

270. Chemistry of the *Coprosma* Genus. Part V. The Anthraquinone Colouring Matters from *Coprosma acerosa*.

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From the bark of *Coprosma acerosa* the following compounds have been isolated by chromatography: lucidin, anthragalol 2-methyl ether, anthragalol 1:2-dimethyl ether, rubiadin 1-methyl ether and 1:6-dihydroxy-2-methylanthraquinone. Rubiadin and 3-hydroxy-2-methylanthraquinone are probably present with another unidentified compound, all three in minute amount. Anthragalol 2-methyl ether, anthragalol 1:2-dimethyl ether, and 1:6-dihydroxy-2-methylanthraquinone, and probably rubiadin and rubiadin 1-methyl ether, are present also as glycosides.

Coprosma acerosa (Cunningham, *Ann. Nat. Hist.*, 1838, 2, 207; Oliver, "The Genus *Coprosma*," *Bernice P. Bishop Museum Bull.*, 132, 1935) is a scrambling shrub with slender interlacing branches forming masses 1—5 feet high. It is characteristic of coastal sand dunes throughout New Zealand and the Chatham Islands to which it is endemic.

A preliminary investigation indicated the presence, in the bark, of both free anthraquinone compounds and their glycosides, and these were extracted separately. The air-dried bark was first extracted with light petroleum. The material which separated after concentration of the extract was dissolved in acetone and adsorbed on calcined magnesia. The light petroleum concentrate contained waxy material and very little of the anthraquinone compounds. The bark was then extracted with benzene from which compound *A* separated on cooling. The residue from the remaining benzene solution was adsorbed from acetone solution on calcined magnesia. Both chromatograms gave the same number of bands of the same colour, and the products of each were then combined. This method of double extraction was preferable to a single extraction with benzene as the light petroleum removed the waxy material which otherwise proved troublesome in the chromatography.

The residual bark was then extracted with alcohol to remove the glycosides. The mixture of yellow solids obtained from the alcoholic solution rapidly changed to a red viscous mass when kept in air, and no crystalline material could be obtained; the material was therefore hydrolysed with dilute sulphuric acid, and the water-insoluble product was then adsorbed on calcined magnesia from acetone solution.

All the chromatograms were produced on a series of separate columns equivalent to one long fully-developed column (cf. Part IV, this vol., p. 1241). The material recovered from individual bands was reabsorbed, as before, on smaller columns, and the process repeated, if necessary, until pure chromatograms were obtained.

Compound *A*, which is insoluble in both light petroleum and benzene, does not appear on the chromatogram. The melting points of the purified free compound and its acetate (and the mixed m. p. thereof), its relative insolubility in all common solvents, and its colour reactions indicated identity with lucidin, a trihydroxy-2-methylanthraquinone of uncertain structure obtained from *C. lucida* (Part IV, *loc. cit.*).

The various bands of the chromatograms of the free anthraquinone compounds yielded the following substances. (i) A non-crystalline substance, present in very small amount, which gives a purple colour with alkali and an insoluble barium salt, and may be identical with compound *C* from *C. lucida* (Part IV, *loc. cit.*). (ii) Anthragalol 2-methyl ether, identified by its colour reactions and the melting points of the free compound, its acetate, and methyl ether, and the mixed melting points of the mono- and tri-methyl ethers; it has been found previously only in *C. lucida* (*loc. cit.*) and has been synthesised by Kubota and Perkin (*J.*, 1925, 1894).

(iii) 1 : 6-Dihydroxy-2-methylanthraquinone, identified by analyses and melting points (289°, 215°, and 192°) of the free compound, its acetate, and methyl ether [Simonsen and Rau (*J.*, 1921, 119, 1339) record m. p.s 281°, 212°, and 182°, respectively, for these compounds, and Bhattacharya and Simonsen (*J. Indian Inst. Sci.*, 1927, 10A, 6) m. p. 192° for the dimethyl ether]. This compound also occurs in *C. lucida* (cf. *loc. cit.*, where other occurrences are recorded). (iv) Anthragallol 1 : 2-dimethyl ether, identified by its mixed melting point and that of the acetate; it occurs in *C. lucida* (*loc. cit.*) and in *Oldenlandia umbellata* (Perkin and Hummel, *J.*, 1895, 67, 823), and has been synthesised by Perkin and Story (*J.*, 1929, 1399). (v) A compound, present in too small amount to be isolated, corresponding in position on the chromatogram and in colour with rubiadin, which is present in much larger amount in *C. lucida* (*loc. cit.*). (vi) Rubiadin 1-methyl ether, identified by its mixed melting point and that of the acetate; it occurs in *C. australis* (cf. *J.*, 1948, 564, where other references to its occurrence and synthesis are given) and *C. areolata* (*J.*, 1948, 568). (vii) Another compound present in too small a quantity for isolation, corresponding in position and colour of the band to 3-hydroxy-2-methylanthraquinone present in *C. lucida* (*loc. cit.*).

The chromatogram of the aglycones from the glycosidic anthraquinones was very similar to that of the free anthraquinone compounds. It consisted of six bands similar to the first six bands of the free compounds, the last band corresponding to 3-hydroxy-2-methylanthraquinone (?) being missing. The amounts isolated sufficed for purification and identification (mixed m. p.s) only in the cases of anthragallol 2-methyl ether, 1 : 6-dihydroxy-2-methylanthraquinone, and anthragallol 1 : 2-dimethyl ether. Two bands corresponded in position and colour with rubiadin and its 1-methyl ether, respectively.

The anthraquinone compounds of *C. acerosa* are thus very similar to those present in *C. lucida*, the principal difference being the presence in *C. lucida* of a much larger proportion of lucidin and a lower level of methylation as shown by the occurrence of free anthragallol and of rubiadin almost entirely unmethylated. This similarity of chemical constituents is in marked contrast with the position of the two species in the phylogenetic relationship proposed by Oliver (*loc. cit.*), which places them in widely differing sections of the genus.

EXPERIMENTAL.

The smooth, reddish bark was collected from mature shrubs in February from Muriwai, both the root and stem bark being used in the investigation. The air-dried, finely ground bark (50-g. lots) was extracted with light petroleum (b. p. 50—70°) in a Soxhlet apparatus for 70 hours, the solvent being kept dry by suspending a small beaker containing calcium chloride at the head of the Soxhlet. The yellow solid which separated from the extract, together with that precipitated from the filtered extract on concentration to 40 c.c., was dissolved in acetone and treated by chromatography on 25-mm. diameter columns of magnesia, freshly heated for 6 hours at a little below dull red heat. Exposure of the magnesia to the atmosphere for 24 hours led to noticeable loss of activity.

The bark was then extracted, as above, with benzene for 70 hours, and the yellow insoluble substance, compound A, filtered off after the extract had cooled. The residue from the benzene extract was dissolved in acetone and subjected to chromatography on freshly calcined magnesia. As the bands were the same in number and colour as those in the chromatogram from the light-petroleum extract, the material from both chromatograms was combined.

The residual bark was then extracted with alcohol for 70 hours to remove the glycosides. The residue from the alcoholic extract was dissolved in cold water and filtered through a bed of kieselguhr. The aqueous solution of the glycosides was hydrolysed by heating on the water-bath for 36 hours with 250 c.c. of 10% sulphuric acid, and the precipitate, black through the presence of asperuligenol, was extracted repeatedly with boiling acetone. The acetone solution was then treated by chromatography on freshly calcined magnesia.

All the chromatograms were made as described in Part IV (*loc. cit.*), the whole of the material being absorbed on a series of developed columns equivalent to one long column. The material from each band was liberated by dissolving the magnesia with hydrochloric acid, reabsorbed from acetone on a smaller column, and, if necessary, the process was repeated until a pure chromatogram was obtained from each constituent.

Identification of the Free Anthraquinone Compounds from the Chromatogram Bands.—The colour, length of band in a 25-mm. diameter column, and approximate weight of material isolated from a 5-g. sample of bark is recorded for each band.

Band I (blue-grey, 5 mm., —) : compound B (unidentified); too little to be purified or identified.

Band II (red, 10 cm., 500—600 mg.) : anthragallol 2-methyl ether. Sublimation at 0.01 mm. afforded yellow needles, m. p. 220°, undepressed by an authentic sample. Only after sublimation could it be crystallised and then only from dioxan. The acetate, from 25 mg., prepared as in Part IV (*loc. cit.*), crystallised from alcohol in yellow prismatic needles, m. p. and mixed m. p. with the diacetate of anthragallol 2-methyl ether, 146°. Likewise, the methylated product from 42 mg., prepared as in Part IV, crystallised from acetone in pale yellow needles, m. p. and mixed m. p. with anthragallol trimethyl ether, 171°.

Band III (dark red, 2 cm., 25—40 mg.) : 1 : 6-dihydroxy-2-methylanthraquinone. After purification by sublimation at 150°/0.01 mm., the material from this band crystallised from alcohol in orange-

yellow needles, m. p. 289° (Found : C, 70.9; H, 4.3; OMe, 0. Calc. for $C_{15}H_{10}O_4$: C, 70.9; H, 3.9%). The diacetate was prepared from the free compound (32 mg.) with acetic anhydride (2 c.c.) and 60% perchloric acid (1 drop) at room temperature during 1 hour. The product obtained on pouring the mixture into water crystallised from alcohol in fine, pale-yellow needles, m. p. 215°, unchanged by recrystallisation (Found : C, 67.35; H, 4.2. Calc. for $C_{19}H_{14}O_6$: C, 67.4; H, 4.1%). The dimethyl ether was prepared as described previously and after two crystallisations from acetone formed yellow rods, m. p. 193° (Found : C, 72.1; H, 5.1; OMe, 17.7. Calc. for $C_{17}H_{14}O_4$: C, 72.3; H, 4.9; 2OMe, 22.0%).

Band IV (orange, 4 cm., 125—150 mg.) : anthragallol 1 : 2-dimethyl ether crystallised from alcohol in yellow, rhombic plates, m. p. and mixed m. p. with authentic material, 238°. The acetate, prepared from 37 mg., as in the case of the 2-methyl ether, crystallised from alcohol in yellow rods, m. p. and mixed m. p. with the authentic acetate, 180—181°.

Band V (dark red, trace, —) : compound *F* (unidentified), present in too small a quantity to be isolated or identified.

Band VI (red, 2 mm., 2.5 mg.) : rubiadin 1-methyl ether. The light yellow crystalline aggregates obtained from this band, on crystallisation from alcohol, had m. p. 282—284°, undepressed by an authentic specimen, m. p. 286—288°. The product (11 mg.) from this band and the corresponding band from the aglycone chromatogram was acetylated with acetic anhydride (2 c.c.) and 60% perchloric acid (1 drop) at room temperature during 45 minutes. The product, isolated as above, crystallised from alcohol in yellow, flattened, prismatic rods (5.5 mg.), m. p. and mixed m. p. with the authentic acetate, 169—170°.

Band VII (pink, 1 mm., —) : compound *I* (unidentified), present in too small a quantity to be isolated or identified.

Identification of the Aglycones of the Anthraquinone Glycosides from the Chromatogram Bands.—The chromatogram of the aglycones of the anthraquinone glycosides contained six bands, I (grey, not eluted with acetic acid-acetone), II (red, 10 cm.), III (dark red, 2 cm.), IV (orange, 4 cm.), V (dark red, 3 mm.), and VI (red, 1—2 mm.). Only from the second, third, and fourth bands was material obtained sufficient for purification and identification. The materials (250 mg.) from the second, the third (40 mg.), and the fourth band (66 mg.) were purified as described previously for the materials isolated from the corresponding bands of the free anthraquinone compounds and were identified with anthragallol 2-methyl ether, 1 : 6-dihydroxy-2-methylanthraquinone, and anthragallol 1 : 2-dimethyl ether, respectively, by mixed melting points.

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