Cancer Chemother Pharmacol (1982) 7:99–101

Cancer Chemotherapy and Pharmacology

Articles

Modification of the Pendant Ring of Podophyllotoxin*

David C. Ayres and Chang Kee Lim[1]

Chemistry Department, Westfield College (University of London), Hampstead, London, NW3 7ST, Great Britain

Summary. *An improved route to quinonoid analogues of podophyllotoxin has been devised. Derivatives have been characterised by HPLC, NMR, and mass spectrometry; these include compounds in which the original reactivity of the lignan is modified by remote substition, which also retain the physiologically active configuration in the presence of bases.*

Introduction

An account of the chemistry of podophyllotoxin, including aspects of its action as an inhibitor of malignant tumours has been given [6]. Conjoint papers [1] detail the considerable current activity in this field. It has been shown [7] that glycosides in which the 4′-position has been demethylated show a new form of activity in that they induce degradation of cellular DNA. The synthesis of variants incorporating basic and/or hydrogen-bonding groups in ring C is therefore an attractive approach to cancer chemotherapy.

Materials and Methods

3′,4′-Dihydroxydemethoxy-3′,4′-dioxopodophyllotoxin (A). Podophyllotoxin (832 mg) in glacial acetic acid (10 ml) was added to a mixture of nitric acid (3 ml) and glacial acetic acid (10 ml) at 0° C. After 5 min the dark red solution was diluted with water (50 ml) and extracted with chloroform. Crude material obtained by drying ($MgSO_4$) and evaporation of this solvent was recrystallised from benzene to afford the *Quinone* (568 mg, 74% yield), m.p. 190–192° C. This was characterised by typical quinonoid peaks in the i.r. spectrum at 1,665, 1,700 and 1,725 cm^{-1} and maximum absorption in the u.v. region at 470 nm. The mass spectrum included peaks at – 400 (M+16, 15%); 386 (M+2, 100%); 384 (M, 60%) and 367 (M-OH, 60%): $C_{20}H_{16}O_8$ requires $M = 384$. The nmr spectrum was consistent with this structure and showed that chemical change was confined to the pendant ring.

The quinone was also obtained in 75% yield by shaking a chloroform solution of 4′-demethylpodophyllotoxin with nitrous acid.

4′-Hydrodemethoxy-4′-oxo-β-apopodophyllic-1′-lactone (D). A mixture of nitric acid (2 ml) and glacial acetic acid (8 ml) was added dropwise to a solution of picropodophyllin (258 mg) in glacial acetic acid (8 ml). The procedure described above was then followed, save that the crude product was recrystallised from aqueous-acetone to give colourless needles (208 mg, 84%), m.p. 223–224° C. The i.r. spectrum showed peaks typical of dienone absorption at 1,620, 1,655 and 1,680 cm^{-1}, with a typical lactone absorption at 1,760 cm^{-1} and modified hydroxyl peaks at 3,520, 3,580 cm^{-1}. The mass spectrum exhibited a molecular ion peak at 398 ($C_{21}H_{18}O_8$ requires M = 398).

2′-Halopodophyllotoxins. These were prepared and characterised as described previously [2].

Quinoxaline Derivative (B) of 3′,4′-dihydrodemethoxy-3′,4′-dioxopodophyllotoxin. A solution of o-phenylene diamine (115 mg) in chloroform (5 ml) was added dropwise to a solution of the quinone (A) in chloroform (5 ml), when the intense red colour of the solution was discharged. After standing for 30 min at room temperature the solution was concentrated to half its volume and the crude product precipitated by the addition of light petroleum (b.p. 40–60° C). The pure quinoxaline (B), m.p. 195° C, was obtained by recrystallisation from aqueous-ethanol with i.r. absorption at 1,610, 1,630, 1,710 and 1,720 cm^{-1}, 1,770 (lactone C = O) and 3,400 cm^{-1} (OH). The mass spectrum showed M^+ 456.1326 (95%, $C_{26}H_{20}N_2O_6$ requires M = 456.1321.

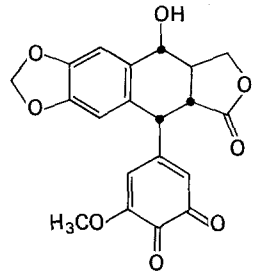
Chromatography. HPLC of these lignans was carried out with a μ-Bondapak C_{18} column whose interaction with polar sites is particularly sensitive to configurational changes. Typically elution was with 40% methanolwater when the retention time of podophyllotoxin was 13.1 min and that of its C2-epimer picropodophyllin was 12.0 min.

Send offprint requests to D. C. Ayres at Westfield College

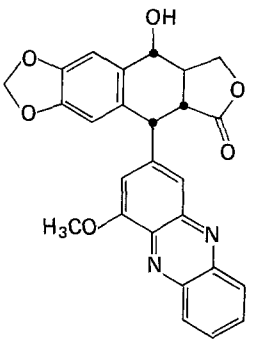
1 *Present address:* Division of Clinical Chemistry, Clinical Research Centre, Watford Road, Harrow, Middlesex, Great Britain

* This paper forms part 17 in the series 'Lignans and Related Phenols'

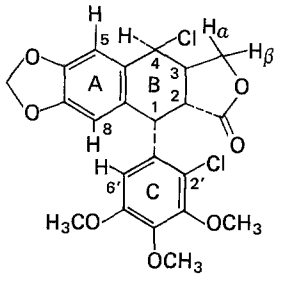
0344-5704/82/0007/0099/$01.00



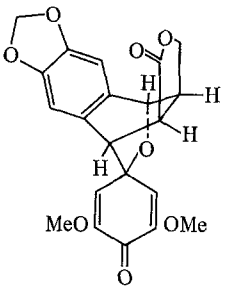
A



B



C



D

Discussion

Quinonoid derivatives of the C ring in podophyllotoxin were first obtained [3] by the action of periodate upon 4′-demethylpodophyllotoxin. Since the yield of this precursor from *Podophyllum emodi* resin is only one twentieth that of podophyllotoxin itself an alternative procedure was sought. We found that an identical product (A) could be obtained by treatment of podophyllotoxin with nitric acid. Under these conditions there was no aromatic substitution but rather acid-induced demethylation to yield 4′-demethylpodophyllotoxin as a transitory intermediate. In a separate experiment we were able to show that the demethylated lignan was oxidised to the quinone by nitrous acid; it is probable that the related nitrosonium ion is responsible for the final oxidation step in the nitric acid/acetic acid medium [4].

Quinones could be used as precursors for numerous podophyllotoxin derivatives since they undergo a wide range of reactions [5]. Substitution of alkylamino groups is possible, alternatively a basic group can be incorporated by condensation with a primary amine. We used this reaction to further characterise the quinone (A) as its quinoxaline derivative (B).

Earlier work [2] showed that 2′-bromo and chlorosubstituents can be inserted by reaction with phosphorus pentahalides. There is an increase in strain in the B ring of these products which leads to an increase in the rates of reaction at C4, presumably by acceleration of the initial ionisation step at this position. Thus on hydrolysis in 12% water/dioxan at 40° C 4α- and 4β-chlorodeoxypodophyllotoxin had half-life times of 215 and 274 min respectively. 2′,4β-Dichlorodesoxypodophyllotoxin (C) reacted very rapidly under these conditions and its reaction rate could only be determined reliably by reducing the temperature to 30° C and the water content to 3% aqueous dioxan. Under these conditions it had a half-life of 478 min, but now there was negligible hydrolysis of the monochloro epimers after 16 h in this medium at 30° C. From a clinical point of view this kind of control of rates and hence of equilibria between interacting species could be valuable.

Physical evidence of strain in 2′-substituted podophyllotoxins is obtained from their nmr spectra. Here the resonance of the only remaining ring C proton occurs at extremely high field: In O-methyl-2′-chloroepipodophyllotoxin/chloroform this resonance coincided with that of the methylenedioxy group. A shift upfield of this size shows that the rotation of ring C is restricted, so that the proton at C6′ resides largely within the shielding zone of ring A. This congestion in the molecule confers a stability to epimerisation by bases which is not shown by the podophyllotoxins themselves for the physiologically active trans-lactone configuration is retained by the 2′-haloderivatives even in the presence of alkoxide ions. The mechanism of this change has always been interpreted as an inversion of configuration at C2, following the formation of the enol at this position by proton abstraction. Preliminary work that we have undertaken shows that the change is more complex. A methanolic solution of podophyllotoxin containing piperidine was analysed by HPLC at intervals of time, when three major intermediates were detected in solution before precipitation of the relatively insoluble picropodophyllin.

It is of interest that although picopodophyllin is an isomer of podophyllotoxin it reacts quite differently with nitric acid under the conditions mentioned above. No quinonoid products were seen, but a colourless product was obtained which was characterised as a dienone. Full details of this work will be published elsewhere but on the existing evidence the structure (D) is favoured for this material.

References

1. Papers submitted to the First International Symposium on the Podophyllotoxins in Cancer Therapy, Southampton, July 1981
2. Ayres DC, Lim CK (1972) Lignans and related phenols. J Chem Soc (Perkin 1): 1350

3. Ayres DC, Harris JA (1973) Lignans and related phenols. Part XIV. Selective oxidation of aryltetrahydronapthalenes. J Chem Soc (Perkin 1): 2059
4. Barton DHR, Gordon PG, Hewitt DG (1971) On the dealkylation of aromatic ethers with nitrous acid. J Chem Soc (C): 1206
5. Finley KT (1974) In: Patai S 'The addition and substitution chemistry of quinones' (ed). The chemistry of quinonoid compounds. Wiley, London, p 877
6. Hartwell JL, Schrecker AW (1958) The chemistry of podophyllum. In: Zechmeister L (ed) Progress in the chemistry of organic natural products. Springer, Berlin Heidelberg New York, p 83
7. Loike JD, Brewer CF, Sternlicht H, Gensler WJ, Horwitz SB (1978) Structure-activity study of the inhibition of microtubule assembly in vitro by podophyllotoxin and its congeners. Cancer Res 38: 2688

Accepted July, 1981