Lignans and Related Phenols. Part XIII.[1] Halogenated Derivatives of Podophyllotoxin

By D. C. Ayres * and C. K. Lim, Chemistry Department, Westfield College, London NW3 7ST

Replacement of the alcoholic hydroxy-group by halogen in podophyllotoxin on treatment with phosphorus pentahalides is followed by halogenation of the pendant aryl substituent; this occurs either by substitution of a hydrogen atom or by displacement of a methoxy-group. The sequence ends with the halogenation of the methylenedioxy-residue. The orientation and stereochemistry of the inserted halogen atoms have been largely assigned by n.m.r. spectroscopy.

SELECTIVE demethylenation of podophyllotoxin (I; X = OH, Y = OMe, Z = H) with boron trichloride has been described [2] but the procedure led to experimental difficulties when we attempted to adapt it for large-scale operation. With the initial collaboration of Mr. J. A. Harris we therefore explored the use of phosphorus pentachloride [3] for the reaction, having noted that methylenedioxy-groups attached to both the A and C rings of lignans had been converted by this means [4,5] into readily hydrolysed carbonates in high yield. Under relatively forcing conditions [5] a lactone function was retained and the methoxy-group in a related compound, 2-methoxy-3,4-methylenedioxybenzaldehyde, was unaffected.[6]

In the initial experiment (by J. A. Harris) phosphorus pentachloride and podophyllotoxin afforded a dichloroderivative which was characterised by its mass spectrum. That one of the substituents was located at C-4 was confirmed by the low-field doublet due to 4-H in the n.m.r. spectrum (Table 2, entry 4 is typical). The weight of evidence available for reactions with halogenating agents other than phosphorus pentachloride suggests that this substituent has the 4β- or *epi*-configuration, and that this is retained on solvolysis; this point will be taken up in the later detailed discussion of stereochemistry. The n.m.r. spectrum showed that the second chloro-substituent had entered ring C, for the three methoxy-groups had become non-equivalent and the methylenedioxy-signal was unchanged. The i.r. spectrum was consistent with this interpretation: the intense carbonyl stretching absorption of the strained lactone was retained but a pronounced peak at 1590 cm^{-1} had disappeared. This lower frequency i.r. peak is generally found in compounds of this class, and subsequent findings established that its disappearance is characteristic of further substitution in the pendant ring.

A puzzling feature of the n.m.r. spectrum was the appearance in deuteriochloroform solution of signals for only two aromatic protons, although three were to be expected in a dihalogenoderivative. The signal for the 'missing' proton was resolved from those of the methylenedioxy-group on addition of hexadeuteriobenzene.

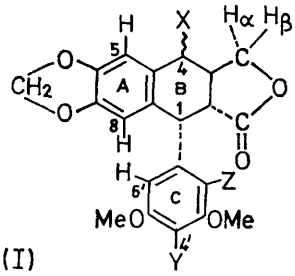
Assignment of aromatic proton signals is straightforward when ring C is not further substituted (Table 1). Here the 5-H signal varies as expected when structural changes are confined to C-4 and the greater intensity of the 2'- and 6'-H resonance distinguishes it from that of 8-H, which lies at lower field. This difference is likely to be accentuated if rotation is restricted by steric hindrance between the lactone carbonyl group and a 2'-substituent, when the balance of ring C rotamers will be weighted in favour of those in which 6'-H lies within the shielding zones of ring A and the carbonyl

[1] Part XII, D. C. Ayres, J. A. Harris, P. N. Jenkins, and L. Phillips, preceding paper.
[2] E. Schreier, *Helv. Chim. Acta*, 1964, **47**, 1529.
[3] G. Barger, *J. Chem. Soc.*, 1908, **93**, 563.
[4] N. S. Bhacca and R. Stevenson, *J. Org. Chem.*, 1963, **28**, 1638.
[5] R. S. Burden, L. Crombie, and D. A. Whiting, *J. Chem. Soc.* (*C*), 1969, 693.
[6] I. R. C. Bick and R. A. Russell, *Austral. J. Chem.*, 1969, **22**, 1563.

group: the exceptionally high-field signal is therefore assigned to the lone ring c proton in a product chlorinated at C-2′ and C-4 (I; X = Z = Cl, Y = OMe).

TABLE 1

Chemical shifts (δ in p.p.m.) of aromatic protons in podophyllotoxin and derivatives at 100 MHz (solutions in $CDCl_3$)



(I)

Compound	4-Substituent	Ring c	8-H	5-H
Podophyllotoxin	α-OH	6·29	6·40	7·01
Epipodophyllotoxin	β-OH	6·19	6·43	6·79
O-Acetylpodophyllotoxin	α-OAc	6·30	6·44	6·67
O-Acetylepipodophyllotoxin	β-OAc	6·21	6·49	6·81
4α-Chloro-4-deoxypodophyllotoxin	α-Cl	6·30	6·43	7·04
4β-Chloro-4-deoxypodophyllotoxin	β-Cl	6·19	6·42	6·80
Podophyllotoxone	(C)=O	6·30	6·62	7·44
O-Methyl-2′-chloroepipodophyllotoxin	β-OMe	5·88 (1H)	6·35	6·67
O-Methyl-2′-bromoepipodophyllotoxin	β-OMe	5·87 (1H)	6·33	6·68
O-Methyl-4′-chloro-4′-demethoxypodophyllotoxin	β-OMe	6·15	6·45	6·72

Continued chlorination of ring c leading to its complete substitution has not been observed and would be difficult under any conditions in view of the steric factors. Minor variations in the experimental procedure did, however, reveal another competing side reaction which also preceded the modification of the methylenedioxy-group. The product formed had a mass number of 436, 30 units less than the product of ring c substitution, which indicated the loss of a methoxy-group and the retention of both protons on ring c. If the first chlorine atom was inserted by the rapid reaction at C-4 it follows that the second enters at C-4′ with the formation of 4β,4′-dichloro-4-demethoxypodophyllotoxin (I; X = Y = Cl, Z = H). The n.m.r. spectra of this product and its C-4 methoxy-derivative (Table 1, last entry; Table 2, entry 1; Table 3) confirm this assignment: the methylenedioxy-group is retained; the 4-H signal is evidently deshielded by chlorine; a 2′- and 6′-H resonance appears at a chemical shift typical of the precursor, and a single peak at δ 3·76 (6H) is assigned to equivalent 3′- and 5′-methoxy-groups.

Analogous dibromo-compounds were formed when podophyllotoxin was treated with phosphorus pentabromide. The rate of solvolysis reactions at C-4 in these compounds was so rapid that they were converted into the methoxy-derivatives for characterisation (Table 2, entry 3; Table 3). Signals for three non-equivalent methoxy-groups (due to ring c substitution) and considerable shielding of the remaining proton confirmed 2′-bromination in one product; signals for two equivalent methoxy-groups and the mass spectrum established C-4′ bromine insertion in the other.

Kofod and Jorgensen [7] first described the bromination of podophyllotoxin with bromide–bromate in chloroform–aqueous acid; there is some uncertainty about their product because of its dual m.p. (155 and 183°). Substitution in ring c was indicated by the isolation of 2-bromo-3,4,5-trimethoxybenzoic acid in low yield following oxidation with alkaline permanganate, partial conversion into bromopodophyllotoxone under these conditions was suspected but was not confirmed. Mechanistic considerations and the n.m.r. spectra (Table 2, entry 3 is typical) of the methanolysis products, show that the alcohols we obtained by hydrolysis of the 2′,4-dibromo-derivative and of the 4,4′-dibromo-4′-demethoxy-compound are C-4 epimers of podophyllotoxin. We have established that demethoxylation does not occur under the conditions employed by Kofod and Jorgensen but find that excess of bromate leads to the formation of 2′-bromopodophyllotoxone in high yield.

Phosphorus pentabromide readily dissociates into phosphorus tribromide and bromine; the ^{31}P n.m.r. spectrum of a solution in benzene [8] shows that the concentration of phosphorus(v) species is small. Substitution of bromine in ring c, with liberation of hydrogen bromide, can therefore proceed in benzene by direct bromination, and displacement at C-4 will follow the interaction of the hydroxy-group with phosphorus tribromide [9] or hydrogen bromide. The displacement of the 4′-methoxy-group has not been observed before, but it probably arises from the well-known [10] acid-induced demethylation, with subsequent reaction between the exposed phenolic hydroxy-group and the halogenating agent; retention of this group in the presence of bromate is consistent with the assessment because hydrogen bromide will not accumulate in the presence of the oxidising agent.

A new mechanistic feature enters into the reactions with phosphorus pentachloride, which does not dissociate as does the pentabromide. The substitution in ring c is analogous to the reaction [11] of pyrogallol trimethyl ether, which is converted into the 4-chloro-derivative on heating with phosphorus pentachloride at 100°; this level of alkoxylation imparts considerable nucleophilic character to the aromatic ring, which accounts for the fact that lignans with fewer oxy-substituents were not chlorinated in this way when subjected to Barger's reaction. Despite considerable

[7] H. Kofod and C. Jorgensen, *Acta Chem. Scand.*, 1955, **9**, 1327.
[8] D. S. Payne, *Topics Phosphorus Chem.*, 1967, **4**, 136.
[9] J. L. Hartwell and A. W. Schrecker, *J. Amer. Chem. Soc.*, 1951, **73**, 2909.
[10] W. H. Hunter and A. A. Levine, *J. Amer. Chem. Soc.*, 1926, **48**, 1608.
[11] M. Kohn and E. Gurewitsch, *Monatsh.*, 1928, **49**, 179.

steric hindrance, the reaction of podophyllotoxin occurred under milder conditions than before [11] and the specificity is in contrast to the other reactions of aromatic compounds without activating substituents, which only occur [12] at high temperatures. Postulation of an ionic mechanism for this aromatic substitution is justified under these conditions, as phosphorus pentachloride is an ionic solid [13] and, to the extent that reaction occurs in solution, ionic character can be induced by complexation with the substrate.[14] The ions PCl_6^- and PCl_4^+ may here be involved in a push–pull mechanism and the Lewis acid is also expected to form a complex of the type $Ar\overset{+}{O}(Me)PCl_4$, which can initiate the competing demethylation reaction. Once an exposed phenolic group has been chlorinated, or brominated, no further insertion of halogen occurs in ring C; this is in itself evidence of an ionic process. Specific substitution into the more nucleophilic ring during this reaction lends support to a recent revision [15] of the orientation of electrophilic substitution in these compounds.

Previous investigators [2,9] of C-4 hydroxy-group displacement did not employ phosphorus pentahalides, but they established a tendency for net inversion in the reactions of podophyllotoxin and for retention of configuration in those of its C-4 epimer with reagents of a similar type. A mechanism involving a common C-4 carbonium ion, stabilised by mesomeric interaction with ring A, and subsequent attack by the nucleophile from the less hindered β * face is satisfactory and in keeping with the ease of solvolysis of these halides. Evidence of inversion during the formation of ' chloro-podophyllotoxin,' or more strictly 4β-chloro-4-deoxypodophyllotoxin, from the reactions with phosphorus trichloride and thionyl chloride comes from the observation [2] of a small coupling constant ($J_{3,4}$ 3 Hz) in its n.m.r. spectrum. Schreier [2] also mentions the isolation in low yield of another chloro-compound from the reaction between DMA-podophyllotoxin and hydrogen chloride. This was not fully characterised but was thought to have the 4α-configuration since $J_{3,4}$ was larger and more typical of a diaxial proton coupling in this rigid model.

By shortening the time of contact between podophyllotoxin and phosphorus pentachloride we were able to isolate the monochloro-derivative with ring C unaffected; this was identical with the 4β-chloro-compound prepared by using thionyl chloride under the original conditions.[9] When the reaction occurred in a less polar medium, that is in a dilute benzene solution, the S_Ni mechanism [16] was favoured and the epimeric 4α-compound was formed as the only product; this modification indicates an approach to other derivatives of podophyllotoxin which retain its more physiologically active configuration.

TABLE 2

Chemical shifts (δ in p.p.m.) of ring B protons in halogenated derivatives of podophyllotoxin (PT) at 100 MHz

		Shifts				J/Hz			
No.		1-H	2-H	3-H	4-H	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{3,\alpha}$
1	*O*-Methyl-4′-chloro-4′-demethoxyepi-PT	4·55	3·40	2·85	4·26	5·5	13·5	*ca.* 2·5	9
2	*O*-Methyl-2′-chloroepi-PT	5·15			4·22	7·0		Small	8
3	*O*-Methyl-2′-bromoepi-PT	5·14	*ca.* 3·40	2·96	4·20	7·0	14	2·5	8·5
4	4β,4′-Dichloro-4-deoxy-4′-demethoxy-PT	4·58	3·25		5·32	4·5		2·8	
5	4β-Chloro-4-deoxy-PT	4·57	3·33	*ca.* 3·00	5·30	4·75	15	3·2	
6	4α-Chloro-4-deoxy-PT *	4·30	2·17	*ca.* 2·50	4·09	5·2	14	*ca.* 8	†

* This compound in hexadeuteriobenzene, the others in deuteriochloroform. † H_α and H_β are nonequivalent.

It was shown (by J. A. Harris) that the methylenedioxy-group was modified at a late stage in the reaction with phosphorus pentachloride, but owing to the complexity of the mixture of products which had then been formed the dichloromethylene group could not be characterised. A clearer result was obtained by using *O*-methyl-2′-bromoepipodophyllotoxin, where no chlorination is possible in ring C; hydrolysis of this product afforded a carbonate and subsequently a catechol derivative.

The compounds listed in Table 2 are representative of all those we describe here, the data reveal where epimerisation has occurred at C-4 and they show that the podophyllotoxin configuration is always retained at the other centres under these conditions. The short account of the spectral analysis which follows is based on the methods described in the preceding paper.[1]

O-Methyl-4′-chloro-4′-demethoxyepipodophyllotoxin (I; X = OMe, Y = Cl, Z = H) (Table 2, entry 1).—The only difference of note between this spectrum and that of epipodophyllotoxin (preceding paper, Figure 5) is that the 4-H signal lies at higher field than that due to 1-H. The 2-H signal was identified by irradiation of the 1-H doublet located at δ 4·53; the quartet partly concealed by the highest field methoxy-peak then simplified to a doublet ($J_{2,3}$). This was confirmed by INDOR analysis, when on monitoring one of the peaks due to 1-H two progressive and two regressive signals were detected, at δ 3·30, 3·35, 3·43, and 3·50; these interchanged when the monitor was set on the other peak of the doublet as was shown for podophyllotoxone (preceding paper, Figure 1). In common with all the compounds examined in deuterio-

* Arbitrarily assigned as that remote from the pendant aryl substituent.

[12] R. D. Kimbrough and R. N. Bramlett, *J. Org. Chem.*, 1969, **34**, 3655.

[13] D. Clark, H. M. Powell, and A. F. Wells, *J. Chem. Soc.*, 1942, 642.

[14] Ref. 8, pp. 126 and 132.

[15] J. F. Manville and G. M. Barton, ' Bi-Monthly Research Notes, Department of Fisheries and Forestry,' Ottawa, 1969, **25**, 22.

[16] C. K. Ingold, ' Structure and Mechanism in Organic Chemistry,' Bell, London, 2nd edn., 1969, p. 536.

chloroform the protons of the lactone group had a chemical shift close to δ 4·30; they were equivalent and gave rise to a doublet ($J_{3,\alpha}$ 8·5 Hz, Figure 1). The

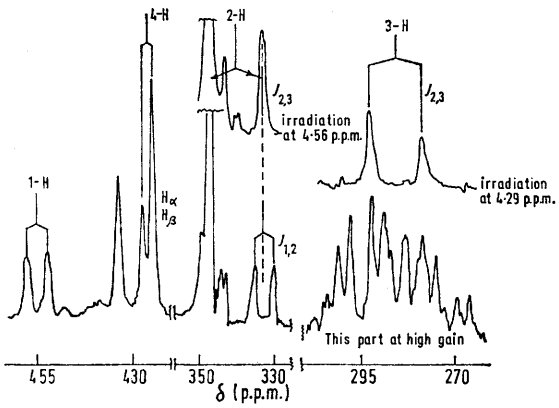


FIGURE 1 *O*-Methyl-4′-chloro-4′-demethoxyepi-PT. Detail of n.m.r. spectrum—location of 2-H and 4-H signals by double irradiation; 3-H by quadruple irradiation

doublet with the smaller coupling constant, partly concealed by the lactone signal, was shown to arise from 4-H, since strong irradiation at δ 4·30 decoupled all but 2-H from the 3-H multiplet.

O-*Methyl-2′-chloroepipodophyllotoxin* (I; X = Y = β-OMe, Z = Cl) (entry 2) *and its 2′-Bromo-analogue* (entry 3).—In these compounds the 4-H and lactone signals are nearly coincident at δ 4·28 (*cf.* entry 1); the 1-H doublet appears at lower field. The further deshielding of the latter signal is due to the neighbouring halogen atom. These assignments were confirmed as for entry 1; thus irradiation at δ 4·28 decoupled H_α, H_β, and 4-H, simplifying the 3-H multiplet to a doublet ($J_{2,3}$) centred at δ 2·96.

We have already referred to the effect of the halogen substituents in restricting rotation and increasing the shielding of the ring C proton; this signal was concealed beneath the methylenedioxy-peak at δ 5·87 in chloroform solutions of both compounds. Addition of about 10% of hexadeuteriobenzene to the chloro-compound shifted the ether peak upfield to δ 3·86, revealing the 6′-H signal at δ 5·80. The signals for the three aromatic protons were all clearly resolved in the spectrum of the bromo-analogue dissolved in pure hexadeuteriobenzene; further the shift of the methylenedioxy-ether signal reached an extreme of δ 5·23 and the two protons were then non-equivalent (J_{AB} 2·5 Hz).

4β,4′-*Dichloro-4-deoxy-4′-demethoxypodophyllotoxin* (I; X = Y = Cl, Z = H) (entry 4).—Three doublets were resolved between the methylenedioxy-peak (δ 5·90) and the methoxy-peaks (δ 3·70). The most intense (2H) signal is normal for the resonance of equivalent protons H_α and H_β at δ 4·29 and that at δ 4·58 as expected for 1-H when remote from the halogen atom (contrast entries 2 and 3); the 4-H signal occurs at lowest field owing to strong deshielding by the 4β-chloro-substituent. This assignment of the 1-H and 4-H resonances could not be confirmed by conventional double irradiation experiments because the 2-H quartet and the 3-H multiplet were too near coincidence in this spectrum; however, substantive evidence was obtained by examination of the closely related 4β-*chloro-4-deoxypodophyllotoxin* (entry 5). On irradiation of the signal at δ 4·57 (1-H) a doublet ($J_{2,3}$) due to the simplified 2-H quartet was distinguishable near δ 3·30. Irradiation at δ 3·00 near the centre of the 3-H signal completely decoupled the doublet at lowest field assigned to 4-H, although the intermediate 1-H doublet was also slightly modified owing to the proximity of the 2-H resonance.

4α-*Chloro-4-deoxypodophyllotoxin* (entry 6).—The spectrum of a deuteriochloroform solution differed considerably from the one (entry 5) just described, and this in itself established the different geometry of the epimeric chlorides: a satisfactory detailed analysis was however only possible on examination of the spectrum of a solution in hexadeuteriobenzene, where the 2-H quartet was clearly resolved at higher field than the 3-H multiplet (compare Table 1 of the preceding paper, entries 3, 6, and 7). Despite this critical resolution, the appearance of both the 1-H and 4-H resonances close to the lower field part of the non-equivalent lactone signals was a complication (Figure 2): however, irradiation at

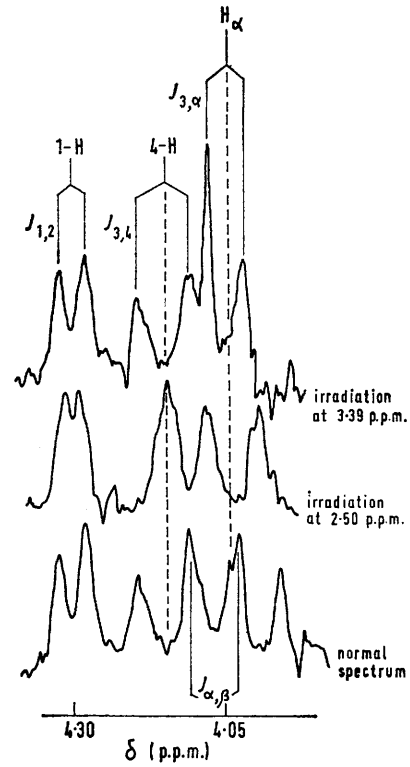


FIGURE 2 N.m.r. spectrum of 4α-chloro-4-deoxy-PT. Identification of 1-H and 4-H signals by irradiation at the 3-H and H_β frequencies

the 3-H frequency collapsed a doublet (centre spectrum) due to 4-H partly coincident with H_α (centred at δ 4·04; $J_{\alpha,\beta}$ 8·5 Hz). The high-field lactone resonance was located near δ 3·39, partly concealed by an ether peak;

irradiation here simplified the H_α signal ($J_{3,\alpha}$ 6 Hz) and $J_{3,4}$ was determined as 8·5 Hz.

EXPERIMENTAL

M.p.s were taken with a hot-stage apparatus. T.l.c. was carried out on Kieselgel G with benzene–ethyl acetate as eluant. I.r. spectra (KBr discs) were recorded with an Infracord 237 spectrometer and u.v. spectra with a Unicam SP 800 instrument. The S.R.C. spectroscopy unit at Imperial College recorded the n.m.r. spectra with a Varian HA 100 spectrometer. The mass spectra were taken by the Physico-Chemical Measurements Unit, Harwell, and by the U.L.I.R.S. at the London School of Pharmacy.

Many of the n.m.r. characteristics of the compounds described are given in Tables 1 and 2; Table 3 summarises the remaining data for all new compounds. A range of absorption is quoted when there are detectable differences between bromo- and chloro-derivatives.

TABLE 3

Typical ^1H n.m.r. absorption of ether groups in halogenated podophyllotoxin derivatives

Chemical shifts (δ in p.p.m.) at 100 MHz; solutions in $CDCl_3$; Me_4Si standard

Ring c substitution	Ring c OMe	O·CH_2·O	4-OMe
No halogen	3·66 (6H), 3·70 (3H)	5·86	
2′-Halogeno-	3·53(3H), 3·78(3H), 3·83 (3H)	5·86	3·36
4′-Halogeno-4′-demethoxy-	3·68 (6H)	5·88—5·90	3·41

2′,4β-*Dichloro-4-deoxypodophyllotoxin* (I; X = Z = Cl, Y = MeO) (prepared by Mr. J. A. HARRIS).—Podophyllotoxin (486 mg) was suspended in benzene (6 ml; sodium-dried) and stirred with phosphorus pentachloride (1·002 g) at 60° in a dry atmosphere. After 1 h the mixture was filtered, benzene was evaporated off under reduced pressure, and the yellow solid residue was *rapidly* washed with ice-cold water containing a little hydrochloric acid. The white residue was dried ($MgSO_4$) in chloroform solution, which yielded an oil on evaporation; this crystallised from a solution in benzene–n-hexane on slow cooling as *needles*, m.p. 186—188° (119 mg, 22%) (Found: C, 56·0; H, 4·4; Cl, 15·5%; M^+, 466 showing isomer peaks typical of dichlorination. $C_{22}H_{20}{}^{35}Cl_2O_7$ requires C, 56·0; H, 4·3; Cl, 15·2%; *M*, 466), $\nu_{max.}$ 1785 cm^{-1} (*trans*-γ-lactone); no OH absorption.

4β,4′-*Dichloro-4-deoxy-4′-demethoxypodophyllotoxin* (I; X = Y = Cl, Z = H) (entry 4).—The mother liquor from the recrystallisation of the foregoing 2′-chloro-compound was refluxed and diluted with an equal volume of ether, and n-hexane was added until the hot solution was slightly turbid. On slow cooling a product separated; further crystallised from chloroform–light petroleum (b.p. 40—60°) this had m.p. 195—196° (95 mg, 18·5%). It was characterised by its n.m.r. spectrum (Table 3) and by solvolysis to the corresponding 4-hydroxy- and 4-methoxy-compounds.

Solvolysis of Chlorides.—4β,4′-Dichloro-4-deoxy-4′-demethoxypodophyllotoxin (266 mg) was dissolved in acetone (5 ml), water (2 ml) was added, and the solution was refluxed for 10 min. The solid obtained on cooling and dilution with water was identified as 4′-*chloro-4′-demethoxyepipodophyllotoxin*, m.p. 182—184° (from benzene–n-hexane; 63% yield) by its i.r. spectrum [$\nu_{max.}$ 3400br (OH) and 1775 cm^{-1} (lactone C=O)] and accurate mass measurement of the molecular ion [418·0813 ($C_{21}H_{19}{}^{35}ClO_7$ requires 418·0809)].

The dichloro-compound was also converted into O-*methyl-4′-chloro-4′-demethoxyepipodophyllotoxin* (Table 2, entry 1) (59%) by warming in methanol (3 ml) and addition of water (2 drops): a crystalline product separated overnight; m.p. 191°. The i.r. spectrum had no OH stretching frequency; the molecular ion mass of 432 corresponds to $C_{22}H_{21}{}^{35}ClO_7$.

In a similar methanolysis, the 2′-chloro-4β-chloride (I; X = Z = Cl, Y = OMe) (363 mg) was converted into O-*methyl-2′-chloroepipodophyllotoxin* (entry 2) (62%), m.p. 189°, M^+, 462·1078 ($C_{23}H_{23}{}^{35}ClO_8$ requires 462·1082). Hydrolysis of the dichloro-compound in wet acetone gave 2′-*chloroepipodophyllotoxin* (60·5%), m.p. 192—193° (from benzene–n-hexane), M^+, 448 corresponding to $C_{22}H_{21}{}^{35}ClO_8$; the i.r. and n.m.r. spectra (Table 3) correlated with those of its 4-*O*-methyl analogue.

Reaction between Podophyllotoxin and Phosphorus Pentabromide.—Podophyllotoxin (528 mg) was treated with phosphorus pentabromide [17] (*ca.* 1·0 g, recryst. from nitrobenzene) in dry benzene as described for the pentachloride. Purification by shaking the benzene solution for *a few seconds* with ice–water does not lead to hydrolysis, although the solution reactions described are complete within seconds at room temperature. The crude material could not be crystallised but a solid (411 mg) was obtained by precipitation from benzene with light petroleum (b.p. 60—80°); t.l.c. indicated that this was a binary mixture and the components were shown to be the 2′-bromo- and 4′-bromo-4′-demethoxy-bromides by solvolysis.

Solvolysis of Bromides.—(*a*) The mixture (302 mg) from the preceding experiment was dissolved in methanol (2 ml) at room temperature. A crystalline product (188 mg), m.p. 184° (from methanol), separated a few seconds after dissolution was complete. The i.r. spectrum lacked the OH peak, and that near 1590 cm^{-1} indicative of 2′-substitution. The material was shown to be O-*methyl-2′-bromoepipodophyllotoxin* (I; X = 4β-OMe, Y = OMe, Z = Br) by its n.m.r. spectrum (entry 3) and the molecular ion mass of 506·0562 ($C_{23}H_{23}{}^{79}BrO_8$ requires 506·0572). A little water was added to the mother liquor from the methanolysis, which was then clarified by heating; a white crystalline product (95 mg) was collected after 2 days. The material had m.p. 187° (from methanol–water) and was characterised as O-*methyl-4′-bromo-4′-demethoxyepipodophyllotoxin* (I; X = 4β-OMe, Y = Br, Z = H). The n.m.r. spectra were similar to those of entry 1 (Table 2) and the ether protons had typical resonances (Table 3). The molecular ion mass of 476 units corresponds to $C_{22}H_{21}{}^{79}BrO_7$.

(*b*) A binary mixture of the dibromo-derivatives was prepared as before from podophyllotoxin (551 mg) and phosphorus pentabromide. The crude solid (324 mg) was dissolved in acetone (5 ml) and water (2 ml) and the solution was refluxed for 10 min. After dilution with water the precipitated mixture of hydrolysis products was collected, dried, and dissolved in hot benzene containing a little n-hexane; 2′-*bromoepipodophyllotoxin* (105 mg) crystallised with m.p. 189—191° (from benzene–hexane). The n.m.r. spectrum was typical of this orientation of substitution (Table 2, *cf.* entry 3 and Table 3), and the molecular ion mass of 492 corresponds to $C_{22}H_{21}{}^{79}BrO_8$.

The hot benzene–n-hexane liquor was diluted with an

[17] A. I. Popov and N. E. Skelly, *J. Amer. Chem. Soc.*, 1954, **76**, 3916.

equal volume of ether followed by sufficient n-hexane to produce turbidity. Slow cooling gave crystals (98 mg) of *4′-bromo-4′-demethoxyepipodophyllotoxin*, m.p. 181—182° [from chloroform–light petroleum (b.p. 60—80°)], M^+, 462·0140 ($C_{21}H_{19}{}^{79}BrO_7$ requires 462·0299).

4β-Chloro-4-deoxypodophyllotoxin (I; X = Cl, Y = OMe, Z = H).—Podophyllotoxin (420 mg) in benzene (6 ml) was stirred with phosphorus pentachloride (450 mg) for 10 min at room temperature. The material obtained on evaporation of solvent, washing with ice-cold dilute hydrochloric acid, and drying ($MgSO_4$) in benzene solution was shown to consist of two components by t.l.c. The minor component (54 mg, 12% yield) was separated from a substantial quantity of unchanged podophyllotoxin by recrystallisation [m.p. 181—182° (from benzene–n-hexane)] and was identical with 4β-chloropodophyllotoxin prepared [9] by use of thionyl chloride diluted with three volumes of benzene.

4α-Chloro-4-deoxypodophyllotoxin.—Podophyllotoxin (1·54 g) was refluxed with thionyl chloride (1·2 ml) in benzene (15 ml) for 15 min and the solute, recrystallised from benzene–hexane had m.p. 92—93°; no attempt was made to optimise the yield of pure *4α-chloro-compound* (550 mg, 34%), M^+, 432·0992 ($C_{22}H_{21}{}^{35}ClO_7$ requires 432·0976).

Evidence for the Formation of Dichloromethylene Ethers in Reactions with Phosphorus Pentachloride.—In the initial stage of work (with J. A. Harris) it was found that no appreciable concentration of the dichloromethylene ether was obtained from podophyllotoxin until the reagents had been refluxed for 4 h in benzene; at the end of this period t.l.c. showed that six products had been formed. Under similar conditions *O*-methyl-2′-bromoepipodophyllotoxin afforded a simpler mixture of products in benzene solution and washing with cold water produced a carbonate ($\nu_{max.}$ 1850s cm^{-1}). Heating the material from the washed benzene layer in acetone–aqueous hydrochloric acid, dilution with water, and drying the benzene extract yielded a solid that could not be further purified by recrystallisation. The mixture gave an intense green colouration with iron(III) chloride solution and the i.r. spectrum in the absence of CO_2H absorption gave additional evidence of a catechol derivative [$\nu_{max.}$ 3400br and 1765 cm^{-1} (lactone)].

2′-Bromopodophyllotoxone.—Podophyllotoxin (75 mg) was dissolved in chloroform (6 ml) and shaken with potassium bromide (25 mg) in 2N-sulphuric acid (20 ml) and N-potassium bromate (5 ml) for 20 min at room temperature. After washing with water the crude *bromo-ketone* (615 mg, 59%) was precipitated from the dried ($MgSO_4$) organic layer with light petroleum (b.p. 60—80°). The material was purified by preparative layer chromatography on Kieselgel G [benzene–ethyl acetate (5 : 1) as eluant] and extraction of the component of higher R_F value with chloroform. Pure material had no i.r. absorption at 3400 cm^{-1} (OH) nor at 1590 cm^{-1} (showing substitution at C-2′), the lactone peak was retained at 1790 cm^{-1} and a second carbonyl peak had appeared at 1690 cm^{-1} similar to that in the spectrum of podophyllotoxone [18] (1670 cm^{-1}). The u.v. spectrum (ethanol) showed maxima at 285 and 326 nm, comparable to the maxima of podophyllotoxone (282 and 322 nm in ethanol). The molecular ion mass was 490 ($C_{22}H_{19}{}^{79}BrO_8$ requires 490).

[1/2244 *Received*, 26*th November*, 1971]

[18] W. J. Gensler and F. Johnson, *J. Amer. Chem. Soc.*, 1955, **77**, 3674.