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PHENYLNAPHTHALENE PIGMENTS OF *LACHNANTHES TINCTORIA*

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Abstract—Extracts of the roots of *Lachnanthes tinctoria* Ell yielded several new phenolic products one of which has been identified as 4-hydroxy-3-methoxy-5-phenylnaphthalic anhydride. Three other components were isolated as their fully methylated derivatives which were identified respectively as di-, tri- and tetramethoxyphenylnaphthalides.

INTRODUCTION

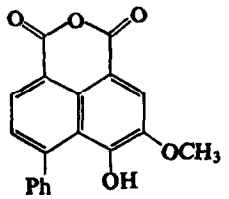
THE RECOGNITION of the root pigment of *Haemodorum corymbosum* Vahl. as a phenalenone derivative[1] naturally aroused interest in the chemistry of other representatives of the family Haemodoraceae. *Lachnanthes tinctoria* Ell was particularly interesting for a number of reasons. Its common names (bloodwort, paintroot, redroot) suggested the probable occurrence of similar pigment and, as it is the only generally recognized representative of its family to be found in the Northern Hemisphere, it was desirable for taxonomic reasons to compare it with *Haemodorum* species which are confined to Australia. Furthermore, repeated reports of toxicity to animals[2–5] suggested a photodynamic effect which might be attributable to phenalenones and their derivatives known to be coloured and strongly fluorescent from our work on haemocorin. Through the courtesy of Dr. E. C. Horning (then at the U.S. National Institutes of Health) we were able to obtain an extract of *Lachnanthes* roots in 1956. Examination of this extract revealed a number of fluorescent components in small amounts. Some of these were soon recognized as phenylnaphthalene derivatives and their possible implication in phototoxicity was suggested.[6] Determination of complete structures was not accomplished until some years later when suitable spectroscopic facilities became available. This work is described below and the structures of two of the compounds have recently been confirmed by synthesis.[7]

RESULTS AND DISCUSSION

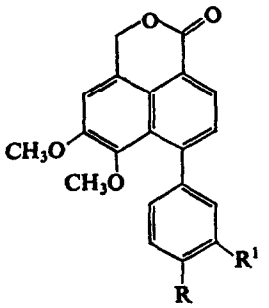
An orange-yellow product, $C_{18}H_{12}O_5$, was easily separated from the extract. Analysis showed the presence of one methoxyl group and the i.r. absorption spectrum showed the presence of a hydroxyl group (3356 cm^{-1}), a phenyl group (708,768 cm^{-1}) and the characteristic twin carbonyl peaks of a naphthalic anhydride[8] at 1727 and 1761 cm^{-1}. It was converted

[1] R. G. COOKE, B. L. JOHNSON and W. SEGAL, *Australian J. Chem.* **11**, 230 (1958).
[2] L. H. PAMMEL, *A Manual of Poisonous Plants*, p. 386, Torch Press, Iowa (1911).
[3] H. F. BLUM, *Photodynamic Action and Diseases Caused by Light*, American Chemical Society, Monograph Series, No. 85, p. 161, Rheinhold, New York (1941).
[4] W. C. MUENSCHER, *Poisonous Plants of the United States of America*, p. 53, Macmillan, New York (1947).
[5] J. M. KINGSBURY, *Poisonous Plants of the United States and Canada*, p. 470, Prentice-Hall (1964).
[6] R. G. COOKE, *Annual Report of Research and Investigation*, p. 308, University of Melbourne (1959).
[7] R. G. COOKE and R. A. H. FLETCHER, manuscript in preparation.
[8] R. G. COOKE, *Chem. Ind.* **142** (1955).

into a methyl ether identical with 3,4-dimethoxy-5-phenylnaphthalic anhydride which was obtained previously by the degradation of haemocorin.[9] These results demonstrated the close phytochemical relationship of *Lachnanthes* and *Haemodorum* and left only the relative positions of the hydroxyl and methoxyl groups to be established. This structural detail was determined by the NMR spectrum of the compound which showed the methoxyl resonance at δ4·06. Synthetic 7,8-dimethoxy-1-phenylnaphthalene[1] showed two resonance peaks at δ3·17 and 3·97, that at higher field is assigned to the 8-methoxyl group which is subject to shielding by the peri-phenyl group. Because of steric interference, the latter is not co-planar with the naphthalene ring system. This effect is well known and several cases of similar shielding have been reported.[10–12] A theoretical treatment of the phenomenon has also been published.[13] The normal methoxyl resonance of the natural anhydride therefore indicated that the hydroxyl group was in the peri-position adjacent to the phenyl group and the structure of the compound was established as 4-hydroxy-3-methoxy-5-phenyl-1,8-naphthalic anhydride (I). The remainder of the PMR spectrum was also in agreement with this structure. A one-proton peak at 6·84 is assigned to the hydroxyl group, a five-proton singlet at 7·4 to the



(I)



(IIa) $R = R^1 = H$
(IIb) $R = OCH_3$, $R^1 = H$
(IIc) $R = R^1 = OCH_3$

phenyl group, a sharp singlet at 8·35 to the proton at the 2-position and an AB quartet at 8·54 and 7·45 (J, 7·5) to the remaining two *ortho*-coupled aromatic protons.

A mixture of several other fluorescent phenolic compounds could not be separated satisfactorily by chromatography so the mixture was methylated. The resulting methyl ethers were separated by a combination of column chromatography and fractional crystallization and three pure products were isolated. The three neutral products dissolved slowly in hot aqueous sodium hydroxide and were recovered on acidification. Each showed a single carbonyl absorption peak near 1715 cm^{-1} consistent with a conjugated δ-lactone structure. It therefore seemed probable that these compounds were 1,8-naphthalides related to the above anhydride. This was confirmed by the PMR spectra which showed that the compounds differed only in the degree of methoxylation in the phenyl group.

Lactone A, $C_{20}H_{16}O_4$, showed two methoxyl resonance peaks at δ 3·17 and 3·95, a five-proton singlet at 7·39 due to the phenyl group and an AB quartet at 7·44 and 8·32 (*J*, 7 Hz), the

[9] R. G. COOKE and W. SEGAL, *Australian J. Chem.* **8**, 413 (1955).
[10] T. GILCHRIST, R. HODGES and A. L. PORTE, *J. Chem. Soc.* 1780 (1962).
[11] V. BALASUBRAMANIYAN, *Chem. Rev.* **66**, 567 (1966).
[12] A. F. A. WALLIS, *Tetrahedron Letters* 5287 (1968).
[13] C. E. JOHNSON, JR. and F. A. BOVEY, *J. Chem. Phys.* **29**, 1012 (1958).

downfield part of which can obviously be assigned to the aromatic proton adjacent to the lactone carbonyl group. The lactone methylene group gave a finely split doublet at 5·78 ($J \sim 1$ Hz) due to coupling with the adjacent aromatic proton which gave a multiplet at 7·28. The structure IIa is therefore assigned to this lactone which was presumably derived from the corresponding hydroxymethoxyphenylnaphthalide recently isolated by Edwards and Weiss.[14] The structure IIa has also been confirmed by synthesis.[7]

Lactone B, $C_{21}H_{18}O_5$, had very similar properties except for the presence of one more methoxyl group which was evidently in the *para*-position of the phenyl group because the five-proton singlet in the NMR spectrum of Lactone A was replaced by an A_2B_2 quartet at δ 6·87 and 7·30. Structure IIb is therefore proposed for Lactone B.

Lactone C was obtained in very small amount and its structure was not so readily established in complete detail. The exact mass of the molecular ion showed the molecular composition $C_{22}H_{20}O_6$ and the NMR spectrum showed the presence of four methoxyl groups δ 3·19, 3·84, 3·90 and 3·92. The remainder of the spectrum (see Experimental) was very similar to those given by the other lactones but the substituted phenyl group gave an unresolved three-proton multiplet. This showed that two methoxyl groups were located in this ring but it was not possible to determine their positions. However, the observation of four distinct methoxyl resonance peaks limited the possibilities and on biogenetic grounds the 3,4-position seemed most probable (structure IIc). Thomas[15] has demonstrated that haemocorin is probably derived from shikimic acid by way of a dicinnamoylmethane or similar skeleton analogous to the structures of the natural curcuminoids.[16] The structures assigned to the three lactones discussed above provide an obvious analogy with curcumin and its congeners which show similar variation of the degree of oxygenation in the aromatic rings. It is probable therefore that the anhydride and the lactones are derived from phenalone derivatives similar to haemocorin or are formed by similar but parallel biosynthetic steps. It will be shown[7] that synthesis confirmed the structure IIc for Lactone C.

Other highly coloured fractions were detected in the plant extract but no other pure compounds could be isolated. This may be explained by the observations of Edwards and Weiss[14] on the instability of the pigments and their distribution in the plant parts

EXPERIMENTAL

M.p.s were determined on a Kofler block and are corrected. Analyses were done by the Australian Microanalytical Service, Melbourne. I.r. spectra were recorded in KCl discs. Nuclear magnetic resonance spectra were recorded in $CDCl_3$ using tetramethylsilane as internal reference. Chemical shifts are given on the δ scale followed by multiplicity, relative intensity and coupling constant in Hz.

Isolation of Constituents of Lachnanthes tinctoria *roots*

The methanol-soluble material supplied by Dr. E. C. Horning was stirred with water and the insoluble fraction was dried. This material was separated with benzene into a more soluble fraction and a less soluble fraction. The former material was purified by chromatography in benzene over silica gel and the brightly fluorescent eluate was concentrated to give orange-yellow crystals of the anhydride (I) which is described below.

The less soluble fraction was a mixture of phenolic materials which could not be separated satisfactorily by crystallization or chromatography. It was therefore methylated with Me_2SO_4 and K_2CO_3 in acetone and the resulting fully methylated products were separated by chromatography on silica gel in benzene, fractional crystallization from mixtures of benzene and light petroleum (40–60°), and repeated chromatography over silica-gel. All fractions were easily located on columns by strong yellow-green fluroescence in u.v. light. The resulting three methylated lactones are described below.

[14] J. M. Edwards and U. Weiss, manuscript in preparation.
[15] R. Thomas, *Biochem. J.* **78**, 807 (1961) and personal communication.
[16] K. R. Srinwasan, (*a*) *Current Sci.* (*India*), **21**, 311 (1952); (*b*) *J. Pharm. Lond.* **5**, 448 (1953).

4-Hydroxy-3-methoxy-5-phenylnaphthalic anhydride (*I*) was purified by vacuum sublimation and crystallization from benzene as orange-yellow plates, m.p. 261–262°. (Found: C, 71·2; H, 4·0; OMe, 9·7. $C_{19}H_{12}O_5$ required: C, 71·2; H, 3·8; OMe, 9·7%.) This material was methylated in acetone with Me_2SO_4 and K_2CO_3 to give dimethyl 3,4-dimethoxy-5-phenylnaphthalate which crystallized from light petroleum in tufts of needles, m.p. 125–126° identical (i.r. spectrum) with an authentic specimen.[9] (Found: C, 69·3; H, 5·4. Calc. for $C_{22}H_{20}O_6$: C, 69·45; H, 5·3%.) This ester was hydrolysed in boiling aqu. NaOH, the solution was filtered and then acidified with HCl. After standing overnight the precipitate was collected and crystallized from ethanol to give 3,4-dimethoxy-5-phenylnaphthalic anhydride, identical (i.r. spectrum) with the specimen previously reported.[9]

Lactone A (*IIa*) crystallized from benzene–light petroleum in pale-yellow plates, m.p. 172–173°. (Found: *m/e* 320·1047. $C_{20}H_{16}O_4$ required: *m/e* 320·1049.) The compound dissolved slowly in hot aqueous NaOH and was regenerated on acidification of the solution with HCl (identical i.r. spectrum), ν_{max} 1715, 755, 700 cm^{-1}.

Lactone B (*IIb*) crystallized from benzene–light petroleum as pale-yellow needles, m.p. 182–183°. (Found: C, 72·1; H, 5·2; OMe, 25·8; mol. wt. (Rast) 352. $C_{21}H_{18}O_5$ required: C, 72·0; H, 5·2; OMe, 26·5%; mol. wt. 350.) NMR spectrum (60 MHz): 3·18, 3·89, 3·91 (singlets, 3H each); 5·76 (doublet, 2H, 1); 7·19 (multiplet, 1H); 6·87, 7·30 (quartet, 4H, 8·5); 7·37, 8·21 (quartet 2H, 7·5). ν_{max} 1706. After dissolving in hot alkali the material was regenerated by addition of HCl (identical i.r. spectrum).

Lactone C (*IIc*) (approx. 3 mg) crystallized from benzene–light petr oleum as yellow plates, m.p. 194–196°. (Found: *m/e* 380·1259. $C_{22}H_{20}O_6$ required: *m/e* 380·1260.) NMR spectrum (100 MHz): 3·19, 3·84, 3·90, 3·92 (singlets, 3H each); 5·73 (doublet, 2H, 1); 6·90 (multiplet, 3H); 7·17 (multiplet, 1H); 7·40, 8·23 (quartet, 2H, 7); ν_{max} 1715.