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SHORT COMMUNICATION

THE PRESENCE OF 3-*O*-METHYLRHAMNOSE IN *ARAUCARIA* RESINOUS EXUDATES

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Abstract—The comparatively rare sugar 3-*O*-methylrhamnose has been detected in the polysaccharide components of the resinous exudates from five *Araucaria* species, and it appears to act as a useful chemical marker in gymnosperms.

ANALYTICAL studies of natural products[1] should be oriented, wherever possible, towards obtaining data which may be of value for chemotaxonomic purposes. The identification of comparatively rare chemical compounds, which can be used conveniently as characteristic taxonomic markers, is particularly important.

3-*O*-methyl-L-rhamnose (6-deoxy-3-*O*-methyl-L-mannose; L-acofriose)[2] is a comparatively rare sugar. It occurs in glycosides from the seeds of *Acokanthera friesiorum*[2] and *A. schimperi*;[3] in cardiac glycosides;[4] and in specific glycolipids from *Mycobacterium avium*.[5] In polysaccharides, 3-*O*-methyl-L-rhamnose was first found[6] in small amount in the extractive-free hemicellulose from the gymnosperm *Picea nigra*; since then, reports of its natural occurrence in polysaccharides appear to have been confined to its presence, as a minor component, in gum exudates from the gymnosperms *Encephelartos latefrons* Lehm. (f.),[7] *E. longifolius* Lehm. (f.),[8, 9] and *Welwitschia mirabilis* Hook. (f.).[7, 8] So far as we can ascertain, 3-*O*-methylrhamnose has not been reported as a component of the exudate from any Angiosperms.

The characteristic resinous exudates from coniferous woods were formerly believed to be terpenoid in character,[10] but it is now known[10, 11] that the resin from the gymnosperm *Araucaria bidwillii* also contains an acidic polysaccharide.

The samples of *A. bidwillii* resin studied[10, 11] were of Australian origin. The genus *Araucaria* contains only 14 species,[12] and to ascertain whether the presence of acidic polysaccharides in the resins is a general feature of this genus, we have investigated the exudates

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from *A. bidwillii* and other species growing in different parts of the world. The resins from *A. bidwillii* and *A. cunninghamii* growing at Kew, from *A. araucana* and *A. excelsa* growing at Edinburgh, and from *A. columnaris* growing in East Africa have all been found to give acidic polysaccharides, which, furthermore, all contain 3-*O*-methylrhamnose.

Chromatographic studies indicated that, of the six species investigated, 3-*O*-methylrhamnose was present in greatest amount (*ca.* 5%) in the exudate from *A. cunninghamii*, and the experimental evidence detailed below was obtained in studies of the polysaccharide isolated from this species.

The presence of 3-*O*-methylrhamnose in the exudate from *Welwitschia mirabilis*[7] has already given useful taxonomic support for the otherwise rather arbitrary classification of this species as a gymnosperm. Whether the presence of 3-*O*-methylrhamnose will prove to be a general taxonomic characteristic for all gymnosperms must await the results of further studies.

EXPERIMENTAL

Origins of Specimens

Resin from *Araucaria columnaris* (Forst.) Hook. (syn. *A. cookii* R.Br. ex. Lindl.) was obtained in October 1962 from Mr. R. L. Willan, silviculturalist at Lushoto Arboretum, Tanganyika. The resin was collected from a tree, 6 yr old, that had been pruned in 1960. Resins from four trees of *A. araucana* (Molina) K. Koch (of which there are 9 synonyms[12]) and from *A. excelsa* (Lam.) R.Br. (syn. *A. heterophylla* (Salisbury) Franco) were obtained, by courtesy of the Regius Keeper, from the Royal Botanic Gardens, Edinburgh, in October 1967 and February 1968 respectively. Resins from single trees of *A. cunninghamii* D. Don and *A. bidwillii* Hook. were obtained in November 1967, from the Royal Botanic Gardens, Kew, by courtesy of the Curator.

The terpenoid material in the *Araucaria* exudates was removed by exhaustive extraction with ethanol until the ethanol gave no turbidity when poured into water. The residual polysaccharide material was dissolved in cold water; the solution was filtered, dialysed, and freeze-dried to give the purified acidic polysaccharide.

Electrophoresis was carried out in 0·05 M borate buffer solution for 2·5 hr at a potential gradient of 20 V/cm. Chromatography was carried out on Whatman No. 1 and 3MM papers in: (*a*) benzene-butan-1-ol-pyridine-water (1:5:3:3, upper layer); (*b*) ethyl acetate-pyridine-water (10:4:3); (*c*) ethyl acetate-acetic acid-formic acid-water (18:3:1:4), (*d*) butan-1-ol-ethanol-water (4:1:5).

Polysaccharide samples (*ca.* 150 mg) were hydrolysed with 1N H_2SO_4 for 7·5 hr on a boiling water-bath. After neutralization ($BaCO_3$) and de-ionization (Amberlite IR-120 H^+ resin), solutions were reduced in volume to a syrup which was examined chromatographically in solvents (*a*) to (*d*). The hydrolysates of all the *Araucaria* specimens studied showed a fast-moving sugar having R_{gal}=2·70 (solvent (*a*)), 2·62 (*b*); 4·3 (*c*); and R_g (relative to 2:3:4:6-tetra-*O*-methyl-D-glucose) = 0·65 in solvent (*d*). This component had the same chromatographic mobility in these solvent systems as an authentic sample (kindly supplied by Professor A. M. Stephen, University of Cape Town) of 3-*O*-methylrhamnose that had been obtained from methylated *Khaya senegalensis* gum.[13]

A similar, large-scale, hydrolysis of the polysaccharide (4g) from *A. cunninghamii*, followed by separation of the fast-moving component by thick-paper chromatography, gave a syrup (109 mg) having $[\alpha]_D$+30° (C, 1·1 (water)) which was not distinguishable from authentic 3-*O*-methylrhamnose on paper chromatography and electrophoresis. The chromatograms gave on spraying a yellow-green colour with *p*-anisidine hydrochloride and a yellow colour with aniline oxalate; this is characteristic[5] of 3-*O*-methylrhamnose, in contrast to its 2-*O*-methyl analogue. De-*O*-methylation with hydriodic acid gave rhamnose and methyl iodide, which was identified in the vapour-phase by i.r. spectroscopy.[14] The syrup was *O*-deuterated and the NMR spectrum was obtained. This showed a singlet, corresponding to three protons, at 6·58 τ; and a doublet, with a coupling constant of 6 cycles, corresponding to three protons, at 8·69–8·78 τ. These features indicate the presence of one *O*-methyl group and a 6-deoxymethyl group.[15]

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