Naturally Occurring Quinones. Part XIII.[1] Anthraquinones and Related Naphthalenic Compounds in *Galium* spp. and in *Asperula odorata*

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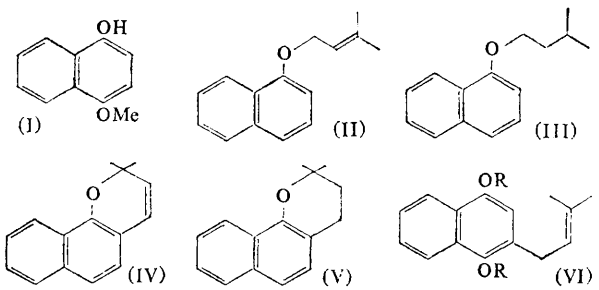
The roots of *Galium* (seven spp.) and *Asperula odorata* contain anthraquinone pigments (<13) and one or more of the following naphthalenic compounds: 4-methoxy-1-naphthol, 1-naphthyl isopentyl ether, 1-naphthyl isopentenyl ether, and 2,2-dimethylnaphtho[1,2-*b*]pyran and its 3,4-dihydro-derivative. The relevance of these products to the biogenesis of the anthraquinones is discussed.

RECENT work on the extractives from *Tabebuia* spp. (Bignoniaceae) [1,2] and from teak (Verbenaceae) [3] strongly suggests that the precursor of the simple anthraquinones in these timbers is a naphthalenic compound bearing a C_5 side chain, most probably 2-($\gamma\gamma$-dimethylallyl)-1,4-naphthaquinone, the implication being that one benzenoid ring is derived from mevalonate. However, as the quinones are present in the heartwood of these tropical trees, obtaining direct proof by appropriate labelling studies is hampered by experimental difficulties, which has led us to look for alternative plant material. The anthraquinones referred to above include 2-methylanthraquinone, 1- and 3-hydroxy-2-methylanthraquinone, and 1-hydroxyanthraquinone and its methyl ether. The only other significant source of quinones of this type, *i.e.*, anthraquinones substituted in only one benzenoid ring (in contrast to the fungal anthraquinones) is the Rubiaceae family, and it has long been apparent that the Rubiaceous quinones are not of polyketide origin (the 'non-acetate' group of Birch and Donovan [4]) and conceivably [5] may arise from naphthalenic precursors. Many of the anthraquinones in the Rubiaceae have no carbon side chain (alizarin, purpurin, etc.) but we were encouraged to turn to this family following a report [6] (which we have confirmed [7]) of the isolation, for the first time, of 2-methylanthraquinone from a Rubiaceous plant. Since the present work was completed it has been shown [8] that the unsubstituted ring, and one of the central carbon atoms, in alizarin are derived from shikimic acid.

For convenience, we have examined first a group of British Rubiaceae, all of which belong to the tribe *Galieae*. Although the roots of *Galium* and of *Asperula* have been used for dyeing purposes,[9] the pigments have received little attention apart from the work of Hill and Richter [10] who identified glycosides of alizarin, rubiadin, and pseudopurpurin in various species of both genera. Our results (see Table) show that up to thirteen pigments are present in various *Galium* spp. and in *Asperula odorata* (woodruff), all of which are normal 'Rubiaceous anthraquinones,' and no further comment is required.†

Of greater interest is the co-existence of five naphthalenic compounds, most species containing at least one of these (see Table). The compounds are 4-methoxy-1-naphthol (I), 1-naphthyl isopentenyl ether (II), 1-naphthyl isopentyl ether (III), the naphthopyran (IV), and its dihydro-derivative (V), which has been found



previously in teak.[14] The new natural products were readily recognised from analytical and spectral data, especially the u.v. absorption which closely resembles that of 1-methoxynaphthalene in several cases, and the n.m.r. spectra for which many analogies exist among prenylated aromatic compounds. The presence of compounds (I)—(V) in *Galium* demonstrates that these Rubiaceae can elaborate a naphthalene nucleus with an oxygen function at C-1, or C-1 and C-4, and can also attach a C_5 unit on to either oxygen or carbon. Although compounds of type (VI) have not been detected it seems reasonable to suggest that a plant such as *G. mollugo*, which produces (I), (II), and (V), may also biosynthesise (VI) which is then rapidly converted, by oxidation and cyclisation, to the anthraquinone pigments.

† The atypical structure 4-ethoxymethyl-1,3-dihydroxyanthraquinone recently assigned [11] to a pigment isolated from *Rubia iberica* C. Koch DC. (Rubiaceae) is incorrect. The compound is actually 2-ethoxymethyl-1,3-dihydroxyanthraquinone, probably an artefact derived from the lucidin present in *R. iberica*.[12,13]

[1] Part XII, A. R. Burnett and R. H. Thomson, preceding paper.
[2] A. R. Burnett and R. H. Thomson, *J. Chem. Soc.* (*C*), **1967**, 2100.
[3] W. Sandermann and M. H. Simatupang *Holz als Roh- und Werkstoff*, 1966, **24**, 190.
[4] A. J. Birch and F. W. Donovan, *Austral. J. Chem.*, 1953, **6**, 360.
[5] R. H. Thomson in 'Comparative Biochemistry,' Academic Press, New York, 1962, vol. 3A, p. 631.
[6] Miss W. H. Hui, personal communication, 1965; W. H. Hui and C. W. Yee, *Phytochemistry*, 1967, **6**, 441.
[7] A. R. Burnett and R. H. Thomson, unpublished.
[8] E. Leistner and M. H. Zenk, *Tetrahedron Letters*, 1967, 475.
[9] E. Bancroft, 'Philosophy of Permanent Colours,' Cadell and Davies, London, 1813, vol. 2, p. 303.
[10] R. Hill and D. Richter, *Proc. Roy. Soc.*, 1937, *B*, **121**, 547; *J. Chem. Soc.*, 1936, 1714.
[11] V. A. Stikhin, A. I. Bankovskii, and M. E. Perel'son, *Khim. prirod. Soedinenii*, 1966, 12.
[12] A. R. Burnett, Ph.D. Thesis, University of Aberdeen, 1967.
[13] V. A. Stikhin, A. I. Bankovskii, and M. E. Perel'son, personal communication.
[14] W. Sandermann and M. H. Simatupang, *Naturwiss.*, 1967, **54**, 118.

Most of these plants were unsuitable for labelling studies but feeding experiments have been carried out on a closely related *Rubia* species.[15]

EXPERIMENTAL

Anthraquinones were identified by direct comparison (mixed m. p., i.r., u.v., and R_F) with authentic specimens.

We describe below the extraction and chromatographic separation of the pigments in *G. verum*, and significant parts of the isolation procedure relating to two other species. Full experimental details supporting all the data given in the Table are described elsewhere.[12]

Anthraquinones and Naphthalenic Compounds in *Galium* spp. and in *Asperula odorata*

	A	B	C	D	E	F	G	H	I	J
2-Hydroxyanthraquinone	—	+	+	+	—	+	—	—	—	+
2-Methoxyanthraquinone [a]	+	+	+	+	—	—	—	—	+	+
1-Hydroxy-2-methylanthraquinone	+	+	+	+	—	+	—	—	—	—
1-Methoxy-2-methylanthraquinone	+	+	+	—	—	—	—	—	—	+
Alizarin [d]	+	+	+	+	+	+	+	+	+	+
Alizarin 1-methyl ether	+	+	+	+	+	+	—	—	+	+
Alizarin 2-methyl ether	+	+	+	+	+	+	—	—	—	+
Xanthopurpurin	+	+	+	+	+	+	—	+	+	+
Xanthopurpurin dimethyl ether [a]	+	+	+	+	+ [b]	—	—	—	—	—
Rubiadin [d]	+	+	+	—	+	+	+	—	+	+
Lucidin	+	+	+	+	+	+	—	—	+	+
Purpurin	+	+	+	+	+	+	+	+	+	+
Pseudopurpurin [d]	+	+	+	+	+	+	+	+	+	+
4-Methoxy-1-naphthol (I) [a]	—	—	—	+	—	—	—	—	—	+
1-Naphthyl isopentenyl ether (III) [a]	—	—	—	+	—	—	—	—	—	—
1-Naphthyl isopentyl ether (II) [a]	—	—	+	—	—	+	—	—	+	+
2,2-Dimethylnaphtho[1,2-*b*]pyran (IV) [a]	+	—	+	—	—	+	—	—	—	—
3,4-Dihydro-2,2-dimethylnaphtho[1,2-*b*]pyran	—	+	—	+	+ [b]	—	—	—	+	—

A *G. aparine* Linn.
B *G. saxatile* Linn.
C *G. verum* Linn.
D *G. mollugo* Linn.
E *G. sterneri* Ehrend.
F *G. pumilum* Linn.
G *G. normani* Dahl.[c]
H *G. fleuroti* Jord.
I *G. saxatile* × *G. sterneri*
J *Asperula odorata* Linn.

[a] New natural product. [b] Not isolated, identified by u.v. spectra and co-chromatography (t.l.c.). [c] Actually *G. normani* ssp. *islandicum*, the Icelandic equivalent of *G. sterneri*. [d] Present both free and as a glycoside.

Extraction of Galium verum.—Fresh roots (500 g.), collected in Aberdeenshire during June and July, 1966, were washed, air-dried, ground to a fine meal, and extracted (Soxhlet) with successive quantities of light petroleum (b. p. 60—80°) (2 × 1·2 l.), benzene (3 × 1·2 l.), and methanol (1·2 l.).

(a) *Light petroleum extract.* The orange-red solution was repeatedly shaken with 2N-sodium carbonate (12 × 200 ml.) and the purple aqueous solution was acidified with 2N-hydrochloric acid to give an orange precipitate (2·1 g.). Extraction of the filtrate with ether (4 × 150 ml.) yielded a further 0·1 g. of this material. The light petroleum solution was then extracted, under nitrogen, with N-sodium hydroxide (2 × 150 ml.); acidification afforded an orange-yellow solid (26 mg.). The light petroleum solution containing the alkali-insoluble fraction was then dried ($MgSO_4$) and evaporated under reduced pressure leaving a brown tar (240 mg.).

(i) The alkali-insoluble fraction was transferred, in chloroform, to silica gel G plates and chromatographed in benzene to give seven main components.

Fraction 1 (R_F 0·89) showed a blue fluorescence in ultraviolet light and yielded a brown oil (40 mg.) which was rechromatographed through a column of Florisil (1 × 10 cm.), in benzene, to give a pale yellow oil (27 mg.); distilled at 125—128° (bath)/0·2 mm. it then formed plates, m. p. 43—44° of 2,2-dimethylnaphtho[1,2-*b*]pyran (IV) (18 mg.) (Found: C, 85·9; H, 6·6. Calc. for $C_{15}H_{14}O$: C, 85·7; H, 6·7%); $\lambda_{max.}$ (EtOH) 288sh, 301, 314, 345, and 358 mμ (log ε 3·91, 4·31, 4·34, 3·78, and 3·73); the 60 Mc./sec. n.m.r. spectrum (in $CDCl_3$) showed a singlet (6H) at τ 8·58 (Me_2C), doublets (1H each) centred at 4·42 and 3·04 (*J* 10 c./sec.) (C*H*=C*H*), and a complex multiplet (6H) at 2—3 (Ar*H*). The picrate formed red needles, m. p. 125—126° (lit.,[16] 123—125°). The pyran (10 mg.) in ethanol (5 ml.) was hydrogenated over 10% palladised charcoal (15 mg.); 1 mol. of hydrogen was absorbed in 15 min.

Chromatography (t.l.c.) of the crude product on silica gel G, in benzene, afforded a main component (R_F 0·86) which was identical (i.r., u.v., and R_F) with an authentic sample of 3,4-dihydro-2,2-dimethylnaphtho[1,2-*b*]pyran (V).

Fraction 2 (R_F 0·78) yielded a yellow oil (29 mg.) which was rechromatographed on silica gel G, in light petroleum (b. p. 60—80°), and then distilled at 145—150° (bath)/0·3 mm. to yield 1-naphthyl isopentyl ether (III) (18 mg.) as an oil, identical (i.r., u.v., and R_F) with a synthetic sample (Found: C, 83·9; H, 8·4. Calc. for $C_{15}H_{18}O$: C, 84·1; H, 8·5%); $\lambda_{max.}$ (EtOH) 275, 286, 313, and 322 mμ (log ε 3·91, 3·67, 2·44, and 1·37).

Fraction 3. The pigment from this yellow band (R_F 0·69) crystallised from ethanol as pale yellow needles, m. p. 196—197° of 2-methoxyanthraquinone (11 mg.).

Fraction 4. The pigment (5 mg.) from this zone (R_F 0·6) crystallised from methanol as pale yellow needles, m. p. 154—156°, identical with 1-methoxy-2-methylanthraquinone.

Fraction 5. The pigment from this bright yellow band (R_F 0·51) was purified by further chromatography on a column of Florisil (1 × 10 cm.), in benzene, to give xanthopurpurin dimethyl ether (6 mg.) as long, bright yellow needles, m. p. 154—155° (from ethanol).

Fraction 6 (R_F 0·32) afforded a light brown solid (20 mg.)

[15] A. R. Burnett and R. H. Thomson, *Chem. Comm.*, 1967, 1125.

[16] R. Livingstone and R. B. Watson, *J. Chem. Soc.*, 1957, 1509.

which crystallised from hexane as yellowish needles, m. p. 214—216°, and gave a positive Liebermann–Burchard reaction.

Fraction 7. The brown tar from this zone (R_F 0·11) also gave a positive Liebermann–Burchard reaction.

(ii) The sodium hydroxide-soluble fraction was chromatographed on layers of silica gel G, in benzene, to give two main bands.

Band 1 (R_F 0·42). This bright yellow zone afforded alizarin 2-methyl ether (7 mg.) as yellow needles, m. p. 234° (from ethanol).

Band 2 (R_F 0·34) afforded 1-hydroxy-2-methylanthraquinone (9 mg.) as yellow needles, m. p. 185—186° (from ethanol). The methyl ether had m. p. 154—155°.

(iii) The sodium carbonate-soluble fraction. A portion (1 g.), dissolved in hot benzene, was added to acid-washed silica gel [1] (8 g.). The solvent was evaporated *in vacuo*, and the pigmented silica gel was poured on to a column containing acid-washed silica gel (100 g.) in light petroleum (b. p. 60—80°). Development with light petroleum–benzene (1 : 1) effected the slow separation of a broad orange band which was finally eluted with light petroleum–benzene (2 : 3) to give an orange solid which crystallised from ethanol as orange-red needles (0·6 g.), m. p. 290—291°, of alizarin. Further development with benzene gave purpurin as red needles (0·2 g.), m. p. 263—264°.

(b) *Benzene extract.* The orange-red solution was reduced in volume to *ca.* 750 ml. On cooling a yellow solid (20 mg.) was deposited. It was dissolved in 2% aqueous sodium hydroxide, filtered, acidified, and the precipitate then repeatedly crystallised from dioxan to give yellow needles, m. p. >330°, of lucidin (10 mg.).

The benzene extract was taken to dryness. Part (1 g.) of the residue (6·1 g.) was redissolved in hot benzene and added to acid-washed silica gel (9 g.). The solvent was evaporated *in vacuo*, and the silica gel with adsorbed pigment was poured onto a column containing acid-washed silica gel (130 g.) in light petroleum (b. p. 60—80°). Separation into six fractions was effected by successive elution with light petroleum, light petroleum–benzene (2 : 3, 1 : 5, 1 : 9), benzene, and chloroform.

Fraction 1 was rechromatographed on silica gel G, in benzene–ethyl acetate (2 : 1), to give two main yellow bands; (i) (R_F 0·41) afforded 2-hydroxyanthraquinone (6 mg.) as yellow needles, m. p. 306° (from ethanol), and (ii) (R_F 0·36) yielded alizarin 1-methyl ether (7 mg.) as orange-yellow needles, m. p. 178—180° (from ethanol). The acetate formed yellow needles, m. p. 210—212° (from ethanol).

Fraction 2, a broad orange band, gave alizarin (0·41 g.) as orange-red needles, m. p. 290° (from ethanol).

Fraction 3 was rechromatographed (t.l.c.) on acid-washed silica gel, in chloroform, to effect the separation of an orange band and a yellow band; the orange band (R_F 0·66) afforded a further quantity (12 mg.) of alizarin, m. p. 288—289° (from ethanol), and the yellow band (R_F 0·61) yielded xanthopurpurin (10 mg.) which crystallised from pyridine as yellow needles, m. p. 269—270°. The dimethyl ether had m. p. 154—155°.

Fraction 4, a yellow zone, was fractionated (t.l.c.) on acid-washed silica gel with chloroform–benzene (1 : 1) to give two yellow bands and an orange band; the orange band (R_F 0·58) gave alizarin (4 mg.), the first yellow band (R_F 0 54) yielded xanthopurpurin (6 mg.), and the main pigment, from the third band, crystallised from acetic acid as yellow plates (12 mg.), m. p. 300—303°, identified as rubiadin. Rubiadin 1-methyl ether formed yellow needles, m. p. 292° (from ethanol).

Fraction 5 was rechromatographed on a column of acid-washed silica gel; development with benzene effected the rapid separation of a red band from which purpurin (130 mg.) was obtained as red needles, m. p. 264—265° (from ethanol).

Fraction 6 yielded pseudopurpurin (38 mg.) as red leaflets, m. p. 222—224° (decomp.) (from ethanol). On sublimation at 180—195° it afforded purpurin, m. p. 263—264°.

The adsorbent from the chromatographic column was extracted (Soxhlet) with acetone (1200 ml.) for 24 hr. Evaporation of the solvent *in vacuo* gave a gummy solid which was triturated with ether and acetone, and then crystallised from dioxan as yellow needles (15 mg.), m. p. >330°, identical with lucidin.

(c) *Methanol extract.* Evaporation of the solvent left a brown tar (7·7 g.) which was redissolved in hot methanol (120 ml.) and treated with 2N-hydrochloric acid (50 ml.) on a steam-bath for 6 hr. The solution was cooled and diluted with water (100 ml.), and the precipitate (3·8 g.) was collected, washed with water and a little methanol, and dried. Part (1 g.) of this material was dissolved in hot benzene and chromatographed through a column of acid-washed silica gel as above, giving three main fractions which yielded alizarin (120 mg.), rubiadin (13 mg.), and pseudopurpurin (20 mg.).

Extraction of G. mollugo.—Air-dried roots (54 g.), collected in Sussex in September, 1966, were successively extracted with light petroleum (b. p. 60—80°), benzene, and methanol. The fraction (14 mg.) of the light petroleum extract soluble in sodium hydroxide, was separated by t.l.c. on silica gel G, in benzene–ethyl acetate (4 : 1), into three main components.

Band 1 (R_F 0·54) afforded 4-methoxy-1-naphthol (I) (1 mg.) as needles, m. p. 124—126° [from light petroleum (b. p. 60—80°)] [Found: *M* (mass spectrum), 174. Calc. for $C_{11}H_{10}O_2$: *M*, 174], $\lambda_{max.}$ (EtOH) 248, 317, and 331 mμ (log ε 4·39, 3·78, and 3·77), identical (u.v., i.r., mixed m. p., and R_F) with an authentic sample.

Band 2 yielded alizarin 2-methyl ether (3 mg.) and Band 3 gave 1-hydroxy-2-methylanthraquinone (3 mg.).

The alkali-insoluble fraction (96 mg.) of the light petroleum extract was chromatographed on silica gel G plates, in benzene, to give seven main components.

Fraction 1 (R_F 0·86) consisted of a dark oil (21 mg.) which was rechromatographed on a column of Florisil (1 × 10 cm.), in benzene, to give 3,4-dihydro-2,2-dimethylnaphtho[1,2-*b*]pyran (V) as a yellow oil, b. p. 135—140° (bath)/0·2 mm. identical (u.v., i.r., and R_F) with an authentic sample; the picrate formed orange needles, m. p. 137—138° (lit.,[14] 135—137°).

(ii) Fraction 2 (R_F 0·81) yielded a dark oil (15 mg.) which was purified by chromatography, in benzene, on a column of Florisil (1 × 10 cm.) to yield a colourless oil (11 mg.), $\lambda_{max.}$ (EtOH) 274, 285, 314, and 324 mμ (log ε 3·74, 3·60, 2·42, and 1·37); the n.m.r. spectrum (in CCl_4) showed a doublet peak (6H) centred at τ 8·38 (*J* 7 c./sec.) (Me_2C), a doublet (2H) centred at 5·54 (*J* 7 c./sec.) (Ar–O–C*H*$_2$), a multiplet (1H) at 4·48 (C=C*H*), and a complex system (7H) at 1·7—3·5 (Ar*H*). This product was identical (i.r., u.v., and R_F) with a synthetic sample of 1-naphthyl isopentenyl ether (II).

The remaining fractions yielded 2-methoxyanthraquinone, xanthopurpurin dimethyl ether, and two triterpenes.

Extraction of Asperula odorata.—Air-dried roots (61 g.) were extracted in the usual way. The fraction of the light petroleum extract soluble in aqueous sodium hydroxide was chromatographed on silica gel G plates, in benzene–ethyl acetate (4 : 1).

(i) Fraction 1 (R_F 0·54) yielded 4-methoxy-1-naphthol (<1 mg.), m. p. 124—125° [from light petroleum (b. p. 60—80°)].

(ii) Fraction 2 (R_F 0·44). This bright yellow band yielded alizarin 2-methyl ether (4 mg.) as yellow needles, m. p. 230—232° (from ethanol).

The green parts (80 g.) of *A. odorata* were macerated in acetone. Removal of the solvent *in vacuo* left a green residue (2·7 g.). Chromatography of part (0·5 g.) on layers of silica gel G, in benzene–dioxan–acetic acid (15 : 5 : 1), afforded vanillin (8 mg.), and *p*-hydroxybenzoic (14 mg.), *p*-coumaric (5 mg.), caffeic (7 mg.), and gallic (4 mg.) acids. No anthraquinones could be detected.

Similarly, the aerial parts of *G. aparine* yielded *o*- and *p*-hydroxybenzoic, *p*-coumaric, caffeic, and gallic acids, but no anthraquinones.

1-*Naphthyl Isopentenyl Ether*.—1-Naphthol (2·9 g.) in anhydrous acetone (60 ml.), γγ-dimethylallyl bromide (3·0 g.), and anhydrous potassium carbonate (7 g.) were refluxed together for 4 hr., and the mixture was then cooled and filtered, and the acetone removed. The residue (4·5 g.) in ether (70 ml.) was washed with 2N-sodium hydroxide (2 × 15 ml.) and water (3 × 15 ml.), dried ($MgSO_4$), and evaporated *in vacuo*. The residual oil was chromatographed on a column of Florisil (2 × 20 cm.), in benzene, and distilled to give 1-*naphthyl isopentenyl ether*, b. p. 154—156° (bath)/0·3 mm. (3·1 g.) (Found: C, 85·1; H, 7·5. $C_{15}H_{16}O$ requires C, 84·9; H, 7·6%).

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