

THE CYCLOHEXITOL OF JUNIPER FRUIT

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Of the nine possible stereoisomers of cyclohexitol (inositol), five have so far been found in nature in the free form or in the form of derivatives: myoinositol, d- and l-inositols, scyllitol, and mycoinositol. The latter has been found only once [1], in the wood of the New Zealand tree *Phyllocladus trichomanoides* in the form of the noncrystallizing 1-O-methyl derivative. Consequently, the detection of this cyclohexitol in a plant growing in the USSR is of interest from the point of view of the chemical taxonomy of plants.

Among the samples of plants collected by P. S. Massagetov for our investigations [2] were the fruit of the juniper *Juniperus foetidissima* W., growing in Armenia. We have investigated the sugars contained in it. Paper chromatography of an extract from this fruit showed the presence in it of only one reducing sugar, which we identified as D-fructose. After the fermentation of the fructose with yeast, we detected by chromatography in the extract three substances revealed by potassium periodate and benzidine [3], but not reacting with the usual reagents for aldoses or ketoses. By evaporating the extract purified with lead acetate and deionized, we obtained a transparent resin which did not crystallize or give crystalline derivatives. When the resin was oxidized with periodate the latter was consumed but no formaldehyde was detected; neither acid nor alkaline hydrolysis caused changes in a paper chromatogram of this resin. After the resin had been heated with hydriodic acid under relatively mild conditions the strongest spot (R_{pinitol} 1.09) disappeared, and in place of it a new spot appeared somewhat below the original one (R_{pinitol} 0.75), but above the spot of d-inositol (R_{pinitol} 0.51); the other two spots remained in their original positions. This permitted the assumption that the spot with R_{pinitol} 1.09 represented an ether derivative of some polyhydric alcohol, and the resin was studied in more detail.

By chromatography on a column of regenerated cellulose, the resin yielded a noncrystallizing chromatographically homogeneous fraction with R_{pinitol} 1.09. Heating this fraction with hydriodic acid led to a crystalline substance with the composition $\text{C}_6\text{H}_{12}\text{O}_6$ (I) with R_{pinitol} 0.75, optically inactive, and giving a number of crystalline derivatives. The melting point of the hexaacetate (II) of this substance coincided with that of the corresponding derivative of mycoinositol, a cyclohexitol synthesized by Dangschat and Fischer [4] and then by Angyal [5] by two different methods starting from natural cyclohexitols, and isolated by Harvey [1] from plant material. However, the melting point of substance (I) was higher than given by the German authors for their semisynthetic product. From the configuration of mycoinositol (1, 2, 4, 5/3, 6) it was to be expected that it could form a diisopropylidene derivative. In actual fact, the acetonation of our substance (I) by Dangschat's method as described by Angyal [6] gave us a di-O-acetyl-di-O-isopropylidenehexitol (III); the careful hydrolysis of this compound yielded a di-O-acetylhexitol (IV), and its saponification yielded a di-O-isopropylidenehexitol (V). In addition, the hexa-O-benzoyl derivative (VI) was obtained.

Since not one of the derivatives of mycoinositol apart from the hexaacetate has been described in the literature, the identification of substance (I) as mycoinositol was kindly carried out, at our request, in the laboratory of Prof. Angyal (Kensington, Australia).

Experimental

Chromatography on paper (Whatman no. 1) was carried out by the ascending method at 20° C in the acetone-1-butanol-water (3 : 1 : 1) system, with the reagents aniline phthalate, α -naphthylamine- H_3PO_4 , and potassium periodate-benzidine. The samples for analysis were dried at 100° C and 1 mm. The analyses were carried out in the microanalytical laboratory of VNIKhFI [All-Union Scientific-Research Institute for Medicinal Plants] under the direction of V. V. Kolpakova. The melting points are uncorrected.

Extraction and working up of the extract. One hundred grams of the dried and comminuted fruit (berries) of the juniper were extracted in a Soxhlet apparatus with 90% methanol for 18 hr. The methanol was distilled off from the filtered extract, finally under a slight vacuum, and the residue (55 g) was diluted with 100 ml of water and shaken with 50 ml of chloroform. After the separation of the chloroform, the aqueous layer was precipitated with a solution of lead acetate, the bulk of the precipitate was filtered off and washed with water, and the excess of lead in the filtrate was precipitated with sulfuric acid. Chromatography of the filtrate on paper showed the presence in it of fructose—absence of a coloration with aniline phthalate, yellow spot with α -naphthylamine- H_3PO_4 at the level of the fructose spot. By Bertrand's method, 4.6 g of fructose was found in the solution. From an aliquot part of the extract a phenylglucosazone with mp 208-209° C (50% ethanol) was obtained. To the extract was added 20 g of compressed yeast; fermentation was terminated after a day and the yeast was filtered off. On a chromatogram the filtrate did not give the spot for fructose, but potassium periodate-benzidine showed three spots: R_{pinitol} 0.46, 1.09, 1.63. The solution was treated with the cation-exchanger K_b-4 (H^+), the anion-exchanger AN-2F (HCO_3^-), and was evaporated in vacuum. This gave 5.6 g of a transparent brown resin.

Separation of the resin. A solution of 2.8 g of the resin in 3 ml of hot water was stirred with 3 g of regenerated cellulose powder, and the semidry mass was placed on the surface of a layer of regenerated cellulose (30 g) filling a column (20 mm); the column was eluted with a mixture of ethyl acetate, methanol, and water (7 : 2 : 1), and 50-ml fractions were collected. Paper chromatography with the system described previously after the evaporation of the individual fractions gave the following results:

<u>Fraction</u>	<u>Spot, R_pinital</u>	
1 and 2	—	—
3	1.63 (weak)	—
4	1.63 (strong)	—
5	1.63 (weak)	1.09 (medium)
6-10	—	1.09 (strong)
11	—	1.09 (weak)
12	—	—

Fractions 6-10 were combined and the solution was treated with a small amount of activated carbon and evaporated in vacuum. This gave a resin with a yield of 1.1 g (or 39%, calculated on the starting material).

The cyclohexitol (I). A mixture of 1.1 g of the resin obtained (R_pinital 1.09) and 5.5 ml of hydriodic acid (45%) was heated in the boiling water bath for 3 hr, after which the solution was diluted with 100 ml of water and neutralized with lead acetate. The lead iodide was filtered off and the filtrate was evaporated in vacuum to a viscous syrup, which immediately crystallized. To eliminate the lead iodide that had remained in the solution and had separated out on evaporation, the mass was dissolved in 40 ml of boiling 75% ethanol and the solution was boiled with carbon. On cooling, nodules of thin prisms separated from the colorless filtrate. Yield 0.51 g, mp 301-303° C (decomp., capillary inserted at 275° C). Literature data for cycloinositol—mp 285° C [4].

Found, %: C 39.91; H 6.75. Calculated for C₆H₁₂O₆, %: C 39.99; H 6.71.

Substance (I) was acetylated with Ac₂O in pyridine by the usual method. Compound (II) was isolated with mp 182° C (75% methanol). Literature data for mycinositol hexaacetate with mp 179-182° C [5].

Found, %: C 50.07; H 5.78; CH₃CO 59.70. Calculated for C₁₈H₂₄O₁₂, %: C 50.00; H 5.60; CH₃CO 59.73.

The hexabenzoate of (I), (VI), was obtained by the action of benzoyl chloride on a solution of (I) in pyridine, mp 254-256° C [methanol-acetone (3 : 2)].

Found, %: C 71.60; H 4.37. Calculated for C₄₈H₃₆O₁₂, %: C 71.64; H 4.51.

Acetonation of substance (I) by Dangschat's method; preparation of the derivative (III). A solution of 1.5 g of (I) in 10 ml of glacial acetic acid was treated with 10 g of fused zinc chloride and 100 ml of acetone, and the mixture was boiled for 5 hr; after cooling, 20 ml of pyridine was added, whereupon a crystalline precipitate immediately deposited, and then the mixture was placed in the refrigerator. After a day, the precipitate was filtered off with suction and washed with acetone. The acetone was distilled off from the filtrate in vacuum and the residual liquid syrup was mixed with 10 ml of acetic anhydride. On the following day, the mixture was diluted with 100 ml of ethyl acetate (in place of the chloroform proposed by Angyal [6]) and 25 ml of water, the aqueous layer was separated off, and the ethyl acetate was washed with water (5 × 30 ml) until the reaction for Zn²⁺ with K₄Fe(CN)₆ was negative, and was dried with MgSO₄. The ethyl acetate was then distilled off in vacuum, the residual crystalline mass was triturated with 5 ml of 75% methanol, and the crystals were filtered off with suction (0.75 g) and recrystallized from 15 ml of 75% methanol. The yield of (III) was 0.5 g, mp 205-206° C.

Found, %: C 55.93; H 6.87; CH₃CO 25.43. Calculated for C₁₆H₂₄O₈, %: C 55.80; H 7.02; 2CH₃CO; 24.99.

Splitting off of the isopropylidene groups from (III); production of (IV). A mixture of 0.5 g of (III) and 10 ml of 30% acetic acid was heated in the boiling water bath for 40 min, the solution was evaporated in vacuum, and the crystalline residue was recrystallized from acetone. The yield of (IV) was 0.25 g, mp 168-170° C.

Found, %: C 45.26; H 6.23. Calculated for C₁₀H₁₆O₈, %: C 45.45; H 6.11.

Saponification of the acetyl groups of (III); preparation of (V). Compound (III) (0.2 g) was treated with 0.2 N CH₃ONa in methanol (5 ml); after 30 min, the solution was neutralized with acetic acid, the methanol was distilled off, the residue was treated with 5 ml of water and 30 ml of ether, the water was bound with sodium sulfate, and the ether was distilled off, whereupon the residue immediately crystallized. The yield of (V) was 0.12 g (mp 185-187° C) (CCl₄-petroleum ether).

Found, %: C 55.31; H 7.85. Calculated for C₁₂H₂₀O₆, %: C 55.36; H 7.75.

Oxidation of (I) with periodate. When (I) was oxidized with an excess of 0.1 M NaIO₄ at 20° C in the absence of light, the consumption of the oxidizing agent after 23 hr was 6.1–6.2 moles per mole of (I). No formaldehyde was formed.

Conclusions

An amorphous substance isolated from the fruit of the juniper *Juniperus foetidissima* W. is a mycoinositol derivative, probably an O-methylmycoinositol. This is the second case of the detection of mycoinositol in plants. A number of derivatives of the cyclohexitol have been described.

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