Lignans and Related Phenols. Part XIV.[1] Selective Oxidation of Aryltetrahydronaphthalenes †

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The selective oxidation of aryltetrahydronaphthalene lignans by periodate and Fremy's salt has been demonstrated. Steric factors were evident in the reactions of the 5,8-quinone (II), which only underwent Thiele acetylation following its isomerisation to the flexible 'picro' derivative (III). U.v. irradiation of the quinone afforded a novel synthesis of a wholly aromatic analogue (V). Configurations were assigned on the basis of c.d. measurements.

It is probable that catabolism of the cancer-inhibitory members of the podophyllotoxin group leads to the formation *in vivo* of intermediates which are closely related to the products of selective oxidation *in vitro*. Derivatives so prepared, which retain the features essential for activity,[2] offer a means of testing the view

† This paper is also Part I of a series to be entitled 'Oxidation of Aromatic Substrates.'

[1] Part XIII, D. C. Ayres and C. K. Lim, *J.C.S. Perkin I*, 1972, 1350.

[2] E. A. Schreier, Abstracts of the 152nd Meeting of the American Chemical Society, 1966, P34.

that catabolites are the effective inhibitory agents. We studied quinones initially because extensive degradation may be avoided during their synthesis and because similar methods are also applicable to the related problem of identifying labelled centres in biosynthetic studies of lignans. Quinones have recently attracted attention as cancer inhibitors [3] and their reactivity could be used with advantage in the preparation of other lignan variants.

Periodate is a valuable reagent for quinone synthesis [4] and the mechanism of its reaction with lignan substrates having a free phenolic hydroxy-group has been discussed.[5] Samples of podophyllotoxin (I; $R^1 = R^2 =$ Me, $R^3 =$ OH, $R^4 =$ H) may develop a red colouration on treatment with this reagent; the response is actually due to the co-occurring [6] 4′-demethylpodophyllotoxin (I; $R^1 =$ H) which can be eliminated by careful recrystallisation. The pure demethyl compound obtained by chromatography [6] afforded an *ortho*-quinone on reaction with periodate. The structure (I; $R^3 =$ OH, R^4 = H, ring C = 3,4-dihydro-5-methoxy-3,4-dioxophenyl) assigned to the product followed from its intense red colour and absorption maximum (470 nm), typical [7] of a 5-alkyl-3-methoxy-*o*-benzoquinone. In the i.r. spectrum additional carbonyl peaks in the region 1620—1695 cm^{-1} arise from the quinone function; retention of the O–H stretching frequency (3400 cm^{-1}) and that of the strained lactone carbonyl (1765 cm^{-1}) indicated that the oxidation had not affected the fused rings. This conclusion was confirmed by the n.m.r. spectrum (Table). The resonances of the methylenedioxy-protons and of 5-H and 8-H were little changed relative to their positions in podophyllotoxin. Modification of ring C was evident from the one residual methoxy-peak and the resonances of 2′-H and 6′-H, which are in the expected [8] range for a quinone residue. The quinone was finally characterised as the triacetate (I; $R^1 = R^2 = R^3 =$ Ac, $R^4 =$ H), formed by reductive acetylation with zinc–acetic anhydride.

It proved difficult to obtain sufficient of the 3′,4′-quinone for antitumour testing because the precursor is a minor constituent of *Podophyllum emodi* resin. However, the large amounts of β-peltatin (I; $R^1 = R^2 =$ Me, $R^3 =$ H, $R^4 =$ OH), available [9] from chromatography of *P. peltatum* resin, make ring A derivatives readily available. The reaction between this compound and Fremy's salt [10] yielded the *para*-quinone (II); the spectroscopic characteristics of this compound resemble those of the periodate oxidation product (see Table and Experimental section). The mass spectrum included an $M + 2$ peak, whereas the quinol obtained by reduction with dithionite gave a normal mass spectrum. It appears that the *para*-quinone (II) captures two hydrogen atoms from water vapour in the spectrometer.[11] The high frequency (1775 cm^{-1}) of the lactone peak in the i.r. spectrum shows that the strained, inflexible system of podophyllotoxin is retained. There is evidence that in this configuration the pendant ring offers steric hindrance to attack at C-8. Thus the 5,8-quinone (II), unlike the analogous *ortho*-quinone, does not undergo reductive acetylation but will do so provided base is added to the reaction mixture. This modification causes epimerisation [12] at C-2 and imparts flexibility to ring B; hence the pendant ring can now move further from the plane of ring A and the 8-acetoxy-group can be accommodated. The product (III; X = OAc) is the first lignan derivative to be described with all ring A positions substituted.

N.m.r. data (δ values, 100 MHz) for solutions in $CDCl_3$

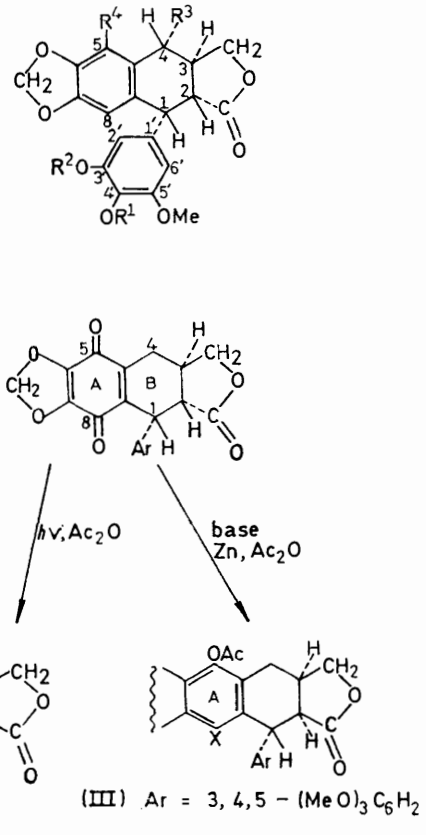
Compound	2′-H	6′-H	5-H	8-H	O·CH_2·O	MeO	OAc
(1) Podophyllotoxin	6·29 (2H)		7·01	6·40	5·88	3·70 (3H); 3·64 (6H)	
(2) 3′,4′-Quinone	6·39 *	5·45	7·08	6·39 *	5·83	3·74 (3H)	
(3) Triacetate (I; $R^1 = R^2 = R^3 =$ Ac, $R^4 =$ H)	6·27 (2H, s)		6·65(s)	6·40(s)	5·85(s)	3·70 (3H)	2·12 2·10 1·98
(4) 5,8-Quinone (II)	6·46 (2H, s)				6·10(s)	3·6 (3H) 3·7 (6H)	
(5) Diacetate (III; X = OAc)	6·29 (2H, s)				5·95(dd)	3·66 (6H) 3·74 (3H)	2·28 1·82

* Coincident signals.

The n.m.r. spectrum of the diacetate (III) shows that the 8-acetoxy-group is relatively shielded (δ 1·82) by the pendant ring, which in turn adopts a quasiaxial position; hence the diequatorial C–H coupling is typified by the 1-H signal at δ 4·68 with $J_{1,2}$ *ca.* 4 Hz. As observed for deoxypodophyllotoxin,[13] the near coincidence of 2-H, 3-H, and 4(α and β)-H signals in the range δ 2·40—3·20 prevented the evaluation of the coupling constants of these ring B protons. The separation (5 Hz) of the methylenedioxy-signal into a double doublet has only been recorded for hindered podophyllotoxin derivatives which are substituted at the 2′-

[3] R. Hardman, *Phytochemistry*, 1969, **8**, 1319.
[4] B. Sklarz, *Quart. Rev.*, 1967, **21**, 3.
[5] E. Adler and S. Hernestam, *Acta Chem. Scand.*, 1955, **9**, 319.
[6] M. V. Nadkarni, J. L. Hartwell, P. B. Maury, and J. Leiter, *J. Amer. Chem. Soc.*, 1953, **75**, 1308.
[7] H. J. Teuber and G. Staiger, *Chem. Ber.*, 1955, **88**, 810.
[8] R. H. Thomson, 'Naturally Occurring Quinones,' Academic Press, London, 2nd edn., 1971, p. 69.
[9] J. L. Hartwell and W. E. Detty, *J. Amer. Chem. Soc.*, 1950, **72**, 246.
[10] H. Zimmer, D. C. Lankin, and S. W. Horgan, *Chem. Rev.*, 1971, **71**, 229.
[11] R. W. A. Oliver and R. M. Rashman, *J. Chem. Soc.* (*B*), 1971, 341.
[12] J. L. Hartwell and A. W. Schrecker, *Progr. Chem. Org. Natural Products*, 1958, **15**, 106.
[13] D. C. Ayres, J. A. Harris, P. N. Jenkins, and L. Phillips, *J.C.S. Perkin I*, 1972, 1343.

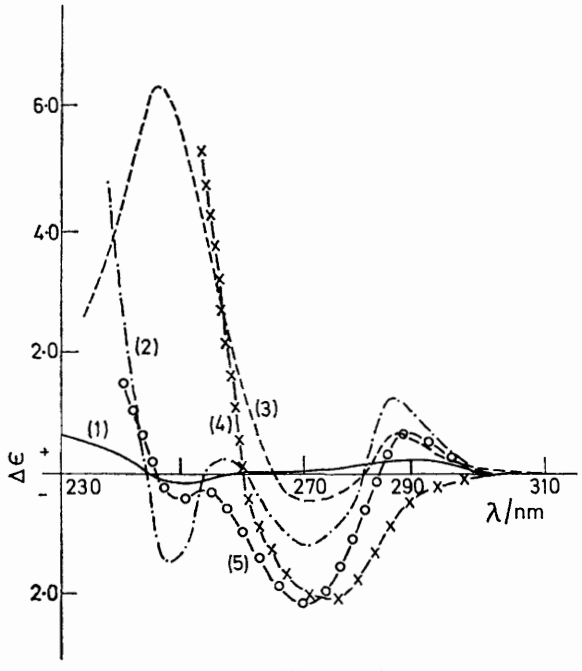
position.[1] The separation is more marked in the diacetate with the picropodophyllin configuration because in this model the pendant group can adopt an



SCHEME
Precursors and reaction products of lignan quinones

extreme quasiaxial position, in which the ether protons are clearly non-equivalent. There was no significant variation in the n.m.r. spectrum of the diacetate (III) between ambient temperature and 115° or in the c.d. of this compound between −10 and −160°, suggesting that the conformation with the quasiaxial aryl substituent at C-1 was considerably more stable than that in which this group was quasiequatorial.

C.d. measurements are of value in characterising diastereoisomers of aryltetrahydronaphthalene lignans, but in peltatin derivatives the differences between the rigid structures (type A, podophyllotoxin group) and their flexible C-2 epimers (type B, picropodophyllin group) are muted by the additional oxy-substitution in ring A. In its general form the curve (Figure,* curve 2)



C.d. of the peltatins and related lignans: (1) 5-*O*-acetyl-β-peltatin, (2) 5,8-diacetoxydeoxypicropodophyllin, (3) β-peltatin (B form), (4) β-peltatin (A form), (5) podophyllotoxin

for 5,8-diacetoxydeoxypicropodophyllin (III; X = OAc) is similar to that of podophyllotoxin [14] (curve 5) and paradoxically this is good evidence for the picro-configuration in the former: this follows because only a change in geometry can compensate for the differences which normally arise on changing the substitution pattern in this way. In the peltatins themselves (curves 3 and 4) an intense positive c.d. maximum at 248 nm obscures any differences of detail in this region but a comparison at longer wavelengths is informative. Here the A form (I; $R^1 = R^2 = Me$, $R^3 = H$, $R^4 = OH$) (curve 4) has only one maximum near 274 nm, whereas the three compounds (curves 1—3) with the picro-configuration show two maxima in this region. For 5-*O*-acetyl-β-peltatin (B) (III; X = H), whose substituents most closely compare with those of the 5,8-diacetate, there is little more than an inflection near 270 nm but the two curves (1 and 2) are so similar overall as to leave no doubt of the common geometry of the two compounds.

The photolysis of the *p*-quinone (II) was of interest to us since trapping of its activated quinol form by interaction with ring C would lead to the model (IV), of value as a reference compound for an n.m.r. study of ring C rotamers. A number of analogous cyclisations

* Measurements on solutions in diethyl ether-ethanol–isopentane (5:2:5) by Mr. R. J. Mullins using a Jouan Dichrographe 185.

[14] P. B. Hulbert, Ph.D. Thesis, London, 1969, also I.U.P.A.C. Congress, Boston, Mass., July 1971.

have been recorded [15] and their dependence on solvent was noted. We found that many products were formed on irradiation in ethanol, but controlled photoreaction of the quinone (II) was possible in acetic anhydride solution; under these conditions the main product was the wholly aromatic lignan (V). Complete dehydrogenation of ring B was indicated by the intense fluorescence of the product which distinguishes it from the dihydro- or apo-derivatives.[16] This provides a new route to lignans of type (V) which is probably sterically preferable to the formation of (IV).

The 5,8-quinone with the podophyllotoxin configuration was tested by Mr. J. L. Everett (Chester Beatty Institute) against the Walker tumour. However, the compound was toxic on intraperitoneal injection at 4 mg kg^{-1}, and no tumour regression was observed at lower dose levels. The 5,8-diacetate was also tested since the axial conformation of the pendant ring is similar to that of the active epimers despite the isomerisation at C-2; here a ratio [17] of tumour weight in controls to that in treated animals of 1·2 was obtained at a dose of 4 mg kg^{-1} but larger doses again proved toxic.

EXPERIMENTAL

M.p.s were taken on a hot-stage apparatus. I.r. spectra (KBr discs) were recorded with an Infracord 237 spectrometer and u.v. spectra with a Unicam SP 800 instrument. The mass spectra were taken by the U.L.I.R.S. at the London School of Pharmacy.

Isolation of 4′-Demethylpodophyllotoxin and β-Peltatin.—The former (300 mg) was obtained [6] from *Podophyllum emodi* resin (35 g) by chromatography; it followed the main fraction of podophyllotoxin (13·1 g). Laporte Spence type H alumina was effective as supplied but the separation may fail with less active material. Pure podophyllotoxin, obtained by precipitation from a concentrated solution in ethanol by addition of benzene, gave no red colouration on treatment with periodate. Demethylpodophyllotoxin, m.p. 251°, was further characterised by comparison of its i.r. and n.m.r. spectra with those of an authentic sample kindly supplied by Dr. E. A. Schreier.

Chromatography on alumina of the resin of *P. peltatum* afforded [9] β-peltatin (A form) (7% yield), m.p. 242°. This was characterised by base-catalysed epimerisation to the B form, m.p. 213° (lit.,[8] 213°), which yielded the 5-*O*-acetate, m.p. 221° (lit., 222°) on refluxing its solution in acetic anhydride.

3′,4′-Dihydrodemethoxy-3′,4′-dioxopodophyllotoxin.—As a test for demethylation it is convenient to shake a solution of the lignan in methylene chloride with the same weight of sodium periodate dissolved in water acidified with a little acetic acid. For preparative purposes 4′-demethylpodophyllotoxin (200 mg, 0·5 mmol) was dissolved in ethanol (10 ml) and stirred with a solution of the periodate (160 mg, 1·5 mmol) in water (10 ml); a deep red colouration developed during 1·5 h. T.l.c. on Kieselgel G (benzene–ethyl acetate as eluant) showed that the reaction was then complete and the dark red *3′,4′-quinone* (147 mg, 72%) was isolated by extraction of the liquor with chloroform (3 × 50 ml). Drying ($MgSO_4$) and trituration of the solute with light petroleum (b.p. 60—80°), gave pure material, m.p. 190—192° (benzene). In addition to its i.r. (see before) and n.m.r. spectra (Table, entry 2), the product was characterised by its molecular ion of mass 384 ($C_{20}H_{16}O_8$).

The zinc–acetic anhydride procedure described later for 5,8-diacetoxydeoxypicropodophyllin, but *without* addition of base, was used to convert the 3′,4′-quinone into the *3′,4,4′-triacetoxy-derivative*. This showed carbonyl and lactone peaks in its i.r. spectrum (1730, 1770, and 1790 cm^{-1}), and the n.m.r. spectrum (Table, entry 3) was typical (Found: C, 61·1; H, 4·9%; M^+, 512. $C_{26}H_{24}O_{11}$ requires C, 60·9; H, 4·7%; *M*, 512).

4-Deoxy-5,8-dihydro-5,8-dioxopodophyllotoxin.—β-Peltatin (A form) (1·0 g, 2·4 mmol) was dissolved in acetone (23 ml) and stirred at room temperature with a solution of Fremy's salt [18] (1·5 g, 5 mmol) in potassium dihydrogen phosphate buffer (0·16 mol; 60 ml). The initial violet colouration changed to orange-brown and at this point deposition of crystals began; no further change was seen after 45 min and the *5,8-quinone* (620 mg) was filtered off, washed with water and dried *in vacuo*. A further quantity (210 mg, 80% overall yield) was obtained by extraction of the aqueous filtrate with chloroform (4 × 100 ml). The main crop of crystals, m.p. 224—227° was pure; $\nu_{max.}$ 1775 (lactone C=O), 1665 (quinone C=O), and 1590 cm^{-1} (aromatic); for n.m.r. spectrum see Table (entry 4) (Found: C, 61·7; H, 4·65%; M^+, 428. $C_{22}H_{20}O_8$ requires C, 61·7; H, 4·7%; *M*, 428).

This quinone had a typical [7] u.v.–visible absorption (freshly prepared solution in chloroform). It was further characterised as the *5,8-quinol*, $\nu_{max.}$ 3300 (OH) and 1760 cm^{-1} (lactone C=O), formed by reduction with sodium dithionite in aqueous ethanol (1 : 5) and extraction with methylene chloride. After recrystallisation from aqueous ethanol the product (85%) was rapidly reoxidised in air to the starting quinone. T.l.c. of a solution showed that this change was about half complete after 3 h at room temperature (Found: C, 61·4; H, 5·05%; M^+, 430. $C_{22}H_{22}O_9$ requires C, 61·4; H, 5·1%; *M*, 430).

5,8-Diacetoxy-4-deoxypicropodophyllin.—The 5,8-quinone (0·50 g, 1·2 mmol) was dissolved in acetic anhydride (3 ml) and shaken with zinc dust (0·5 g), but in contrast to the reaction of the 3′,4′-quinone, no change (t.l.c.) occurred. On the addition of one drop of triethylamine the red colour of the quinone began to fade immediately and had disappeared after shaking for 5 min. After heating under reflux for 2 min the zinc dust was removed and washed with hot acetic acid (5 ml), and a saturated solution of the product in aqueous acetic acid was obtained by refluxing the total filtrate with water (15 ml). The crystalline *diacetate* (200 mg, 33%) which separated had $\nu_{max.}$ 1760br cm^{-1} (lactone and ester C=O); the acetyl groups were distinguished in the n.m.r. spectrum (Table, entry 5) and as fragments in the mass spectrum (Found: C, 60·5; H, 5·1%; M^+, 514. $C_{26}H_{26}O_{11}$ requires C, 60·7; H, 5·1%; *M*, 514).

5,8-Diacetoxydidehydroanhydropodophyllotoxin (V).—(*a*) The 5,8-quinone (250 mg, 0·58 mmol) was dissolved in ethanol and irradiated with a Hanau Q 81 lamp for 30 min; the red colour was then discharged but t.l.c. on Kieselgel G

[15] A. Schönberg, 'Preparative Organic Photochemistry,' Springer, Berlin, 2nd edn., 1968, p. 426.
[16] D. C. Ayres and J. W. Mundy, *Chem. Comm.*, 1967, 222; ref. 12, p. 123.
[17] V. M. Rosenoer in 'Experimental Chemotherapy,' eds. R. J. Schnitzer and F. Hawking, Academic Press, New York, 1966, vol. 4, p. 16.
[18] W. Moser and R. A. Howie, *J. Chem. Soc.* (*A*), 1968, 3039.

[eluant benzene–ethyl acetate (1 : 2)] showed that a complex mixture of products had been formed.

(*b*) On substituting acetic anhydride (25 ml) as solvent, numerous products were again detected but one substance with an intense blue fluorescence predominated. Water (100 ml) was added to hydrolyse the anhydride and the resulting mixture was extracted with dichloromethane (4 × 100 ml). The combined extracts were washed with saturated sodium hydrogen carbonate solution and water, dried ($MgSO_4$), and evaporated to yield an oil (152 mg), which was purified by preparative layer chromatography [Kieselgel G (1 mm); elution with dichloromethane]. Extraction of the fluorescent zone with acetone gave a white solid (11 mg from a 25 mg sample), $\nu_{max.}$ 1760br cm^{-1} (lactone and acetate C=O). This was characterised as the *diacetyldidehydroanhydro-derivative* by its mass spectrum, *m/e* 43 (strong, $CH_3{}^+CO$) and daughter peaks typical of acetoxy-groups (Found: C, 61·5; H, 4·3%; M^+, 510. $C_{26}H_{26}O_{11}$ requires C, 61·2; H, 4·3%; *M*, 510).

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