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PHENALENONE PIGMENTS OF THE ROOT SYSTEM OF *LACHNANTHES TINCTORIA**

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Abstract—The root system of *Lachnanthes tinctoria* (Haemodoraceae) contains lachnanthoside, a glycoside of trihydroxy-9-phenylphenalenone, lachnanthofluorone, a red-fluorescent compound having a novel naphthoxanthenone structure, and a naphthalide related to the phenalenones.

MANY plants of the small monocotyledonous family Haemodoraceae are distinguished by colorful root systems.[1] This is true of all the species of the Australian *Haemodorum*[2] (the only relative large genus of the family), the South African *Wachendorfia* and *Dilatris*, the South American *Pyrrhorhiza*, and the North American *Lachnanthes tinctoria* (redroot), the single species of the family to occur in the Northern temperate zone. From the bright red tubers of *Haemodorum* spp., Cooke *et al*[2] have isolated the cellobioside haemocorin (**1**), the first phenalenone pigment found in a higher plant† In earlier papers of this series, we have described the isolation of several 9-phenylphenalenones, and of compounds presumably derived from them, from the roots,[3] flowers[4] and pericarp[5] of *L. tinctoria*; during unpublished work to be reported elsewhere, we have observed similar pigments in species of *Xiphidium*, *Wachendorfia* and *Anigozanthus* of the same family. Of these plants, we have studied *L. tinctoria* in detail, since fresh material is available in abundance in the Pine Barrens area of New Jersey, and since a remark in Darwin's "Origin and Species",[6] suggestive of phototoxicity[7] to pigs, made this plant appear particularly interesting. Photodynamic activity against *Staphylococcus epidermidis* has indeed been observed[8] in various extracts from red-root.

* Part V in the series "Pigments of *L. tinctoria* Ell." For Part IV see Ref 4

† Isolation of haemodorin, a second plant pigment of this type, from *Haemodorum* sp., was reported by BICK, I R C and BLACKMAN, A J (1973) *Australian J Chem* **26**, 1377, while the present communication was being prepared

[1] EDWARDS, J M, CHURCHILL, J A and WEISS, U (1970) *Phytochemistry* **9**, 1563

[2] COOKE, R G and SEGAL, W (1955) *Australian J Chem* **8**, 107, 413; COOKE, R G, JOHNSON, B L and SEGAL, W. (1958) *Australian J Chem* **11**, 230

[3] WEISS, U and EDWARDS, J M. (1969) *Tetrahedron Letters* 4325

[4] EDWARDS, J M and WEISS, U (1972) *Tetrahedron Letters* 1631

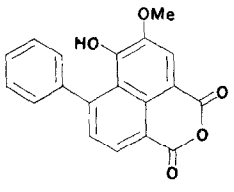
[5] EDWARDS, J M and WEISS, U (1970) *Phytochemistry* **9**, 1653

[6] DARWIN, C (1952) *The Origin of Species*, p 11, Encyclopedia Britannica

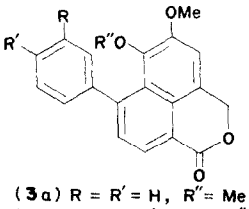
[7] BLUM, H F (1964) *Photodynamic Action and Diseases Caused by Light*, p 161, Hafner, New York

[8] KORNFELD, J M and EDWARDS, J M (1972) *Biochem Biophys. Acta* **286**, 88.

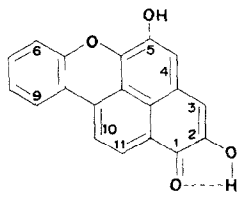
In a study of extracts from the roots of *L. tinctoria*, Cooke[9] isolated 4-hydroxy-3-methoxy-5-phenylnaphthalic anhydride (**2**) and, after methylation, the three naphthalides (**3**a–c): isolation of the naturally-occurring phenolic precursors of these latter compounds was not reported, nor were any phenalenone pigments encountered



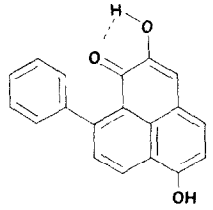
(**2**)



(**3a**) R = R' = H, R" = Me
(**3b**) R = H, R' = OMe, R" = Me
(**3c**) R = R' = OMe, R" = Me
(**5**) R = R' = R" = H



(**6**)



(**7**)

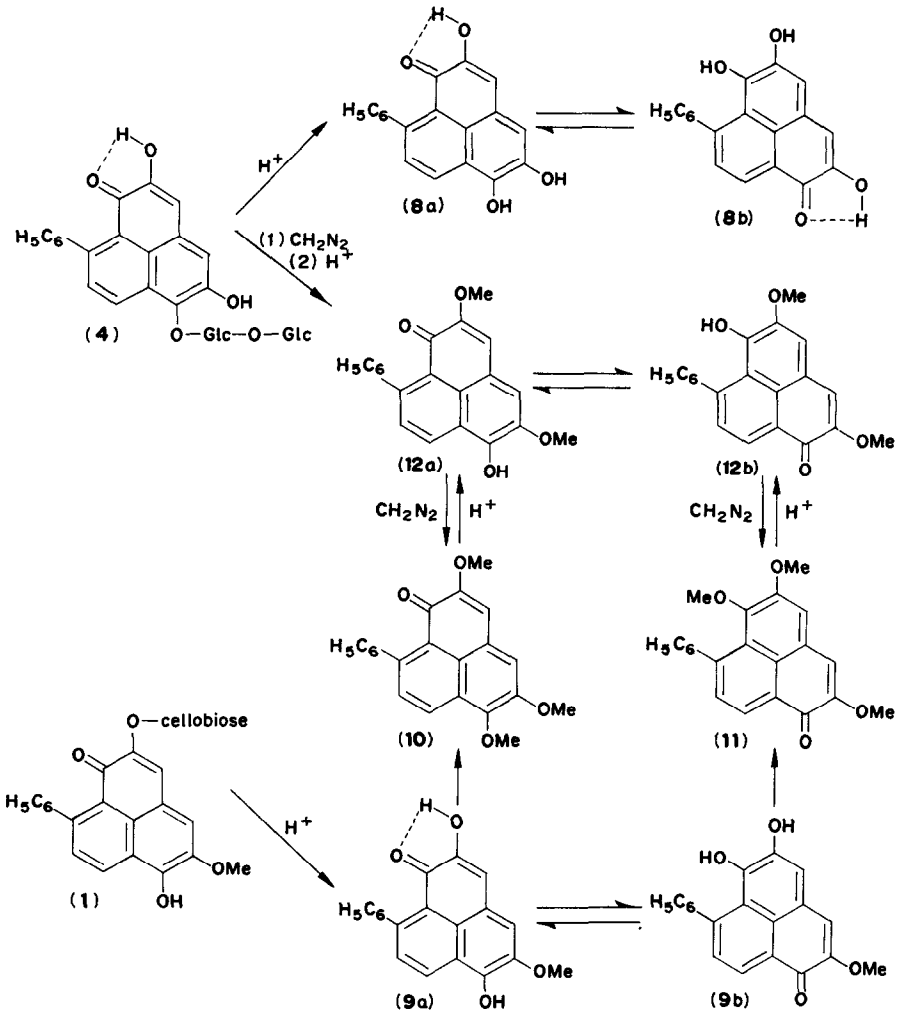
We now wish to present the results of an investigation of the *fresh* root system of *L. tinctoria*. The short roots of the plant and the inside of a small, flattened, bulb-like aneurism at the common base of the leaves are bright orange; the underground runners (usually close to the surface) have a thin purple outer layer and an orange core The pigment mixture, obtained by extraction of the fresh root system with acetone, was separated into water- and chloroform-soluble fractions. From the former, a red–brown phenalenone bioside, lachnanthoside (**4**), was isolated. The chloroform–extract gave a number of colored fractions on chromatography over SiO_2 (see Experimental), from which two major components have so far been isolated the naphthalide (**5**), related to Cooke's substances (**3**a–c),[9] and the red-fluorescent compound lachnanthofluorone (**6**),[3] with a structure closely analogous to that of a compound obtained[3] on irradiation of lachnanthocarpone (**7**), the main phenalenone of the pericarp[5] of *L. tinctoria*

Water-soluble fraction

Lachnanthoside (**4**), $C_{31}H_{32}O_{14}.2H_2O$, is readily cleaved by dilute acid [cf the behavior[2] of (**1**)] into a purple aglycone $C_{19}H_{12}O_4$ (**8**), m p 219–222 and a sugar fraction which on more energetic hydrolysis yields only glucose. The properties of (**8**) suggest a phenylphenalenone closely related to (**9**), the aglycone of (**1**); detailed spectroscopic investigation (see Experimental) proved it to be 2,5,6-trihydroxy-7(or 9)-phenylphenalenone (**8**a or b), the tautomerism of compounds of this type is well known [2,10] This formulation was further proved by the methylation of (**8**) to the two isomeric trimethyl ethers (**10**) and (**11**), identical (m p., m m.p , TLC) with the two dimethyl ethers[2] of (**9**)*, this aglycone is thus the 5-methyl ether of (**8**) Methylation of (**4**) with CH_2N_2, followed by hydrolysis, gave a crystalline dimethyl ether of (**8**), identical with 6-hydroxy-2,5-dimethoxy-7-(or 9)-phenylphenalenone (**12**a, b). This compound ("haemocorin aglycone monomethyl ether C") was obtained independently by Laundon and Morrison[11] through demethylation (HCl) of synthetic (**10**) or (**11**). The structure of our sample was indicated by spectroscopic findings The ν (CO) 1645 cm^{-1} shows that the carbonyl is not hydrogen-bonded Both methoxyl resonances (δ 3 92,

* See Acknowledgements

[9] COOKE, R G (1970) *Phytochemistry* **9**, 1103
[10] REID, D H (1965) *Quart Rev* **19**, 274
[11] LAUNDON, B and MORRISON, G A (1971) *J Chem Soc C*, 1694



3·83) are shifted upfield by *ca* 0·5 ppm on addition of benzene to a $CDCl_3$ solution of (**12**); both methoxy groups must therefore have unsubstituted *ortho*-positions.[12] The tautomerism (**12a**) ⇌ (**12b**), which in itself proves the structure, is shown by the methylation to both (**10**) and (**11**), and the formation of two different mono-acetates (of which, however, we isolated only one); both reactions have already been described.[11] Further evidence for this tautomerism is provided by the occurrence of M^+ and $(M-1)^+$ peaks of about equal intensity in the MS of (**12**). The appearance of $(M-1)^+$ peaks in the spectra of phenylphenalenones having phenyl and carbonyl in *peri*-relationship is well documented.[3,5,13] This peak is the base–peak of the spectrum in those compounds where tautomerism is impossible or is restricted by, e.g. hydrogen-bonding, as in (**7**); in compounds which can tautomerize readily, such as (**8**), the situation resembles the one found in (**12**).

[12] FALES, H. M and WARREN, K S (1967) *J Org Chem* **32**, 501
[13] BUCKLE, D R and WAIGHT, E S (1968) *Organic Mass Spectrometry* **1**, 273

The formation of the *di*-methyl ether (**12**) shows that (**4**) is a bioside rather than a diglucoside of (**8**). The sugar present in (**4**) has not yet been identified, chromatographic evidence indicates that it is not cellobiose, the biose of the closely related (**1**). From the formation of (**12**), it follows that the sugar in (**4**) must be attached to position 1 or 6. All attempts to decide between these possibilities through chemical transformations or MS failed but NMR evidence suggests that (**4**) is a 6-bioside of (**8**) This tentative conclusion is based on the finding, made on suitable pairs of phenalenones, that the signal of the proton at C-7 occurs between δ 8 57 and 8·68 (in CD_3OD), in those compounds which have an OMe group at C-6 [e.g at 8 57 in (**10**) and at 8·66 in the corresponding ether of (**7**)], while it is consistently found further downfield by about 0 1 ppm in the isomers with a carbonyl at C-6 (8·70 and 8·78 in the isomeric ethers corresponding to the examples quoted above) In (**4**), this signal occurs at δ 8 46 This value suggests that the carbonyl group is *not* at C-6, and hence that (**4**) is a 6-bioside Admittedly, however, methyl ethers are less than ideally suited as model compounds for a glycoside

Chloroform-soluble fraction

A chromatogram (TLC, SiO_2–EtOAc) of the chloroform-soluble root pigments shows the presence of non-glycosidic colored compounds, the major colored zones were: (1) a yellow band, R_f 0 8, which proved to be the naphthalide (**5**), (2) a purplish red, intensely red-fluorescing band, R_f 0 73, lachnanthofluorone (**6**), and (3) a green band, R_f 0 47, which was due to a chelate of the aglycone (**8**). There were, in addition, two minor components which turned from mauve to blue on exposure to NH_3 vapor (a reaction typical of 2-hydroxyphenalenones) and small amounts of several other, more polar compounds which have not yet been investigated, two of these exhibited an intense red fluorescence similar to that of (**6**)

The phenylnaphthalide (**5**), which is clearly the naturally occurring compound from which (**3a**) was generated in the work of Cooke,[9] was readily isolated by chromatography of the non-glycosidic mixture over SiO_2 with C_6H_6 as solvent Methylation of (**5**) yielded (**3a**);* hence the only question was the position of the methoxyl group in (**5**) The NMR spectrum of (**5**) contains a single OMe resonance at δ 3·95, and further methylation introduces a second signal at δ 3 17, the free hydroxyl group in (**5**) is thus at C-6, since the introduced methoxyl group at this position in (**3a**) is shielded by the phenyl ring in the *peri*-position.

Lachnanthofluorone (**6**, dark purple crystals, m p > 300 , is a naphtho-(8,1,2-*jkl*)-xanthenone which shows a very striking bright-red fluorescence The compound analyzed for $C_{19}H_{10}O_4$ and exhibited the expected chemical reactions and IR characteristics (see Experimental) of a 2-hydroxyphenalenone, the formation of one single dimethyl ether demonstrated the presence of two phenolic hydroxyl groups and showed that (**6**) could not tautomerize in the manner observed with (**8**), (**9**), and other suitably substituted phenalenones.[2,5,14] Since (**6**) is stable when refluxed with dilute acid or alkali, the fourth oxygen function is assigned to an ether linkage The structure (**6**) is supported by the NMR spectrum, which shows the expected AB quartet (δ 7 92, 6 64, J 9 5 Hz) for the protons at C-11 and C-10, two broadened singlets δ 8 20 and 7 66 (*peri*-coupling), a complex aromatic band (3H), and a doublet at δ 9 10 (J 8 Hz) which is attributed to the proton at C-6 This unexpected low-field resonance has been shown by double-resonance experiments to

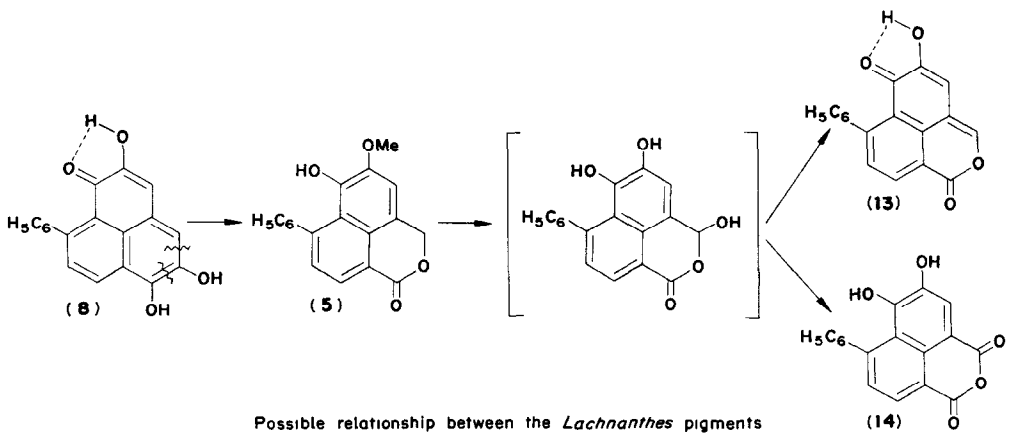
* See Acknowledgements

[14] JARCHO M (1968) *J Am Chem Soc* **90**, 4644

be associated with the aromatic system; however, its assignment to the C-6 proton is only tentative. A similar doublet has been observed in the analogous photoproduct derived from (**7**).[3] Controlled photolysis of (**8**) produces (**6**) in low yield.[3] This oxidative ring closure, which parallels the ion-reaction observed in the MS^3, is less pronounced in the case of (**8**) than in that of (**7**); this is expected, since (**8**) can exist in both tautomeric forms (**8a** and **8b**), whereas the (a) form is favored for (**7**) because of the possibilities for hydrogen-bonding; indeed, the base peak in the MS of (**8**) is the molecular ion (with the M-1 peak *ca* 85%), while it is the $(M-1)^+$ peak in the case of (**7**).

The green bands seen in the TLC examination of the root pigments demonstrated the presence of free (**8**), which has a very strong affinity for metal ions. Attempts to chromatograph the compound on commercial SiO_2 or Al_2O_3 resulted in the formation of the polar green complexes from which (**8**) could not readily be regenerated; these bands had the same R_f values as those from the plant extract. It was not possible to decide whether (**8**) exists as such in the extracts or whether it is an artifact formed from (**4**) during workup.

The root system of *Lachnanthes* thus contains as its major constituents a bioside (**4**) of trihydroxy-9-phenylphenalenone (**8**), a compound (**6**), which also forms in the laboratory from (**8**) on irradiation, and a naphthalide (**5**), obviously related to the same ring system. Although we have observed that the concentration of (**5**) is greatest in the roots of very young plants, the compound is presumably derived from a 9-phenylphenalenone oxygenated at **5** and **6** by oxidative clearage of ring C. The distribution of pigment types in *Lachnanthes* is unexpected: the roots contain the pigments just enumerated; the flowers have yielded pigments (e.g. **13**) in which C-5 is replaced by O or N,[4] and the naphthalic anhydride (**14**)[15] but, so far, no compounds containing the intact 9-phenylphenalenone skeleton; in contrast, the pericarp contains large quantities of (**7**) and smaller amounts of its 5-methoxy derivative (**9**).[15] The pigmentation at the flowering stage is thus less close to that of the root system than is that of the subsequent seed-bearing stage. In view of the formation of naphthalic anhydrides by irradiation of phenalenones *in vitro*,[16] it might be suspected that the naphthalides, naphthopyrans (**13**) and naphthoic anhydrides could be artifacts, however, the abundant occurrence of (**5**) in extracts from fresh roots rapidly worked up militates against this. The various oxidative stages occurring in the *Lachnanthes* pigments are shown below



Possible relationship between the *Lachnanthes* pigments

[15] Bazan, A. C. and Edwards, J. M. (1973) Unpublished work.
[16] Narasimhachari, N., Joshi, V. B. and Krishnan, S. (1968) *Experientia* **24**, 538.

EXPERIMENTAL

Isolation of the root pigments The washed fresh roots and rhizomes of Lachnanthes tinctoria were extracted 3× with acetone in a Waring Blendor The acetone was removed and the aq residue extracted with $CHCl_3$ The $CHCl_3$ was dried and evaporated and the residue was chromatographed over SiO_2 The aqueous phase was continuously extracted with EtOAc for ten days to yield the glycoside (**4**)

Isolation of (**4**) *and* (**8**) Attempted recrystallization of the crude glycosidic fraction from EtOH gave *lachnanthoside* (**4**) as a red–brown hygroscopic solid, m p *ca* 200° Chromatography over polyamide with EtOAc and increasing amounts of MeOH, followed by recrystallization from EtOAc, yielded crystals m p 265 268° retaining solvent of crystallization The compound was homogeneous on TLC (MeOH, polyamide, R_f 0 65) and PC (*n*-PrOH–H_2O, 3 1), R_f 0 8 MeOH–*n*-BuOH–H_2O (2 4 5), R_f 0 85, ν_{max} 1625 cm^{-1}, λ_{max} 465 372, 365 *sh* 335, 258 and 220 *sh* nm, (Log ϵ 3 76, 4 11, 3 82, 3 80, 4 37, and 4 42) δ ($CDCl_3$) 8 46 (*d*, *J* 8 Hz, 1H), 7 50 (*d*, *J* 8 Hz, 1H), 7 32 (*s*, 5H), 7 27 (*s*, 1H), 6 92 (*s*, 1H) (Found C,55 9, H,5 5 $C_{31}H_{32}O_{12}$ 2H_2O requires C 56 0 H 5 5%) Acetylation of (**4**) gave a yellow *peracetate*, recrystallized from C_6H_6–hexane m p 178 (Found C,59 8, H,5 2, MW, 1029 by osmometry $C_{49}H_{20}O_{22}$ requires C,59 4, H,5 1, MW,991) The glycoside was hydrolyzed by boiling with 10% HCl for 5 min The cooled soln was extracted with $CHCl_3$ and after evaporation the residue was chromatographed over dry TLC cellulose with C_6H_6 as eluant The purple *aglycone* (**8**) was recrystallized from $CHCl_3$–hexane m p 217 222 (decomp), ν_{max} 1620 cm^{-1}, λ_{max} 545, 383, 364 300 *sh* 276 and 237 nm (Log ϵ 3 74 3 72 3 59, 3 95 4 07 and 4 34), δ 8 64 (*d*, *J* 8 Hz, 1H), 7 52 (*d*, *J* 8 Hz, 1H), 7 48 (*s* 1H) 7 39 (*s*, 5H), 7 36 (*s*, 1H) (Found C,74 8, H,4 2, MW,304 0728 $C_{19}H_{12}O_4$ requires C,75 0, H,4 0, MW,304 0734) The aglycone (**8**) gave a blue solution in NaOH, unchanged on addition of dithionite, a red-fluorescent solution in concentrated H_2SO_4, and a black ppt with $CuSO_4$

6-Hydroxy-2 5-dimethoxy-7(or 9)-phenylphenalenone (**12**) Methylation of (**4**) with an excess of CH_2N_2, followed by hydrolysis (10% HCl), extraction with $CHCl_3$, chromatography over SiO_2 in C_6H_6 with 10% EtOAc, and recrystallization from EtOH gave 6-hydroxy-2,5-dimethoxy-7(or 9)-phenylphenalenone (**12**) as purple crystals m p 170–172 °, ν_{max} 1645 cm^{-1}, λ_{max} 478, 369, 352, 308 *sh*, 275 and 245 nm (Log ϵ 3 72 3 84, 3 82 4 10, 4 40, 4 36), δ, 8 64 (*d*, *J* 8 Hz, 1H), 7 53 (*s*, 1H), 7 32 (*s*, 5H), 6 85 (*s*, 1H), 6 80 (*d*, *J* 8 Hz, 1H), 3 98 (*s* 3H), 3 92 (*s*, 3H) (Found MW = 332 1042 $C_{21}H_{16}O_4$ requires MW = 332 1044) The compound was homogeneous by TLC (SiO_2, EtOAc, R_f 0 5) and gave a bright-blue color with NH_3 vapor Acetylation of (**12**) gave a mixture of acetoxy derivatives from which 2 5-dimethoxy-6-acetoxy-9-phenylphenalenone[11] was isolated by recrystallization from cyclohexane Compound (**12**) was also prepared by demethylation of (**11**)[11] The product of this demethylation was spectroscopically and chromatographically identical with the compound derived from lachnanthoside

Isolation of (**5**) Chromatography of the $CHCl_3$-soluble fraction over silica gave the naphthalide (**5**) as the least polar colored compound, eluted with C_6H_6 Recrystallization from MeOH yielded the *lactone* as yellow crystals m p 170–173°, ν_{max} 3635, 1720 cm^{-1}, λ_{max} 388, 342 327, 268 and 213 nm (Log ϵ 3 79, 3 60, 3 56, 4 47 and 5 2) δ, 8 30 (*d*, *J* 7 5 Hz, 1H), 7 40 (*s*, 5H), 7 20 (*d*, *J* 7 5 Hz, 1H), 5 98 (*s*, 1H), 5 78 (finely split singlet, 2H), 3 97 (*s*, 3H) (Found M = 306 0859 $C_{19}H_{14}O_4$ requires MW = 306 0892) The compound gave a green solution with NaOH Methylation with an excess of CH_2N_2 gave a yellow monomethyl ether m p 165 168 from cyclohexane identical (IR spectrum) with the compound (**3a**) isolated by Cooke[9]

Isolation of Lachnanthofluorone (**6**) Elution with 2% MeOH in C_6H_6 gave a bright-red fluorescent soln from which *lachnanthofluorone* (**6**) was isolated by recrystallization from MeOH and sublimation (270°, 0 1 mm) as mauve crystals m p > 300 °, ν_{max} 3600 3450 broad, 1620 cm^{-1}, λ_{max} 542, 375, 360, 281 and 237 nm (Log ϵ 3 81 3 23 3 31, 3 92 and 4 23), δ, 10 86 and 10 00 (broad *s*, 2H, exchangeable with D_2O) 9 10 (*d*, *J* 9 Hz, 1H), 8 30 (*s*, 1H) 8 08 (*d*, *J* 9 5 Hz, 1H), 7 15 (*s*, 1H), 7 32 (*m*, 3H), 6 82 (*d* *J* 9 5 Hz, 1H) (Found MW = 302 0567 $C_{19}H_{10}O_4$ requires MW = 302 0579) Methylation with CH_2N_2 gave *a dimethyl ether*, purified by preparative TLC (SiO_2 C_6H_6, EtOAc 20 1) and recrystallized from MeOH m.p 287–289 ν_{max} 1625 cm^{-1}, δ 8 90 (*d*, *J* 9 Hz, 1H), 8 40 (*s*, 1H), 7 68 (*d* *J* 8 Hz, 1H), 7 52 (*s*, 1H), 7 3 (*m*, 3H), 6 82 (*d*, *J* 8 Hz, 1H) 4 10 (*s*, 3H), 4 20 (*s*, 3H) Irradiation of (**8**) in non-degassed MeOH (Pyrex glass, high-pressure Hg lamp) resulted in the formation of (**6**), isolated by TLC, in low yield (3%), the reaction was very rapid and the major part of (**8**) was converted to yellow products presumably naphthoic anhydrides[16]

Acknowledgements— The authors wish to thank Professor R Thomas, University of Surrey, for samples of compounds (**10**) and (**11**)

The comparison between our methylated product and (**3a**) was kindly undertaken by Professor R G Cooke, University of Melbourne