Lignans and Related Phenols. Part XII.[1] Application of Nuclear Magnetic Double Resonance to the Aryltetrahydronaphthalene Class

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Use of double resonance and particularly of the INDOR method has provided the first complete assignment of proton signals from aryltetrahydronaphthalenes. The information has been applied to the conformational analysis of these biologically active lignans.

THE first n.m.r. measurements on these lignans were made [2] in 1962; a number of subsequent papers [3] present n.m.r. results but no total assignment has ever been made. Our interest has been centred on the geometry of the reduced ring B, as this is linked with biological acitivity; here the low effective concentration of 'protons' and screening of fine structure by intense methoxy-signals were obstacles to progress. Further the application of Shoolery's Rules [4] can be misleading in crowded molecules of this type: thus the 2-H and 3-H resonances are close together (see Table 1) and the latter may occur at lower field despite the effect of the carbonyl group on its neighbour. For these reasons assignment of resonances by conventional double resonance techniques was not always possible, but the problem was solved by use of INDOR spectroscopy.

The INDOR experiment, first described by Baker,[5] has only recently [6] been applied to the determination of structure. N.m.r. spectral lines are usually made up of a number of transitions between energy levels of the nuclear spin system. Transition in any one spectral line may have an energy level in common with transitions which are present in other lines in the spectrum; such transitions are said to be connected, and two modes of connection may be distinguished: (*a*) the common level is intermediate in energy between the other two, a progressive connection; (*b*) the common level lies outside the other two in energy, a regressive connection. If the secondary irradiating field is swept across one pair of progressively related transitions, the other increases in intensity; if it is swept across one of a pair of regressively related transitions, the other decreases in intensity. The behaviour to be observed in second-order and first-order spin systems in which several transitions make up any one line may be complex, and has been the subject of a recent detailed investigation; [7] however, in the work to be described the analysis of the INDOR spectra may be performed on the basis of predictions concerning single-transition lines.

Podophyllotoxone (1).—The 100 MHz spectrum and assignments based upon INDOR experiments are shown in Figure 1. The signal due to 1-H was chosen as an 'observing' or monitored line; this was assigned to the doublet appearing at δ 4·78, for its shift is in keeping with that expected for a diarylmethane derivative, and it is coupled to a lone proton. With the monitoring field set upon the low-field branch of the doublet, the secondary perturbing field was swept through the

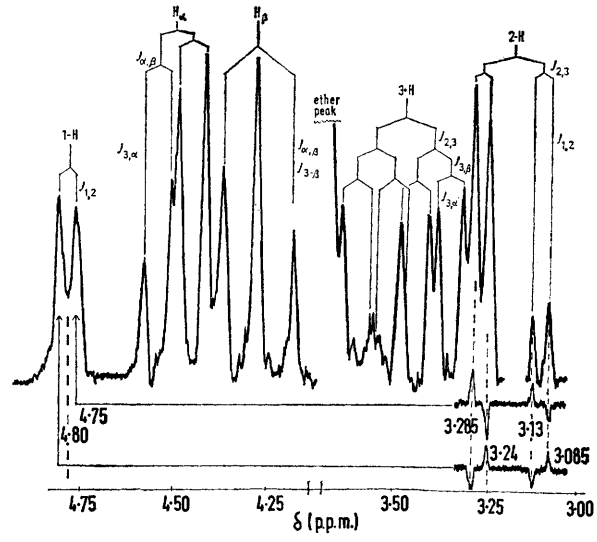


FIGURE 1 Assignment of chemical shifts and coupling constants to the B ring protons of podophyllotoxone, showing INDOR signals for the coupled 1-H and 2-H resonances (the Figure numbers correspond to the compound numbers in Table 1)

remaining spectrum and caused intensity variations only when it passed through a pair of doublets at δ 3·11 and 3·26, hence values of $J_{1,2}$ (5 Hz) and $J_{2,3}$ (15 Hz) were estimated. Monitoring the high-field branch of the 1-H doublet confirmed this, and gave an INDOR spectrum in which the signals had the opposite sense to those obtained previously; this is as expected because of the different connectivity relationships between the transitions involved. Monitoring the branches of the 2-H quartet in their turn revealed additional coupling with a multiplet centred at δ 3·47 due to 3-H; a further

[1] Part XI, D. C. Ayres, J. A. Harris, and P. B. Hulbert, *J. Chem. Soc.* (*C*), 1970, 1111.
[2] T. Gilchrist, R. Hodges, and A. L. Porte, *J. Chem. Soc.*, 1962, 1780.
[3] Especially (*a*) E. Schreier, *Helv. Chim. Acta*, 1964, **47**, 1529; (*b*) Y. Kato, *Chem. and Pharm. Bull.* (*Japan*), 1964, **12**, 512; (*c*) M. Kuhn and A. von Wartburg, *Helv. Chim. Acta.*, 1969, **52**, 948.
[4] See L. M. Jackman and S. Sternhell, 'Applications of N.m.r. Spectroscopy in Organic Chemistry,' Pergamon, Braunschweig, 2nd edn., 1969, p. 181.
[5] E. B. Baker, *J. Chem. Phys.*, 1962, **37**, 911.
[6] F. W. van Deursen and A. C. Udding, *Rec. Trav. chim.*, 1968, **87**, 1243; O. Sciacovelli, W. von Philipsborn, C. Smith, and D. Ginsberg, *Tetrahedron*, 1970, 4589; D. H. R. Barton, P. N. Jenkins, R. Letcher, and D. A. Widdowson, *Chem. Comm.*, 1970, 391; F. W. van Deursen, *Org. Magnetic Resonance*, 1971, **3**, 221.
[7] P. N. Jenkins and L. Phillips, to be published.

experiment showed that this was additionally coupled to the lactone group (α-H,β-H) centred at δ 4·40. Seven peaks appear in this region: they include a pseudo-triplet ($J_{\alpha,\beta}$,$J_{3,\beta}$) at δ 4·26 arising from H_β and a quartet due to H_α($J_{\alpha,\beta}$,$J_{3,\beta}$) at δ 4·48.

Monitoring each line of the lactone multiplet individually showed the 3-H signal to consist of two overlapping quartets, whose extremes were coincident with the high-field part of the ether peak on one side and

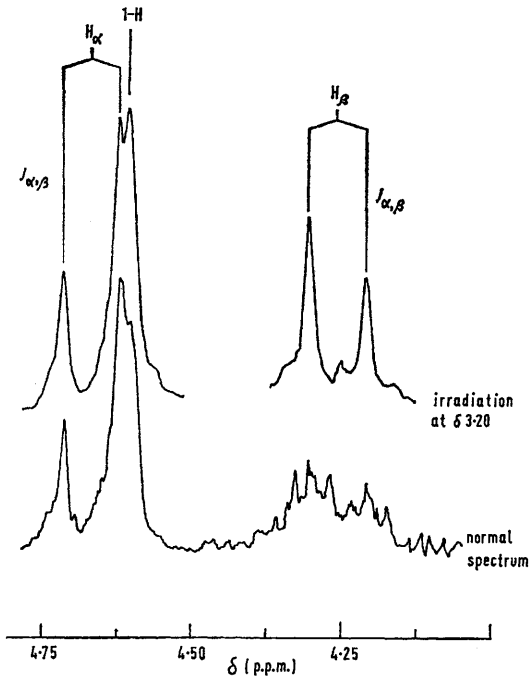


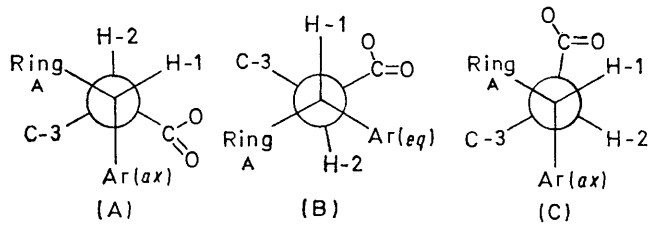
FIGURE 2 Simplification of the lactone and 1-H signals on irradiation of 2-H and 3-H frequencies in picropodophyllone

with the low-field part of the 2-H signal on the other (Figure 1). Interaction with the latter accentuates the high-field part and here the separation due to $J_{3,\alpha}$ and $J_{3,\beta}$ can be seen in addition to the large coupling $J_{2,3}$. The coupling constants evaluated (Table 2) for this rigid *trans*-lactone are sensibly in agreement with those predicted by the Karplus relationship, although conformational changes must be taken into account for flexible models; the C-2 epimer, picropodophyllone(2), affords an illustration of this.

Picropodophyllone (2).—INDOR experiments based on the resolved part of the lactone H_α signal and the concealed 1-H signal (Figure 2) defined the main regions of absorption. However, a complete analysis is not possible because the 2-H and 3-H signals are closer together than in the spectrum of podophyllotoxone and the individual peaks were less well resolved. Irradiation of the frequencies of the 2-H and 3-H signals (Figure 2) simplified the lactone and 1-H peaks to a quartet ($J_{\alpha,\beta}$) with the 1-H signal detectable although largely coincident with that of H_α; $J_{3,\alpha}$ appears to be zero, but the multiplicity of the H_β signal suggests that this is probably due to the second-order nature of the spectrum (ABCDEF) and may be ' deceptive simplicity ' (*cf.* Figure 2). The magnitude of $J_{1,2}$ cannot be evaluated accurately but it must be small and close to that (2 Hz) expected for $J_{eq,eq}$; it follows that the conformation in which the pendant ring is axial [Scheme (C)] predominates.* In a preliminary communication [8] it was shown that the relative shielding of protons on the pendant ring was caused by their proximity to the keto-group in the axial conformation.

The most significant of the reduction products of these two ketones is the naturally occurring alcohol podophyllotoxin (Table 2, entry 5a) and valuable n.m.r. data on some of its derivatives have been published by Shreier.[3a] In the alcohols themselves the proximity of the 1-H, 4-H, and lactone signals precluded an assignment at 60 MHz (Figure 5 shows a typical 100 MHz spectrum), but $J_{3,4}$ was determined in 4-acetoxy-derivatives where acetylation characterised the 4-H resonance by shifting it to lower field; otherwise double-resonance experiments are essential if reliable assignments are to be made.

Podophyllotoxone Picropodophyllone



SCHEME Newman diagrams showing dihedral relationships at C(1),C(2)

Podophyllotoxin Acetate (3) *and Epipodophylloxtoxin Acetate* (4).—In the absence of the hydroxy-proton coupling, the doublets due to 4-H were well resolved and the values of $J_{3,4}$ (Table 2) are similar to those reported.[3] Irradiation at the 4-H frequency of the former compound caused changes in the multiplet near δ 2·80, which must therefore include the 3-H signal, but this was too close to that of 2-H for exact assignment. However, irradiation at δ 2·80 decoupled both these protons, revealing (Figure 3) a singlet due to 1-H and an AB quartet from the lactone group.

In the epi-acetate (4) rotation of the acetyl group, now axially substituted, deshields 2-H relative to 3-H as confirmed by irradiation at the frequency (δ 6·05) of the 4-H doublet: the 2-H signal appeared as a double doublet which simplified to a doublet ($J_{2,3}$) on irradiation at

* Arguments for regarding the flexible compounds of type II (Table 1) as boat forms were first published by A. W. Schrecker and J. L. Hartwell (*J. Amer. Chem. Soc.*, 1954, **76**, 752); the values we find for the coupling constants of ring B protons are consistent with this. There will be restraints on the twisting of the boat by the fused ring A and by the lactone ring; the term ' axial ' here refers to a bond parallel to the main axis of the molecule. The prefix ' pseudo- ' has been omitted for chair forms since fusion of ring A makes this implicit for the whole group.

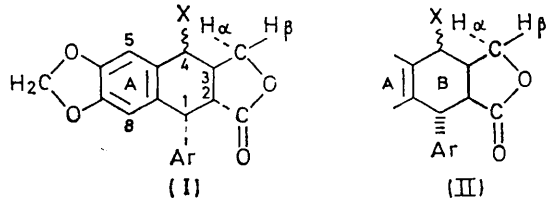
[8] D. C. Ayres and J. A. Harris, *Chem. Comm.*, 1969, 1135.

δ 4·56 (1-H). The 3-proton was shown to be coupled to a quartet centred at δ 4·25 (Figure 4) arising from one of the protons of the lactone group; the signal for the other lay beneath the ether peaks and it was delineated by an INDOR experiment. Monitoring the two wing peaks (1,2) of the low-field quartet showed that the high field lactone proton (H_β) formed a pseudo-triplet ($J_{\alpha,\beta} \simeq J_{3,\beta}$) centred at δ 3·83 with the high field part

pected, but because of another near coincidence of the 2-H and 3-H resonances in podophyllotoxin, its epimer was analysed first. The broad signal at δ 4·75 (Figure 5) arises from 4-H, since on addition of D_2O it simplifies to a doublet ($J_{3,4}$) and the OH peak near δ 2·70 disappears. Of the two remaining doublets, one (intensity 2H) has the same chemical shift (δ 4·25) as part of the lactone resonance in the acetates (3) and (4) and arises from the

TABLE 1

Chemical shifts (δ in p.p.m.) of reduced ring protons in rigid [type (I)] and flexible [type (II)] lactones



Ar = 3,4,5-trimethoxphenyl

Compounds (1)—(6) and (9) in $CDCl_3$; (8) in [$^2H_6$]dimethyl sulphoxide at 100 MHz. In chloroform the methoxy-peaks were at 3·73 ± 0·03 (3H) and 3·70 ± 0·04 (6H) p.p.m. from tetramethylsilane; in dimethyl sulphoxide the two bands interchanged within the same range of chemical shift. Acetate resonances at 2·03 ± 0·07 p.p.m. did not hinder interpretation but where other signals were coincident they are marked by an asterisk. For comparison the range of all the multiplets is given. Where they overlap the whole frequency range is shown under braces, and chemical shifts, if known, appear in parentheses. A number of factors including solvation effects and second-order characteristics make it difficult to distinguish between the chemical shifts of the lactone protons; H_α and H_β were arbitrarily assigned to low and high field, respectively.

No.	Compound (type)	X subst.	1-H (d)	2-H (m)	3-H (m)	4-H (d) or (t)	H_α (m)	H_β (m)
(1)	Podophyllotoxone (I)	keto	4·78	3·60 (3·19)	3·00 (3·47)	—	4·60 (4·48)	4·10 (4·26)
(2)	Picropodophyllone (II)	keto	4·60 *	3·35	3·00	—	4·67 *	(*ca.* 4·25)
(3)	Podophyllotoxin acetate (I)	α-OAc	4·43	broad signal at 2·80		5·80	4·40 (4·26)	4·00 (4·06)
(4)	Epipodophyllotoxin acetate (I)	β-OAc	4·56	3·25—3·05 (3·14)	3·10—2·75 (2·93)	6·05	4·35—4·10 (4·25)	part concealed (3·83)
(5)	Epipodophyllotoxin (I)	β-OH	4·50	3·40—3·18 (3·28)	3·05—2·70 (2·91)	4·75	equivalent (4·25)	
(5a)	Podophyllotoxin (I)	α-OH	*ca.* 4·60 *	3·00	2·70	4·86	*ca.* 4·60 *	4·20—3·95
(6)	Deoxypodophyllotoxin (I)	H	4·58	3·20 (*ca.* 2·70)	(*ca.* 2·70)	2·60 (*ca.* 3·00)	4·55—4·30 (4·40)	*ca.* 3·95—3·75 (3·86)
(7)	(6) in C_6D_6 (I)	H	4·38	<2·30	(2·30)	—	3·86—3·60 (3·78)	3·29—3·02 (3·13)
(8)	Picropodophyllin (II)	α-OH	3·88	3·35—3·12 (3·25)	*ca.* 2·50	4·38 *	4·73 (4·64)	4·22 * (4·40)
(9)	Picropodophyllin acetate (II)	α-OAc	4·32 *	3·30—3·12 (3·23)	3·05—2·80 (2·92)	5·70	4·50 (4·35)*	4·10 (4·19)

concealed below the ether peaks: Figure 7b illustrates a similar experiment where $J_{2,\beta}$ and $J_{3,\alpha}$ differed sufficiently to allow detection of the four separate INDOR peaks. Under these adverse conditions an instrumental limitation caused spurious regressive responses to be obtained; these are attributable to overloading of the detector used to derive the INDOR signals by the strong MeO signal.

Epipodophyllotoxin (5) *and Podophyllotoxin* (5a).—Insofar as these structures are superimposable, similar chemical shifts and coupling constants are to be ex-

equivalent lactone protons in this molecule; the other, at δ 4·50, can only be due to 1-H. These assignments were confirmed by irradiation at the frequency of 1-H, when the double doublet (2-H) at δ 3·28 was resolved as a doublet ($J_{2,3}$). Finally, the broad signal at highest field was allocated to 3-H; irradiation near its centre at δ 2·91 caused changes in the lactone resonance but this did not collapse completely, probably because the irradiated spin system was too wide to allow complete decoupling with the power available.

The spectrum of podophyllotoxin (5a) was largely

defined by observation of its obvious features, by the addition of deuterium oxide, and from a comparison with the peaks allocated in the epimer.

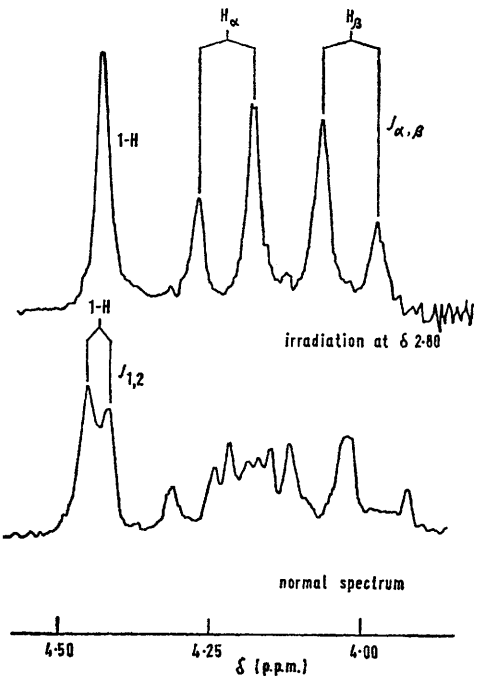


FIGURE 3 1-H and lactone resonances of podophyllotoxin acetate

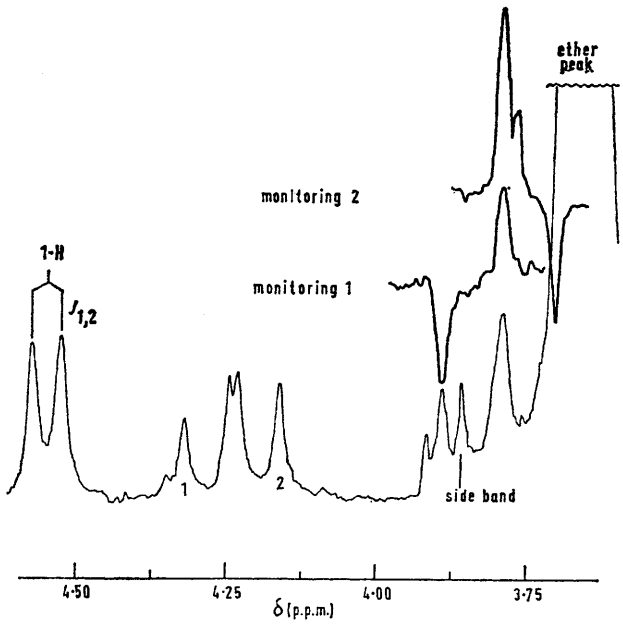


FIGURE 4 1-H and lactone resonances of epipodophyllotoxin acetate showing INDOR response

Deoxypodophyllotoxin (6) *and* (7).—In the spectrum of the deuteriochloroform solution the resonances of the lactone and B-ring protons were as tabulated (entry 6).

[9] J. L. Hartwell and A. W. Schrecker, 'Progress in the Chemistry of Organic Natural Products,' Springer, Vienna, 1958, vol. 15, pp. 146—149.

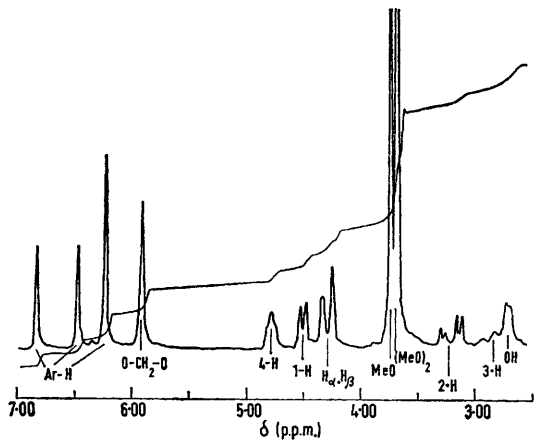


FIGURE 5 Spectrum of epipodophyllotoxin

INDOR experiments showed that the higher-field lactone signal was concealed under the ether peaks (Figure 6) and that apart from 1-H all the other ring protons were in the complex at δ 2·60—3·20, but under these conditions no coupling constants could be obtained in this range.

Aryltetrahydronaphthalene lignans form stable inclusion compounds with benzene and other solvent molecules,[9] and owing to crowding in the acceptor the number of possible orientations of the included molecule must be limited. Dissolution in a strongly anisotropic solvent, small enough to be included, should therefore offer the best chance of changing *relative* chemical shifts. An illustration of this effect and its application to analysis is afforded by a solution of deoxypodophyllotoxin in deuteriobenzene (Figure 7a) rather than chloroform. The protons of the methylenedioxy-group become non-equivalent, the less hindered methoxy-protons remain equivalent but are shifted farther upfield than is the

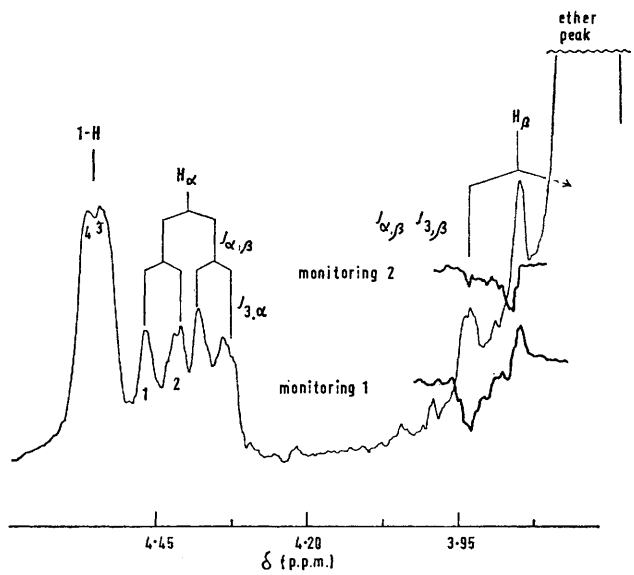
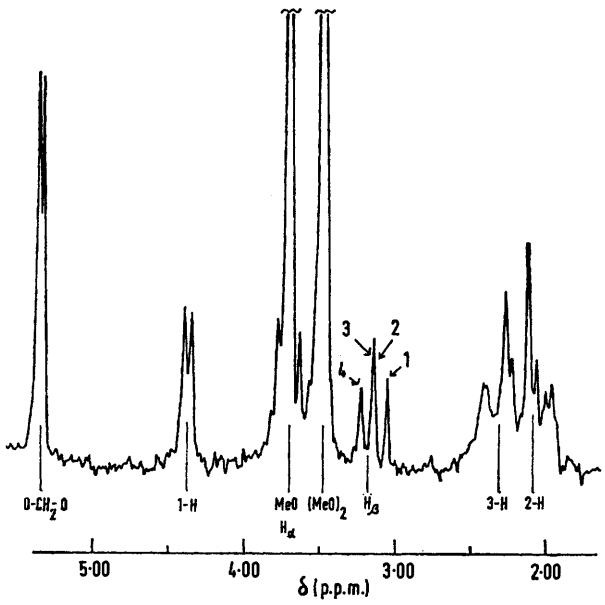
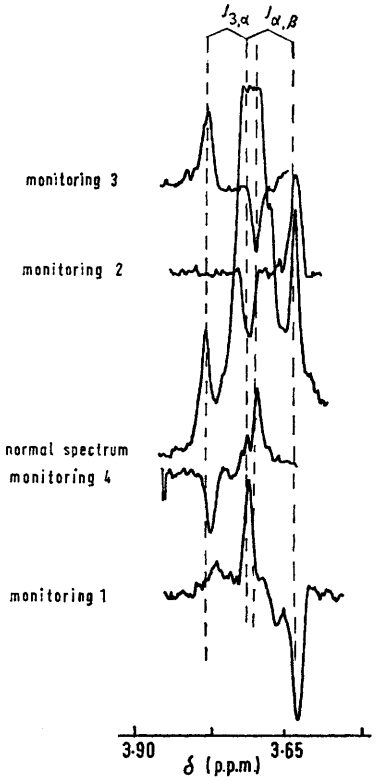


FIGURE 6 1-H and lactone signals of deoxypodophyllotoxin in $CDCl_3$

4-methoxy-peak, and the high-field lactone resonance is shifted to δ 3·13, clearly resolved from the ether peaks.



(a) Spectrum of deoxypodophyllotoxin in deuteriobenzene



(b) INDOR evaluation of concealed H_α signals

FIGURE 7

This pseudo-triplet (H_β) was linked to the concealed lactone signal (H_α) by monitoring successively at points 1—4 (Figure 7a), giving the INDOR signals recorded in Figure 7b (*cf.* Figure 6); $J_{3,\alpha}$ is appreciably smaller than $J_{\alpha,\beta}$; consequently two progressive and two regressive peaks mark the centre of the quartet. When the lowest-field part of the signal due to the lactone group was monitored (δ 3·78), a sweep showed responses in the high-field part and located the 3-H signal near δ 2·30 and below that of 2-H. Strong irradiation at δ 2·10 (2-H) had little effect on the lactone signals but completely decoupled the doublet at δ 4·38, which must therefore arise from 1-H ($J_{1,2}$). The chemical shifts of the 4-protons, their coupling constants, and a value for $J_{2,3}$ remain undetermined.

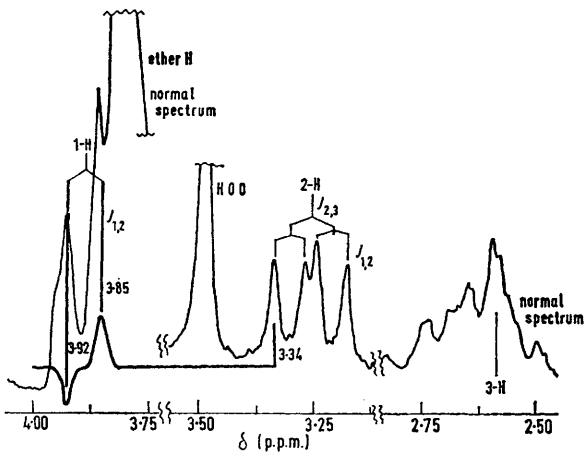
The addition of anisotropic solvents during n.m.r. experiments should simplify the problem of structure evaluation in lignan chemistry; a recent paper [10] on non-equivalent methylenedioxy-groups in the aryl-naphthalene class indicates some possible lines of enquiry. The correlation made there, between the non-equivalence of the protons of a methylenedioxy-group attached to ring C and a proximate lactone carbonyl group, should be treated with caution in view of our observed induction of non-equivalence in a group attached to ring A and remote from the lactone function.

Picropodophyllin (8) *and its Acetate* (9).—Owing to its low solubility, picropodophyllin had to be examined in [$^2H_6$]dimethyl sulphoxide, when the partially protonated solvent obscured the 3-H signal near δ 2·50 and the water peak overlay that due to 2-H. Addition of deuterium oxide shifted the HOD peak and revealed (Figure 8a) a quartet ($J_{2.3}$,$J_{1.2}$) of similar structure to that found for 2-H in the other compounds examined. Monitoring the two lower field peaks of this double doublet in an INDOR experiment showed that the 1-H doublet was concealed by the ether resonances; the INDOR signal separation (*cf.* Figure 8a) established $J_{1.2}$ as 7·25 ± 0·25 Hz and the chemical shift as δ 3·88. The 4-H signal is broadened in dimethyl sulphoxide solution by coupling with OH, but addition of deuterium oxide resolves it as a doublet ($J_{3.4}$) at δ 4·38, where it coincides with the high-field lactone peaks: when the 3-H frequency was irradiated this region was simplified as shown (Figure 8b), confirming these assignments and allowing the determination of $J_{\alpha.\beta}$, $J_{3,\alpha}$, and $J_{3.\beta}$.

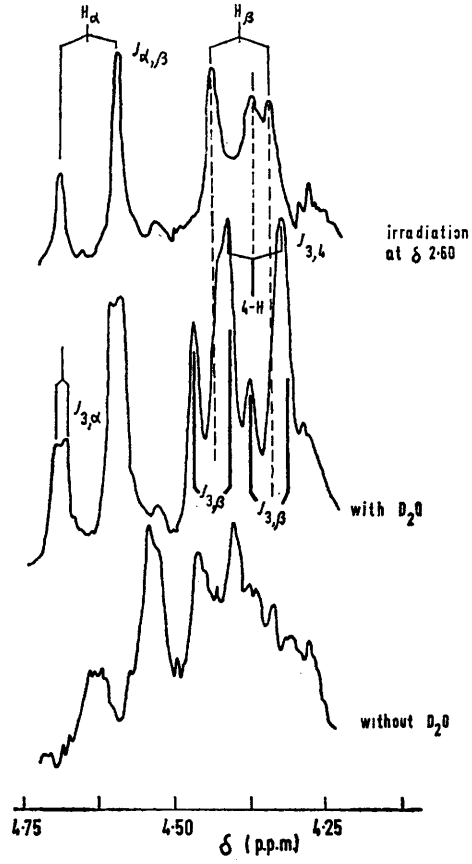
In contrast to the parent alcohol, picropodophyllin acetate is the most soluble compound of this group and consequently its spectrum in deuteriochloroform can be compared with the others. The signals arising from 4-H (doublet), 2-H (double doublet), and 3-H (multiplet) are listed in Table 1; they were identified by reference to previous results and also by double resonance experiments. Irradiation at δ 3·20 collapsed a doublet due to 1-H in the low-field part of the lactone complex centred at δ 4·35; a high irradiation power level at this point decoupled all three of these protons leaving the 2-H signal as a doublet ($J_{2.3}$) and that of 3-H as a double doublet ($J_{2.3}$ and $J_{3.4}$) (Figure 9a). Figure 9a also shows the 3-H complex with 4-H decoupled by irradiation

[10] T. R. Govindachari, K. Nagarajan, N. Viswanathan, and H. Fuhrer, *Indian J. Chem.*, 1971, **9**, 546.

at δ 5·70. As mentioned for epipodophyllotoxin, the broader 3-H signals could not be satisfactorily decoupled from the lactone system, but in picro-compounds $J_{2,3}$ is



(a) Picropodophyllin in $(CD_3)_2SO$–D_2O showing detection of 1-H by monitoring at δ 3·34 (2-H)



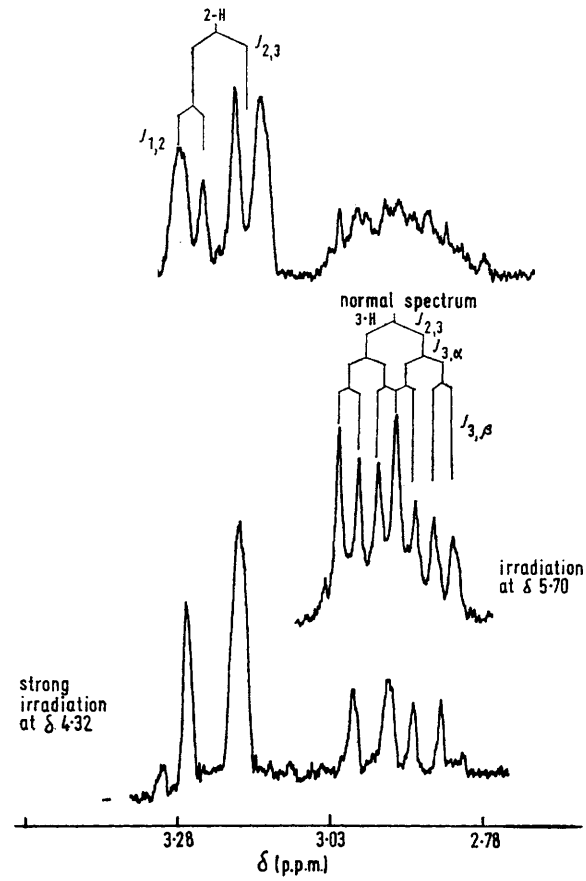
(b) Lactone and 4-H signals showing simplification by addition of D_2O and by irradiation

FIGURE 8

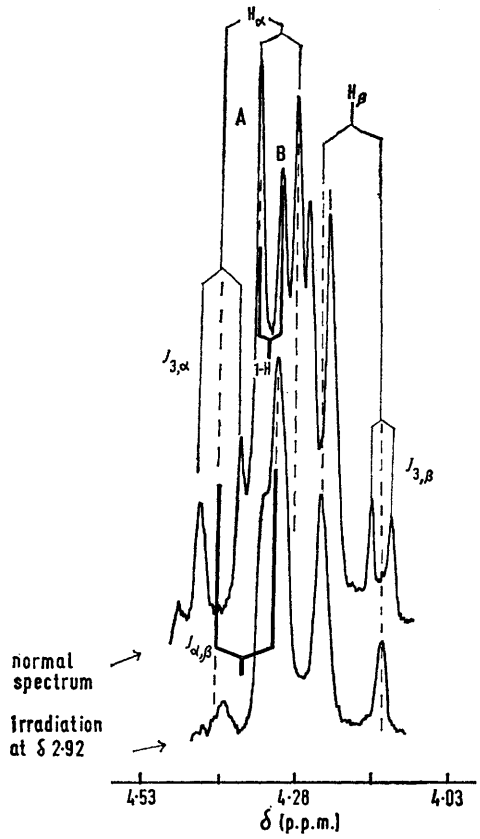
smaller and irradiation of the narrower band (*ca.* 25 Hz) was more effective.

Figure 9b shows the lactone complex, including the 1-H signal which is centred at δ 4·32, with its low-field peak (A) coincident with H_α and the high-field part of the doublet at B. Irradiation of 3-H collapses H_α,H_β to a quartet, but A and B remain superimposed on the collapsed peak at δ 4·31: this location of the 1-H signal was confirmed by irradiation here, which simplified the 2-H signal.

From a knowledge of the relevant coupling constants the geometry of all the diastereoisomers can be confirmed by use of the Karplus relationship; further, an estimate of the conformational equilibrium in flexible models can be obtained. The coupling $J_{1,2}$ is most useful for purposes of conformational analysis, whilst the magnitude of $J_{3,4}$, although modified by an electronegative C-4 substituent, provides a second point of reference. In the rigid models [Table 2, type (I)] small but significant variations in $J_{1,2}$ occur; these cannot arise from any electronic effect by the remote 4-substituent and they are ascribed to torsional effects initiated by changes in hybridisation or epimerisation at this position. The typical dihedral angles summarised in Table 3 were evaluated from Dreiding models, which cannot define small variations due to torsional changes, although these can sometimes be inferred. In epipodophyllotoxin and its acetate the axial X-substituent introduces a 1,3-interaction which in turn reduces the 1-H,2-H dihedral angle; in consequence the similar



(a) Detail of the 2-H and 3-H signals



(b) Location of the 1-H signal

FIGURE 9 Picropodophyllin acetate

values of $J_{1,2}$ in this pair are greater than those found in the epimers.

The three flexible models [Table 2, type (II)] form a series, where, for reasons given above, picropodophyllone lies near the extreme in which the conformation (C) (Scheme) predominates. Taken as they stand, the

TABLE 2

Coupling constants (H,H), evaluated by first-order approximation

No.	Compound	1,2	2,3	3,4	3,α	3,β	α,β
	Type (I)						
1	Podophyllotoxone	5	15		7	8	9
2	Podophyllotoxin	*ca.* 3		8			
3	Podophyllotoxin acetate	3·5		8·0			9·0
4	Epipodophyllotoxin	5·0	14	3·5	9·25		0
5	Epipodophyllotoxin acetate	4·75	14	3·5	7·25	*ca.* 9	9·0
6	Deoxypodophyllotoxin	5·0			5·75	9·5	8·0
	Type (II)						
7	Picropodophyllone	*ca.* 2			0		9·0
8	Picropodophyllin	6·75	9·0	9	1·5	4·5	9·5
9	Picropodophyllin acetate	3·75	9·5	5·0	7·0	3·25	9·75

constants found for the other two compounds (Table 2; entries 8 and 9) well illustrate the confusion that can arise in this field when conformational equilibria are ignored. Despite their different configurations at C(1),C(2) picropodophyllin acetate (entry 9) has the same J value as podophyllotoxin (entry 2) and its acetate (entry 3). The latter pair and picropodophyllin (entry 8) have the *trans*-configuration at C(3),C(4) and $J_{3,4}$ is comparable, yet in picropodophyllin acetate

TABLE 3

Typical dihedral angles (°) of ring B C–H bonds

Compound	Vicinal pair 1-H and 2-H	Vicinal pair 3-H and 4-H
Podophyllotoxone	55—60	
Picropodophyllone B (Ar *eq.*)	160	
Picropodophyllone C (Ar *ax.*)	70	
Podophyllotoxin	50	175
Epipodophyllotoxin	50	50
Picropodophyllin B (Ar *eq.*)	160	160
Picropodophyllin C (Ar *ax.*)	75	70

(entry 9) this constant is closer to the value found for the C-4 epimers (entries 4 and 5).

Some reduction in the contribution of the (C) conformer (Scheme) is to be expected when the carbonyl group of picropodophyllone (entry 7) is supplanted by the axial acetoxy-group of picropodophyllin acetate (entry 9). Evidence of the consequent 1,4 aryl–acetoxy interaction in this boat form is the shielding of acetate protons (at δ 1·96) relative to their position in podophyllotoxin and epipodophyllotoxin acetates (entries 3 and 5; δ 2·08). A larger shielding is not expected because the interaction can be ameliorated by the rotation of both groups in the (C) conformer and by flipping into the (B) conformer. The balance between these conformers was estimated taking $J_{eq,eq} = 1{\cdot}5$ Hz for (C) and $J_{ax,ax} = 9{\cdot}5$ Hz for (B): the weighted mean of these values corresponds to the observed $J_{1,2}$ of 3·75 when the axial aryl contribution is $70 \pm 7\%$. A similar calculation for picropodophyllin (entry 8) in [$^2H_6$]dimethyl sulphoxide indicates that the axial conformer forms $35 \pm 5\%$ of the mixture. This further decrease in the axial component at ambient temperature indicates a greater 1,4-interaction in picropodophyllin than in its acetate (entry 9); this is reasonable because the alcohol will be strongly solvated in dimethyl sulphoxide.

We have not been able to use the observed $J_{3,4}$ values as a quantitative check on the conformational analysis because the electronegative 4-substituent makes for uncertainty in the expected coupling constants of pure conformers. However, a major contribution by conformer (B) in picropodophyllin leads to a predominance of $J_{ax,ax}$ for which a large coupling similar to that found in the podophyllotoxin group is expected. In the acetate (entry 9), $J_{eq,eq}$ will predominate, consistent with the smaller constant comparable with that of epipodophyllotoxin (Table 2, entry 4).

EXPERIMENTAL

The compounds listed in Table 2 were prepared by established procedures from podophyllotoxin, which was

available [11] from *Podophyllum emodi* rhizomes. Base-catalysed epimerisation at C-3 yielded picropodophyllin; [12] alternatively conversion into the chloride and hydrolysis led to inversion at C-4 and isolation of epipodophyllotoxin,[13] which afforded deoxypodophyllotoxin on hydrogenolysis.[14] The acetylation procedure was that described by Hartwell and Schrecker [13] and the 4-ketones were obtained [15] by oxidation with manganese dioxide.

The n.m.r. spectra themselves serve to characterise these compounds but each one was independently characterised by its m.p. and i.r. spectrum (KBr disc).

The n.m.r. spectra were recorded with a Varian HA 100 instrument modified for INDOR as described by Jenkins and Phillips.[16] These experiments were conducted at an ambient temperature of *ca.* 32° within a concentration range of 40—60 mg ml^{-1}.

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