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THE OCCURRENCE OF D-*RIBO*HEXULOSE IN *ITEA ILICIFOLIA ITEA VIRGINICA*, AND *ITEA YUNNANENSIS*

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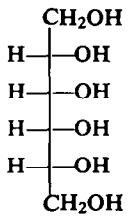
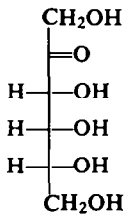
Abstract—D-*ribo*Hexulose (D-allulose; D-psicose) occurs together with allitol as a major component of the leaves, stems, and flowers of *Itea* plants. The isolation of crystalline allitol in high yield from the leaves of *I. ilicifolia* is described. Sucrose and *myo*-inositol have been isolated as minor constituents of the flowers of *I. ilicifolia*.

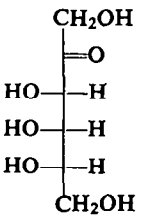
THE OCCURRENCE of about 1·6 per cent (dry weight) of allitol (I) in the leaves, and 0·5–1·2 per cent in the stems of *I. ilicifolia* and *I. virginica*, evergreen shrubs (family, Saxifragaceae; group, Escallonia) native to Eastern Asia and North America respectively, has been reported by Plouvier,[1] who employed an acetone extraction procedure to remove the hexitol. When we isolated allitol directly from an alcoholic extract of *I. ilicifolia* leaves, a 6 per cent yield of pure, crystalline material was obtained based on the dry weight of the leaves, and this plant is clearly an excellent source of this heretofore rare hexitol. Furthermore, paper chromatography of de-ionized, alcoholic extracts of the leaves, stems, and flowers of this plant indicated the presence of an hexulose with the characteristics of *ribo*hexulose (II and III) as a major component in addition to allitol (I). The occurrence of *myo*-inositol, sucrose, glucose,



(I)



(II)



(III)

fructose, and glycerol (?) as minor components was also indicated. Conclusive identification of the unknown hexulose as D-*ribo*hexulose (II), a syrup, was achieved by the isolation of crystalline di-*o*-isopropylidene, phenylosazone, and phenylosotriazole derivatives.

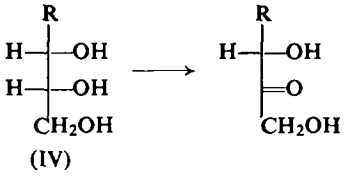
Comparison of alcoholic extracts of the flowers and leaves of *I. ilicifolia* showed that the former contained less allitol, but more sucrose and glucose, in relation to *ribo*hexulose. Separation of an alcoholic extract of the flowers on a column of cellulose by partition chromatography led to the isolation and characterization of D-*ribo*hexulose (II), sucrose, and *myo*-inositol. Somewhat less allitol was detected in the leaves of *I. virginica* than in those of *I. ilicifolia*, but the proportions of *ribo*hexulose were similar; the hexulose was characterized

[1] V. PLOUVIER, *Compt. rend.* **249**, 2828 (1959).

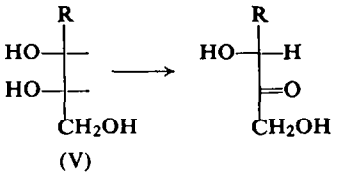
by the preparation of crystalline di-*o*-isopropylidene D-*ribo*hexulose directly from an alcoholic extract.

Unlike the other 2-hexuloses, *ribo*hexulose has not previously been found in Nature, although it has been detected in trace amounts in distillery slops,[2] where it probably arises as an artefact by the action of lime used in sugar manufacture. Several workers[3–7] have demonstrated that D-*ribo*hexulose is formed under mildly alkaline conditions from D-glucose, probably through the 2,3-enediol of D-fructose.

Itea plants are exceptional in that the leaves contain unusually small quantities of sucrose, glucose, and fructose and in that allitol and D-*ribo*hexulose, which have not been found in any other genus, occur together as major components. *Itea* must therefore have an unusual metabolic system and in this respect the occurrence of D-*ribo*hexulose (II) rather than its L-isomer (III) is somewhat unexpected when the known biochemical oxidation of allitol is considered. Steiger and Reichstein[8] prepared L-*ribo*hexulose (III) by the oxidative fermentation of allitol by the sorbose bacterium, *Acetobacter xylinum*, none of the D-isomer (II) being formed. According to the Bertrand rule,[9] the oxidation to a keto group of a hydroxyl group on the penultimate carbon of a polyol requires the presence, on three contiguous carbon



(IV)



(V)

atoms, of two secondary hydroxyl groups, with either the D-erythro (IV) or L-erythro (V) configuration, and a primary hydroxyl group. In the case of allitol (I), where both of the required configurations are present, oxidation of the D-erythro hydroxyls by *A. xylinium* is clearly preferred to the L-erythro configuration. Oxidation of allitol (I) by *A. suboxydans* would also be expected to give L-*ribo*hexulose (III) since this organism is specific for the D-erythro (IV) configuration.[10,11] D-*ribo*Hexulose may well therefore be the precursor of allitol in *Itea*, rather than the reverse, and this biosynthetic problem is now under investigation.

[2] F. W. ZERBAN and L. SETTLER, *Ind. Eng. Chem.* **34**, 1180 (1942).
[3] C. A. LOBRY DE BRUYN and W. ALBERDA VAN EKENSTEIN, *Rec. trav. chim.* **14**, 195 (1895).
[4] H. C. S. DE WHALLEY, N. ALBON and D. GROSS, *Analyst* **76**, 287 (1951).
[5] F. SCHNEIDER and G. A. ERLEMANN, *Zucker-Beih.* **3**, 41 (1951).
[6] F. W. ZERBAN, L. SATTLER, G. ROSENTHAL and A. GLAUBACH, *Sugar* **47**, [2], 33 (1952).
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[8] M. STEIGER and T. REICHSTEIN, *Helv. Chim. Acta* **18**, 790 (1935).
[9] G. BERTRAND, *Ann. Chim. Phys.* [8], **3**, 181 (1904).
[10] R. M. HANN, E. B. TILDEN and C. S. HUDSON, *J. Am. Chem. Soc.* **60**, 1201 (1938).
[11] J. V. KARABINOS, *Advances in Carbohydrate Chem.* **7**, 104 (1952).

EXPERIMENTAL

Spurs of *I. ilicifolia* bearing flowers on pendulous racemes were collected in early October at The Old Down House, Tockington, near Bristol, by kind permission of Mrs. R. Bernays; within 2 hr the samples were placed in boiling ethanol to de-activate enzymes. Cuttings of *I. virginica* and *I. yunnanensis*, not bearing flowers, were kindly supplied by The Director, Royal Botanic Gardens, Kew.

Partition chromatography was carried out by the descending method on Whatman No. 1 filter paper using butan-1-ol–pyridine–water (10:3:3 v/v) as mobile phase. The following spray reagents were used for the detection of polyols, reducing sugars, and ketoses respectively: (1) a 4% w/v solution of silver nitrate in ammonium hydroxide,[12] (2) a 2% w/v solution of *p*-anisidine hydrochloride in butan-1-ol containing a trace of stannous chloride,[13] and (3) a 5% w/v solution of orcinol in butan-1-ol containing 2% v/v concentrated hydrochloric acid.[13] The relative amounts of components present were estimated visually by comparing the size and intensity of the spots on the paper chromatograms and in order of increasing magnitude the symbols used are *t* (trace), +, + +, and + + +.

Unless stated otherwise, all evaporations were carried out under reduced pressure. Optical rotations were determined at 24°.

The Characterization of D-Ribo*hexulose in Leaves of* I. ilicifolia

The leaves (6 g, fresh weight) were cut into small pieces and extracted with boiling methanol (3 × 100 ml) over 24 hr. The filtered extract was then concentrated to a small volume (ca. 1 ml), diluted with water (15 ml), and extracted with ether (3 × 15 ml) to remove fats and other extraneous matter. A portion (8 ml) of the residual, acidic aqueous solution (*A*) was passed through a column of Amberlite IR-45 (OH^-) ion-exchange resin and the neutral effluent concentrated to a syrup which partially crystalized. The crystals were isolated by trituration with alcohol and after recrystallization from aqueous alcohol, allitol (24 mg) was obtained with m.p. 149–150° (lit.[1,14] 149°, 150–151°). The alcoholic washings were clarified by centrifugation and then evaporated to a dry syrup (70 mg). Subsequent paper chromatography of this syrup suggested the presence of *myo*-inositol (*t*), sucrose (+), glucose (*t*), allitol (+), fructose (*t*), *ribo*hexulose (+ + +), and glycerol (*t*), by comparison with authentic specimens. The syrup was treated with a mixture of anhydrous acetone (4·5 ml), anhydrous copper sulphate (0·5 g), and concentrated sulphuric acid (0·01 ml) with continuous shaking for 48 hr.[8] After clarification on the centrifuge, the solution was neutralized by shaking with potassium carbonate (0·3 g) for 2 hr. again centrifuged, and then evaporated to a syrup. A solution of this syrup in ether (20 ml) was shaken with 20% w/v potassium hydroxide solution (3 × 2 ml), then dried over anhydrous sodium sulphate and evaporated to a brown syrup (44 mg) which was distilled at 0·005 mm/90° (bath temp.) on to a cold finger. The colourless syrup cyrstallized on triturating with n-heptane and after recrystallization from light petroleum (b.p. 40–60°) gave di-*o*-isopropylidene-D-*ribo*hexulose (7·8 mg), m.p. 57–58·5°, $[\alpha]_D$-88° (*c*, 0·16 in acetone), with an infra-red spectrum identical to that of an authentic specimen (m.p. 57–58·5°; $[\alpha]_D - 98°$)[14] (Found: C, 55·5; H, 7·57. Calc. for $C_{12}H_{20}O_6$: C, 55·4; H, 7·69%).

Another portion (8 ml) of the aqueous solution (*A*) was mixed with an excess of Bio-Deminrolit mixed-bed ion-exchange resin and boiled under reflux for 6 hr in order to destroy

[12] S. M. PARTRIDGE, *Nature* **158**, 270 (1946).
[13] L. HOUGH, J. K. N. JONES and W. H. WADMAN, *J. Chem. Soc.* 1702 (1950).
[14] M. STEIGER and T. REICHSTEIN, *Helv. Chim. Acta* **19**, 184 (1936).

reducing sugars and sucrose.[15] After filtration and concentration of the solution, a light brown syrup was obtained which partially crystallized. Trituration with ethanol (3 ml) afforded allitol (42 mg) with m.p. 149°. Paper chromatography of the alcohol solubles indicated the presence of allitol (+ + +), *myo*-inositol (*t*), and an unidentified polyol (*t*) with $R_{allitol}$ 1·8.

The Separation and Identification of Various Components of the Flowers of I. ilicifolia

The flowers (30 g, fresh weight) with stalks removed were extracted with boiling ethanol (200 ml) and then boiling methanol (200 ml) to give an insoluble residue (7·2 g, air-dried) and a dark syrup (3·2 g) on evaporation of the combined alcoholic extracts. A portion (2·9 g) of this syrup was shaken with water (20 ml), the mixture clarified on the centrifuge, and the supernatant passed through a column of Amberlite IR-400 (CO_3^{2-}) ion-exchange resin. The effluent was concentrated to a small volume (*ca.* 5 ml) and examined by paper chromatography when the presence of *myo*-inositol (*t*), sucrose (+), glucose (+), allitol (+), fructose (*t*), and *ribo*-hexulose (+ + +) was suggested; no glycerol was detected. To the aqueous concentrate (5 ml), ethanol (20 ml) was added and the amorphous precipitate removed on the centrifuge to give a clear green supernatant. The latter again clouded on concentration and, after the addition of more ethanol (20 ml), was clarified on the centrifuge. By repeating this process a third time, a clear green–brown syrup (2·2 g) was obtained. A portion (1·6 g) of this syrup was dissolved in a little 10% aqueous ethanol, mixed with fresh cellulose powder to give a slurry, and then applied to the top of a cellulose column[13] (50 cm long; 3·2 cm diameter). The mixture was fractionated by passing the mobile phase, water–propan-1-ol (1:4 v/v), through the column at a rate of 10–11 ml/hr. After examining the eluate on paper chromatograms, six fractions were obtained:

Fraction 1. Concentration gave a syrup (503 mg) containing essentially *ribo*hexulose. A portion (100 mg) was dissolved in water (2 ml) containing sodium acetate (300 mg), phenylhydrazine hydrochloride (300 mg), and a trace of sodium hydrogen sulphate and heated in a boiling-water bath. A pale yellow precipitate appeared after 6 min and after a total of 15 min the phenylosazone (69 mg) was isolated and recrystallized from 30% aqueous ethanol when it had m.p. 165–170°, $[\alpha]_D$ −60° (c, 1·0 pyridine) and an infra-red spectrum that was identical with that of an authentic specimen of D-ribohexose phenylosazone (lit.[14, 16, 17] m.p. 173–174°, 162–163°, 178°; lit.[16] $[\alpha]_D$ −78·1 → 67·1° (pyridine). The phenylosotriazole derivative was obtained on treating the phenylosazone (20 mg) with a solution of copper sulphate (15·4 mg) in water (2·4 ml) at 100° for 30 min and then concentrating the solution. Recrystallized from water, the product (3 mg) had m.p. 133·5° (lit.[16, 18] m.p. 132–134°, 134–135°).

Another portion (300 mg) of the syrupy D-allulose was converted as described above into the crystalline di-*o*-isopropylidene derivative (136 mg, 31%), m.p. 56·6–58·5°, mixed m.p. 57–58°, $[\alpha]_D$ −92° (*c*, 1·0 in acetone).

Fractions 2, 3, and 4. These contained, as indicated by paper chromatograms, mixtures of glucose, allitol, fructose, and *ribo*hexulose and were not further examined.

Fraction 5. Concentration yielded a syrup which crystallized on keeping and gave,on recrystallization, sucrose (72 mg) with m.p. and mixed m.p. 186°; the infra-red spectrum was identical with that of an authentic specimen.

[15] J. D. ANDERSON, P. ANDREWS and L. HOUGH, *Biochem. J.* **81**, 149 (1961).
[16] M. L. WOLFROM, A. THOMPSON and E. F. EVANS, *J. Am. Chem. Soc.* **67**, 1793 (1945).
[17] F. W. ZERBAN, *J. Assoc. Offic. Agr. Chemists* **24**, 656 (1941).
[18] W. T. HASKINS, R. M. HANN and C. S. HUDSON, *J. Am. Chem. Soc.* **67**, 939 (1945).

Fraction 6. Crystals (7 mg) were obtained on concentration and after recrystallization from aqueous ethanol had m.p. 221–222° and gave an infra-red spectrum identical with that of *myo*-inositol.

The Isolation of Allitol from I. ilicifolia

The leaves (80 g, fresh weight) without stems were exhaustively extracted with methanol (4 × 500 ml) to give an insoluble residue (25·4 g) and, upon evaporation, a dark green, syrupy extract (10·8 g). Trituration of the latter with warm ethanol (80 ml) gave crystalline material which was removed on the centrifuge. The crystals were stirred with several portions of cold ethanol and the supernatant liquors decanted. The crude product (3·1 g), m.p. 148–149°, was purified by recrystallization from aqueous ethanol giving white crystals of allitol (2·8 g) with m.p. 149–150°. Reaction of the hexitol with benzaldehyde in the presence of hydrochloric acid gave di-*o*-benzylidene-allitol, m.p. 242–245° (lit.[19] m.p. 249–250°). Acetylation of the hexitol with acetic anhydride–pyridine yielded crystalline allitol hexa-acetate with m.p. 61–62° (lit.[20] m.p. 61°) (Found: C, 50·0; H, 6·05. Calc. for $C_{18}H_{26}O_{12}$: C, 49·8; H, 6·0%).

The Characterization of D-Ribo*hexulose in Leaves of* I. virginica

Three leaves were extracted with boiling methanol, the extract evaporated to a syrup to which water (10 ml) was added and the mixture extracted continuously with ether. The acidic aqueous solution was then passed through a column of Amberlite IR-400 (CO_3^{2-}) ion-exchange resin and the eluate concentrated to a neutral syrup (49 mg). Paper chromatography indicated the presence of *myo*-inositol (+), sucrose (+), allitol (+ +), glucose (*t*), fructose (*t*), ribohexulose (+ + +), and traces of two unknown compounds with $R_{glucose}$ 0·97 and 1·3 respectively both of which might be heptuloses since they gave a characteristic blue colour with the orcinol spray reagent. The syrup was treated with acetone–copper sulphate–sulphuric acid as described above and di-*o*-isopropylidene-D-*ribo*hexulose isolated. Recrystallized from light petroleum (b.p. 40–60°), this derivative had m.p. 56–58·5°, $[\alpha]_D - 85°$ (*c*, 0·4 in acetone) and an infra-red spectrum identical with that of an authentic specimen.

The Detection of Allitol and D-Ribo*hexulose in Leaves of* I. yunnanensis

The leaves (6·0 g, fresh weight) were cut into small pieces and exhaustively extracted with boiling methanol, giving an insoluble residue (1·4 g) and, on concentration of the extract, a dark green syrup (0·83 g). Paper chromatography of the extract suggested the presence of *myo*-inositol (*t*), sucrose (+), glucose (+), allitol (+ + +), fructose (*t*), and *ribo*hexulose (+ + +). The syrup was shaken with a mixture of ethanol (2 ml) and water (15 ml) and the cloudy solution extracted with chloroform (3 × 15 ml). The acidic aqueous solution was passed through a column of Amberlite IR-45 (OH^-) ion-exchange resin and then concentrated to a small volume (0·5 ml) when crystallization occurred. The crude crystalline material was recrystallized from water and then aqueous alcohol to give allitol (60 mg) with m.p. 149–150°;* its infra-red spectrum was identical with that of allitol isolated from leaves of *I. ilicifolia*.

* A 6% yield of allitol was obtained in a subsequent experiment when an aqueous extract of the methanol soluble material was passed through a pad of activated charcoal —Hyflosupercell, evaporated, and the product recrystallized from aqueous alcohol.

[19] R. Lespieau and J. Wiemann, *Bull. soc. chim.* [4], **53**, 1107 (1933).
[20] J. Wiemann, *Ann. chim.* [11], **5**, 267 (1936).

The combined mother liquors from the above crystallization of allitol were concentrated to a dry syrup (230 mg), from which di-*o*-isopropylidene-D-*ribo*hexulose (9 mg) was prepared as described previously. The isopropylidene derivative had m.p. 57–59°, $[\alpha]_D - 88°$ (*c*, 0·3 in acetone) and an identical infra-red spectrum to that of an authentic specimen.

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