612. *The Colouring Matters of the Bark of* Rhamnus alaternus *L.*

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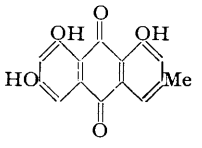
Two colouring matters, emodin and a new tetrahydroxymethylanthraquinone, probably the 3 : 4 : 5 : 7-tetrahydroxy-2-methyl compound, have been isolated from the hydrolysed extract of this bark.

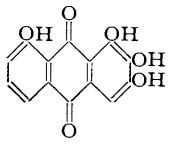
Rhamnus alaternus L. has already been examined for hydroxyanthraquinones (Maurin, *Bull. Sci. pharmacol.*, 1924, **31**, 135; Vutyrakis, *Ann. Fac. franç. méd. pharm. Beyrouth*, 1937, **6**, 257; Olaechea, *Rev. Fac. Cienc. quím. La Plata*, 1945, **20**, 229; Marangoni, *Boll. Soc. Ital. Biol. sperim.*, 1946, **21**, 262; Raimondi, *Fitoterapia*, 1947, **18**, No. 3, 1) but, so far, only emodin (I) has been isolated (cf. Vutyrakis, *loc. cit.*). The tree is endemic to the Mediterranean countries but now grows wild in parts of New Zealand.

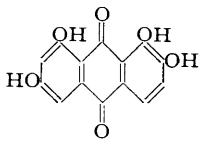
After preliminary extraction of the dried bark with light petroleum, exhaustive extraction with acetone yielded up to 22% of partly crystalline material consisting apparently of water-soluble hydroxyanthraquinone glycosides. Separation of this material into its constituents by chromatography was not readily achieved but the mixture of aglycones obtained after hydrolysis could be readily separated by chromatography on magnesia columns by the displacement technique (Briggs and Nicholls, *J.*, 1949, 1241). The chromatogram consisted of two main bands and a series of smaller bands (amounting to less than 1% of the total length). From the lower main band emodin (I) was isolated and, from the top purple band, a new tetrahydroxymethylanthraquinone, $C_{15}H_{10}O_6$. The latter formed a tetra-acetate and a tetramethyl ether, by the action of methyl sulphate and potassium carbonate in acetone solution, indicating that the four hydroxyl groups are phenolic. As a by-product in the methylation, a dimethyl ether was obtained; we therefore conclude that two of the hydroxyl groups are β since β-hydroxyl are much more readily methylated than α-hydroxyl groups (cf. Perkin, *J.*, 1907, **91**, 2066). The two remaining α-hydroxyl groups must be in the 1 : 8-(or 4 : 5-)positions since the infra-red absorption spectrum exhibits bands at 1681 and 1613 cm.$^{-1}$, characteristic of an unco-ordinated carbonyl group and a carbonyl group co-ordinated with an α-hydroxyl group, respectively (Flett, *J.*, 1948, 1441, and forthcoming communication). The presence of a band at 431 mμ

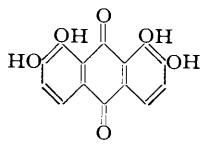
(log ε 4·03) in the ultra-violet absorption spectrum is also in harmony with the presence of two α-hydroxyl groups. Emodin (I) also exhibits a peak at 438·5 mμ (log ε 4·05) characteristic of two α-hydroxyl groups, while the tetramethyl ether of the new anthraquinone has a peak at 367 mμ (log ε 3·80), characteristic of anthraquinones with no free α-hydroxyl groups (cf. Briggs, Nicholls, and Paterson, *J.*, 1952, 1718). A 1 : 8-(or 4 : 5-)structure is also to be expected on phytochemical grounds since all hydroxyanthraquinones isolated from the Rhamnaceae which contain at least two hydroxyl groups have them in the 1 : 8-(or 4 : 5-)positions (cf. Mayer and Cook, " The Chemistry of Natural Coloring Matters," Reinhold Publ. Corp., New York, 1943, pp. 117—137). The above infra-red evidence excludes 1 : 5- and 1 : 4-dihydroxy-structures while 1 : 4-dihydroxy-structures are also excluded by the fact the anthraquinone does not exhibit the fluorescence characteristic of this grouping (Raistrick, Robinson, and Todd, *Biochem. J.*, 1934, **28**, 559).

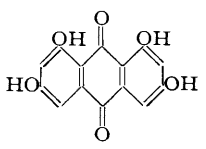
The anthraquinone contains a methyl group (Kuhn–Roth determination); in all anthraquinones derived from plants this is in the β-position. If this methyl group is ignored there are 22 possible tetrahydroxyanthraquinones. Of these, ten are excluded because they do not contain two α- and two β-hydroxy groups; a further eight isomers containing either 1 : 4- or 1 : 5-dihydroxy-structures are eliminated by the infra-red evidence. Four isomers, (II), (III), (IV), and (V) remain. The tetrahydroxymethylanthraquinone does not give the Bargellini test for vicinal trihydroxy-groups (*Gazzetta*, 1919, **49**, ii, 47; see also Seshadri and Rao, *Proc. Indian Acad. Sci.*, 1943, **17**, *A*, 20; Briggs and Locker, *J.*, 1949, 2159) and is not oxidised in alkaline solution, thus eliminating (II). It has dyeing properties on mordanted wool and is not eluted by acetic acid from a magnesia column, both properties characteristic of vicinal dihydroxyanthraquinones, thus eliminating (V). 1 : 2 : 7 : 8-Tetrahydroxyanthraquinone (IV) is known (cf. Wöbling, *Ber.*, 1903, **36**, 2941; Bistrzycki and Krauer, *Helv. Chim. Acta*, 1923, **6**, 769) and consists of red needles which give a blue colour in sodium hydroxide, a violet colour in sodium carbonate and ammonia solutions and a violet-red colour in concentrated sulphuric acid, becoming blue on the addition of boric anhydride. A β-methyl group would not be expected to have a great effect on these properties. The new anthraquinone, however, consists of brick-red plates giving red sodium hydroxide and concentrated sulphuric acid solutions. For these reasons we consider that (IV) can be eliminated, leaving the single possibility (III) for the tetrahydroxyanthraquinone. The methyl compound must then be a β-methyl derivative of (III), *i.e.*, (VI) or (VII).

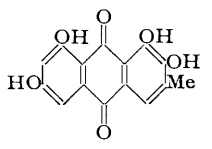

(I)

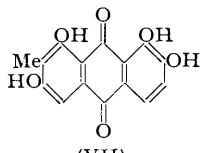

(II)


(III)


(IV)


(V)


(VI)


(VII)

Since (VI) represents a hydroxy-emodin, we consider that on phytochemical grounds (VI) best represents the new tetrahydroxyanthraquinone.

Experimental

Some of the analyses are by Dr. T. S. Ma, Otago University, Dunedin, and Mr. R. N. Seelye of this department. We are indebted to Mr. R. M. L. Paterson for the absorption spectra recorded in this paper; they were measured in approximately 0·00005M-alcoholic solution with

a Beckman spectrophotometer, Model DU. We thank Dr. B. Cleverley for the measurement of the infra-red spectrum in a Nujol mull with a Beckman spectrophotometer, Model IR2.

The bark of a mature tree of *Rhamnus alaternus* L., growing on Rangitoto Island, was cut into small pieces, sun-dried, and ground to a powder. This was extracted exhaustively with light petroleum, which removed little, if any, of the colouring matters, and then with acetone in a Soxhlet apparatus; the extract was taken to dryness (yield, 22%). Chromatography of an acetone solution of this extracted material on a magnesia column was not satisfactory; two main bands were present but difficulties arose in the isolation of the water-soluble mixture of glycosides. When aqueous extracts of the two bands were poured into organic solvents only uncrystallisable oils were obtained. For these reasons our attention was directed to the aglycones.

The acetone extract almost completely dissolved in water, and the small amount of insoluble material was filtered off. For this reason, in proceeding directly to the aglycones, the preliminary extraction with light petroleum could be omitted. Concentrated hydrochloric acid was added to the aqueous solution (1 c.c. per 100 c.c. of water), which was then heated at 100° for 12 hr. The precipitate which formed on cooling was filtered off and washed with water until it was neutral (litmus) (yield of aglycone, 8%). The aglycone mixture was dissolved in acetone and chromatographed on freshly ignited magnesia; development with acetone was followed by displacement with acetic acid–acetone (cf. Briggs and Nicholls, *loc. cit.*) and further development with acetone for 18—24 hr. The chromatogram consisted of two main bands and a series of very small bands at the bottom. The pigments were recovered from the two main bands by dissolving the magnesia with hydrochloric acid and washing the residue with water until free from mineral salts. Each product was purified by rechromatographing it on 4 smaller columns of magnesia until a homogeneous chromatogram was obtained.

3 : 4 : 5 : 7-*Tetrahydroxy-2-methylanthraquinone.*—The pigment isolated from the purple band, which was not displaced by acetic acid, formed brick-red plates, m. p. 310° (constant; slow heating in a sealed tube), from glacial acetic acid (charcoal). It could also be purified by sublimation at 220°/0·01 mm. (Found: C, 62·6; H, 3·7; *C*-Me, 4·0. $C_{15}H_{10}O_6$ requires C, 62·9; H, 3·5; *C*-Me, 5·2%). The *tetrahydroxymethylanthraquinone* exhibited maxima in the ultra-violet at 230·5, 284, 317·5, and 431 mμ (log ε 4·28, 4·41, 3·94, and 4·03, respectively), and in the infra-red at 3367, 1686, 1613, 1572, 1477, 1425, 1370, 1319, 1277, 1206, 1167, 1107, 1087, 1033, 995, 935, 886, 863, 826, and 759 cm.$^{-1}$, with an inflexion at 3195 cm.$^{-1}$.

Tetra-acetate. The anthraquinone (30 mg.) was acetylated with acetic anhydride (2 c.c.) and 60% perchloric acid (1 drop) during 1 hr. The product obtained on pouring the mixture into water was chromatographed in acetone on magnesia. The material recovered from the pink main band crystallised from glacial acetic acid in lemon-yellow needles, m. p. 224° (Found: C, 61·4; H, 3·9; Ac, 35·8. $C_{23}H_{18}O_{10}$ requires C, 60·8; H, 4·0; 4Ac, 37·9%).

Methylation. The anthraquinone (203 mg.), in dry acetone (10 c.c.), was heated with methyl sulphate (1 c.c.) and anhydrous potassium carbonate (3 g.) for ½ hr. The mixture was poured into water, heated at 100° for ½ hr., and cooled, and the precipitate dissolved in acetone and chromatographed on magnesia. On development with acetone three bands and a coloured eluate were obtained. From the purple band unchanged anthraquinone was recovered. The material (30 mg.) isolated from the red band crystallised from glacial acetic acid (charcoal) in fine orange needles, m. p. 220° (constant; with sublimation) (Found: C, 65·3; H, 4·8; OMe, 24·5. The methoxyl determination was carried out on 1·563 mg., the only sample available, and is therefore not particularly accurate. $C_{17}H_{14}O_6$ requires C, 65·0; H, 4·5; 2OMe, 19·7%). The *dimethyl ether* is insoluble in sodium carbonate solution but with sodium hydroxide solution a very insoluble dark red salt is formed. With concentrated sulphuric acid and ferric chloride solution it gives ruby red and brown colours respectively.

The *tetramethyl ether* (93 mg.), recovered from the eluate after evaporation of the solvent, crystallised from glacial acetic acid in yellow needles, m. p. 181—182° (Found: C, 66·4; H, 5·1. $C_{19}H_{18}O_6$ requires C, 66·7; H, 5·2%); it exhibited maxima at 225·5, 278, and 367 mμ (log ε 4·39, 4·45, and 3·80, respectively).

Emodin.—The pigment recovered from the red band of the main chromatogram could be purified either by sublimation at 175°/0·01 mm., followed by crystallisation from alcohol, or directly by crystallisation from glacial acetic acid (charcoal). Emodin was obtained in both cases as long orange needles, m. p. and mixed m. p. 255°. We are indebted to Professor Raistrick for an authentic specimen. The colour reactions in alkaline and sulphuric acid solutions were identical with those described for emodin while the triacetate, prepared with acetic anhydride and 60% perchloric acid, separated from glacial acetic acid in lemon-yellow

needles, m. p. 197° (Kögl and Postowsky, *Annalen*, 1925, **444**, 1, record m. p. 197° for this derivative). Emodin exhibited maxima in the ultra-violet at 253, 266, 289·5, and 438·5 mμ (log ε 4·23, 4·23, 4·29, and 4·05, respectively).

Methylation. A solution of emodin (55 mg.) in dry acetone (15 c.c.) was heated with methyl sulphate (1 c.c.) and anhydrous potassium carbonate (2 g.) for 15 min. at 100°. The mixture was cooled, filtered, and chromatographed on magnesia. After development with acetone, the chromatogram consisted of an orange small upper band, not readily eluted, a pale orange main band, and a pale yellow lower band. The material from the middle band crystallised from glacial acetic acid in orange-yellow needles, m. p. 201—202° [Raistrick, Robinson, and Todd, *J.*, 1937, 80, record m. p. 203—204° for emodin 7-methyl ether (physcion)].

In a similar experiment, in which the reaction time was 2 hr., the main band was the pale yellow one which was readily eluted. Emodin trimethyl ether was recovered from the eluate, and crystallised from glacial acetic acid in yellow needles, m. p. 222—223° (Oesterle and Tisza, *Arch. Pharm.*, 1908, **246**, 432, record m. p. 225°).

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