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^{13}C NMR ANALYSIS OF PODOPHYLLOTOXIN AND SOME OF ITS DERIVATIVES

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Key Word Index—^{13}C NMR spectra; podophyllotoxin; epipodophyllotoxin; picropodophyllin; podophyllotoxin acetate; epipodophyllotoxin acetate; picropodophyllin acetate; podophyllotoxone; deoxypodophyllotoxin.

Abstract—The ^{13}C NMR spectra of podophyllotoxin and some of its derivatives were recorded and the signals assigned. Based on these assignments and on comparison with previously reported ^{1}H NMR data, information regarding the stereochemistry and conformations of the products under study was obtained.

INTRODUCTION

Podophyllotoxin **1a** and related products have recently received considerable attention from the synthetic and spectral point of view [1, 2]. In this connection and as part of a project on ^{13}C NMR spectral analysis of lignans [3], it was decided to carry out an analysis of some members of this group of biologically active natural products,† combined with the previously reported ^{1}H NMR data, in order to elucidate features of their stereochemistry and conformation.

RESULTS AND DISCUSSION

Table 1 lists the carbon shifts of podophyllotoxin **1a**, epipodophyllotoxin **1c**, picropodophyllin **2a** and their corresponding acetates **1b**, **1d** and **2b**, respectively, assigned by standard chemical shift theory, comparison with reference compounds, analysis of the single frequency off-resonance decoupled (sford) spectra, specific proton decoupling and ^{13}C–^{1}H long-range couplings.

Of all the signals in the ^{13}C NMR spectrum of **1a**, that at 174.6 ppm was assigned to the CO of the lactone moiety, and those at 152.1 and 147.2 ppm to the nonprotonated, oxygenated aromatic carbons C-3′ and C-5′, C-6 and C-7, respectively. For the low-field signal (152.1 ppm), 1,2,3-trimethoxybenzene **3**, was used as the model [4]. The low intensity signal at 136.6 ppm was assigned to C-4′ based on the knowledge that intermediate-sized polar molecules relax by a dipole–dipole mechanism, ^{13}C-H; C-4′, without H in any *ortho* positions, shows a longer relaxation time and therefore a lower intensity signal, [5, 6].

For the assignments of the nonprotonated, nonoxygenated aromatic carbons, which show signals at 130.6, 133.1 and 135.4 ppm, the following arguments were used. In the fully coupled spectrum, the signals at 135.4 and 133.1 ppm appear as broad singlets, while the signal at 130.6 ppm is an asymmetric doublet (J = 5.7 Hz). By irradiation at the frequencies of the aliphatic protons [2], the changes shown below are detected in the multiplicity of these signals:

		130.6 ppm	133.1 ppm	135.4 ppm
Irradiation at	4.86 ppm	*d* (J = 3.6 Hz)	*d* (J = 1.8 Hz)	*sh. s*
	4.60 ppm	*d* (J = 4.4 Hz)	*d* (J = 2.7 Hz)	*sh. s*
	3.00 ppm	*br. s*	*d* (J = 4.4 Hz)	*sh. s*
	2.70 ppm	*br. s*	*d* (J = 3.9 Hz)	*sh. s*

As can be observed, in all cases, the signal at 135.4 ppm simplified while the remaining ones appeared as doublets or broad singlets. Analysis of the three bonds C—H couplings in the structure of **1a**, shows that of the 3 carbons under study, C-1′ is the only one that could have ^{3}J just with aliphatic protons, therefore, by irradiation in this region, its signal, assigned then to the one at 135.4 ppm, would be transformed into a sharp singlet. The remaining two signals, attributed to C-9 and C-10, after decoupling of the aliphatic protons would keep their ^{3}J with the aromatic protons at C-5 and C-8, respectively. Attempts to assign the signals at 130.6 and 133.1 ppm to C-9 and C-10 or vice versa were made by specific irradiation at 6.4 and 7.01 ppm, frequencies of absorption of protons at C-8 and C-5, respectively [7]. When irradiation

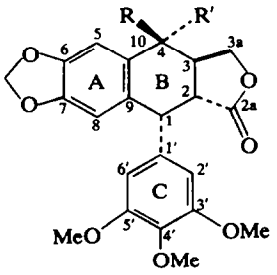
† The numbering used in this paper is the one reported by Seikel, M. K., Hostetler, F. D. and Niemann, G. J. (1971) *Phytochemistry* **10**, 2249.

Table 1. Carbon shifts of compounds studied*

Carbon No.	1a	1b	1c	1d	1e	1f	2a	2b
1	44.0	43.6	43.8	43.5	44.5	43.7	43.8	44.4
2	45.0	45.6	40.4	41.2	46.5	47.4	45.4	45.4
2a	174.6	173.2	174.9	173.8	172.7	174.6	178.0	176.7
3	40.0	38.6	38.3	36.4	43.3	32.7	42.6	29.8
3a	71.3	71.1	67.6	67.1	66.8	72.0	69.7	70.5
4	72.1	73.4	66.5	67.8	188.0	33.1	68.3	72.4
5	106.2	106.8	108.9	109.2	105.5	108.1	104.8	109.8
6	147.2	147.3	148.2	148.4	147.8	146.8	146.6	147.2
7	147.2	147.8	147.2	147.0	152.9	146.5	146.5	148.4
8	109.3	109.4	110.2	110.1	110.2	110.3	108.5	108.3
9	130.6	132.1	131.7	132.4	141.3	130.5	130.3	131.3
10	133.1	128.1	131.7	127.3	128.0	128.1	132.8	126.2
1'	135.4	134.6	134.9	134.1	131.9	136.0	139.1	138.8
2'	108.1	108.0	108.1	107.7	107.5	108.1	105.6	106.3
3'	152.1	152.0	152.3	152.2	152.8	152.3	153.2	153.3
4'	136.6	137.0	136.9	137.1	137.4	136.9	136.6	136.6
5'	152.2	152.0	152.3	152.2	152.8	152.3	153.3	153.0
6'	108.1	108.0	108.1	107.7	107.5	108.1	106.3	106.3
—OCH_2O—	101.1	101.4	101.3	101.5	102.2	101.0	101.2	102.0
—OMe	56.0	56.0	56.1	56.1	56.1	56.2	56.4	56.0
	60.5	60.5	60.6	60.6	60.6	60.6	60.3	60.7
$\underline{CH_3}$		20.9		21.0				20.8
—O—C=O		171.0		170.3				170.0

*The spectra were obtained at 25.2 MHz in Fourier transform mode in $CDCl_3$ solutions. The ^{13}C resonance of $CDCl_3$ was used as internal reference and converted to TMS scale by the following correction: $\delta(TMS) = \delta(CDCl_3) + 76.9$.

at 6.4 ppm was made the signal at 133.1 ppm became simpler and increased in intensity. Unfortunately, irradiation at 7.01 ppm did not show results which were clearly different. In order to differentiate between these signals, the effects observed by the acetylation of benzylic alcohols on the aromatic carbons were used, **4** → **5** [4]. Acetylation of **1a**(**1a** → **1b**) induces shielding and deshielding of C-10 and C-9, respectively, by magnitudes similar to those observed in related carbons of model **6** upon the same transformation, **6** → **7**.



1a, R = H; R' = OH

1b, R = H; R' = OCMe

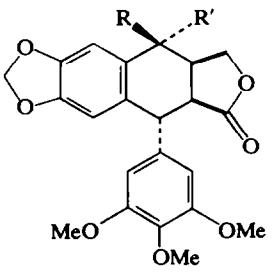
1c, R = OH; R' = H

1d, R = OCMe; R' = H

1e, R + R' = O

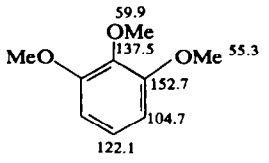
1f, R = R' = H

The protonated aromatic carbons show signals at 106.2, 108.1 and 109.3 ppm. The signal at 108.1 ppm was assigned to C-2' and C-6', based on its intensity and its multiplicity in the fully coupled spectrum (*dd*, $J = 159.8$ and 4.5 Hz), while those at 106.2 and 109.3 ppm were attributed to C-5 and C-8 respectively, by comparison with related carbons of



2a, R = H; R' = OH

2b, R = H; R' = OCMe



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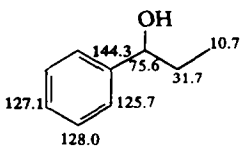

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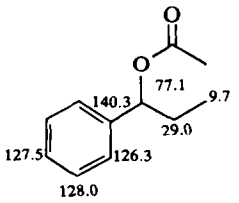

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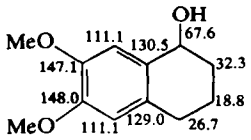

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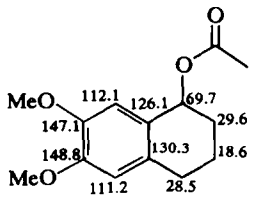

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epipodophyllotoxin **1c**. As a result of the partial elimination of the γ-effect imposed by the OH at C-4, which goes from a pseudo-equatorial to a pseudo-axial position (**1a** → **1c**), C-5 suffers a deshielding effect, while C-8 is much less affected in the transformation.

Of the 5 sp^3 carbon signals of ring B and the lactone moiety, those at 71.3 and 72.1 ppm that appear as a triplet and a doublet in the sford spectrum, were assigned to C-3a and C-4 respectively, and the remaining ones, all doublets, at *ca* 40 ppm, to C-1, C-2 and C-3. The signal at 44.0 ppm was assigned to C-1, since by irradiation at 4.5 ppm, frequency of absorption of C-1 proton [2], it becomes a singlet and those at 40.0 and 45.0 ppm, which are shielded and practically unaffected upon acetylation, to C-3 and C-2, respectively.

As expected, the OMe groups on C-3′ and C-5′ resonate at *ca* 55 ppm and the one on C-4′ at lower field, *ca* 60 ppm. Finally, the assignment of the methylenedioxy group at 101.1 ppm is trivial.

The shift assignments of epipodophyllotoxin **1c** are based on similar arguments. The low field signal at 174.9 ppm was readily assigned to the lactone CO and amongst the nonprotonated, oxygenated aromatic carbon signals, the one at 152.2 ppm to C-3′ and C-5′, the low intensity signal at 136.9 ppm to C-4′, leaving those at 147.1 and 148.1 ppm for C-6 and C-7 or vice versa. The protonated aromatic carbons show signals at 108.1, 108.9 and 110.9 ppm. Based on its intensity and multiplicity, the signal at 108.1 ppm was assigned to C-2′ and C-6′, while those at 108.9 and 110.9 ppm to C-5 and C-8 respectively, as was discussed for **1a**. The nonprotonated, non-oxygenated aromatic carbons with signals at 131.9 and 134.9 ppm were assigned to C-9 and C-10 and to C-1′, by comparison with **1a**, and further confirmed by acetylation. As expected, C-10 and C-9 suffer shielding and deshielding effects, and C-1′ is practically unaffected in the transformation.

In **1c**, the sp^3 carbons of ring B and the lactone moiety also show two groups of signals, those at *ca* 65 ppm (66.5 and 67.6) and at *ca* 40 ppm (38.2, 40.4 and 43.7). The signals at 66.5 and 67.6 ppm were attributed to C-4 and C-3a respectively, based on their multiplicity in the sford spectrum, and the signals of the second group, all doublets, were assigned to each methine carbon by specific irradiation experiments. The ^{1}H NMR signals of protons at C-1, C-2 and C-3 appear at 4.5, 3.28 and 2.91 ppm respectively [2], and irradiations at these frequencies transform the doublets at 43.7, 40.3 and 38.2 ppm into singlets allowing the unequivocal assignments of these signals to C-1, C-2 and C-3 respectively. The OMe groups and the methylenedioxy moiety show shifts identical to those of podophyllotoxin **1a**.

The shift assignment of picropodophyllin **2a** was made on a similar basis. The signals at 178.0 and 153.2 ppm were assigned to the CO of the lactone moiety and to C-3′ and C-5′ respectively, and those at 146.5 and 146.6 ppm to C-6 and C-7 or vice versa. Again and due to its low intensity, the signal at 136.6 ppm was asigned to C-4′. The assignment of the nonprotonated, non-oxygenated aromatic carbons was carried out by analysis of the fully-coupled spectrum and irradiation at the frequencies of absorption of the aliphatic protons. In the fully-coupled spectrum of **2a**, the signals at 130.3 and 139.1 ppm appear as asymmetric doublets ($J = 6.9$ *and* 5.1 Hz, respectively). While that at 132.8 ppm is a broad singlet. On irradiation at the frequencies of absorption of some of the aliphatic protons*, the following changes are observed:

Irradiation at	130.3 ppm	132.8 ppm	139.1 ppm
4.38 ppm *d* ($J = 4.6$ Hz)		*d* ($J = 3.3$ Hz)	*sh. s*
3.88 ppm *d* ($J = 4.9$ Hz)		*br. s*	*sh. s*
3.25 ppm *d* ($J = 5.4$ Hz)		*d* ($J = 4.2$ Hz)	*sh. s*

On the basis of the same arguments used for **1a**, the signal at 139.1 ppm was assigned to C-1′. On acetylation, picropodophyllin **2a** showed changes similar to those observed in the transformation of **1a** → **1b**, allowing the assignment of the signals at 130.3 and 132.8 ppm to C-9 and C-10, respectively.

The protonated aromatic carbons show signals at 104.8, 105.6 and 108.5 ppm. The signal at 105.6 ppm, on the basis of its intensity and multiplicity in the fully coupled spectrum was assigned to C-2′ and C-6′. The remaining signals at 104.8 and 108.5 ppm were assigned to C-5 and C-8, respectively.

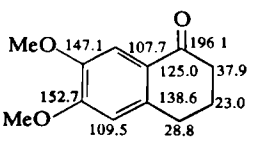
As in the cases of **1a** and **1c**, the sp^3 carbon signals are split into two groups. Again on the basis of their multiplicities in the sford spectrum, the signals at 69.7 and 68.3 ppm were assigned to C-3a and C-4, respectively. Irradiations at the frequencies of absorption of the C-1 and C-2 protons, at 3.88 and 3.25 ppm,* transform the doublets at 43.8 and 45.4 ppm to singlets, while the signal at 42.6 ppm, assigned to C-3, remains as a doublet. Confirmatory evidence of this

* The frequencies of irradiation were selected from the ^{1}H NMR spectrum of **2a** recorded in DMSO-d_6 solution (ref. [2a]), assuming that any difference with those in $CDCl_3$ would not significantly affect the results.

assignment was obtained by analysing the effects of acetylation on C-4 and C-3 and by specific irradiation experiments. In fact, in picropodophyllin acetate **2b**, the mentioned carbons suffer deshielding and shielding effects compared with similar sites of **2a**, and further by irradiation at 4.35 and 3.25 ppm [2] the doublets at 44.4 and 45.4 ppm become singlets.

Comparison of the spectra of podophyllotoxin **1a** and epipodophyllotoxin **1c**, both compounds having a *trans*-fused lactone moiety which imposes on ring B a rigid half-chain conformation, clearly shows the influence of the different position of the OH group on the chemical shifts of the neighboring carbons [8]. In **1c**, with the OH group at a pseudoaxial position, C-4, C-3 and C-10, and C-2, α, β, and γ respectively to the oxygenated function are shielded, while C-3a and C-9 are shielded and deshielded respectively, compared with related carbons of **1a**. The reasons for the deshielding effect observed on C-5 of **1c** was discussed above, and finally, the shifts shown by ring C carbons are, as expected, very similar in both compounds. Picropodophyllin **2a**, on the other hand, has a *cis*-fused lactone ring which fixes ring B in flexible half-boat forms, each of which can flip between two conformers. It was suggested, based on the J value of H-1 and H-2, that the most probable conformation of **2a** in DMSO-d_6 solution has ring C and the OH group at pseudo-equatorial positions [2a]. This is in agreement with the ^{13}C NMR spectrum of picropodophyllin, which shows shielding of C-5 and deshielding of C-1′ and C-3, probably due to a more effective eclipsing of the OH group and H-5 and elimination of the 1,3-diaxial interaction between ring C and the H carried by C-3 in podophyllotoxin **1a**. The deshielding effect observed on C-5 of picropodophyllin **2a** upon acetylation (**2a** → **2b**) appears to be consistent with the conformation of **2b** in which the acetoxy group is at a pseudo-axial position, as was suggested by analysis of its ^{1}H NMR spectrum [2a]. If this is so, in **1a** and **2b** ring C carbons show practically the same shifts at either a pseudo-equatorial or a pseudo-axial position.

The transformation of **1a** into **1e**, having also a rigid conformation, produces the expected changes on rings A and B carbon shifts and further a clear shielding effect on C-1′. The shielding and deshielding effects observed on C-10, and on C-5 and on C-9, C-7 and C-3 respectively, are readily explained by the influence of the CO at C-4 and confirmed by comparison with models **6** and **8**. On C-1′, however, a long range effect produced by the CO group, similar to those observed in some cyclo-aklanons [9], could be invoked to explain its chemical shift. Further support for this long range effect, potentially useful for the conformational analysis of related products to **1e**, was obtained by analysing the shifts of deoxypodophyllotoxin **1f**. As expected, C-1′ shows a shift comparable to the one observed in **1a**. The assignments of the remaining signals of **1f**, based on similar arguments and comparison with compounds **1a**, **1b**, **1c** and **1d**, and those of **1e** are also listed in Table 1.



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EXPERIMENTAL

The compounds **1b**–**1f**, **2a** and **2b** were prepared from podophyllotoxin (**1a**) by previously reported procedures [1, 10–13].

Podophyllotoxin (**1a**), dried under vacuum at 110°, mp 182–184°, $[\alpha]_D^{25}$ − 124° (*c* 1.0, $CHCl_3$), lit. [10] mp 183–184°, $[\alpha]_D^{20}$ − 132°. Podophyllotoxin acetate (**1b**), mp 208–210°, $[\alpha]_D^{25}$ − 136° (*c* 1.0, $CHCl_3$), lit. [10] mp 209.5–210.5°, $[\alpha]_D^{20}$ − 143°. Epiopodophyllotoxin (**1c**), mp 156–158°, $[\alpha]_D^{25}$ − 68° (*c* 0.5, $CHCl_3$), lit. [10] mp 159.4–161.2°, $[\alpha]_D^{20}$ − 75°. Epipodophyllotoxin acetate (**1d**), mp 171–172°, $[\alpha]_D^{25}$ − 135° (*c* 1.0, $CHCl_3$) lit. [10] mp 172.6 − 173.2°, $[\alpha]_D^{20}$ − 141°. Podophyllotoxone (**1e**), prepared with CrO_3–C_5H_5N complex [1], mp 190–192°, $[\alpha]_D^{25}$ − 138° (*c* 1.0, $CHCl_3$), lit. [11] mp 190–191.5°, $[\alpha]_D^{20}$ − 125°. Deoxypodophyllotoxin (**1f**), mp 165–166°, $[\alpha]_D^{25}$ − 110° (*c* 1.0, $CHCl_3$) lit. [12] mp 165–166°, $[\alpha]_D$ − 115°. Picropodophyllin (**2a**), mp 226–229°, $[\alpha]_D^{25}$ 0° (*c* 0.6, $CHCl_3$), lit. [13, 14] mp 227–230°, $[\alpha]_D^{20}$ + 4.8°; mp 223–224.5° $[\alpha]_D^{25}$ 0°. Picropodophyllin acetate (**2b**), mp 212–214°, $[\alpha]_D^{25}$ + 19° (*c* 1.0, $CHCl_3$) lit. [10] mp 214.2–215.6°, $[\alpha]_D^{25}$ + 19.4°.

The model compounds **6** and **7** [15] were prepared by $NaBH_4$ reduction of ketone **8** [16] and acetylation (Ac_2O and C_5H_5N), respectively.

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