chloride system recommended by Vining and Taber[9]; 3-ml fractions were collected. Polyhydric alcohols were localized spectrophotometrically[27].

Arabinitol and xylitol were identified by p.c. (systems *B*, *D*, *E*, and *F*), and arabinitol also by g.l.c. (systems 2 and 4) and by paper electrophoresis.

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