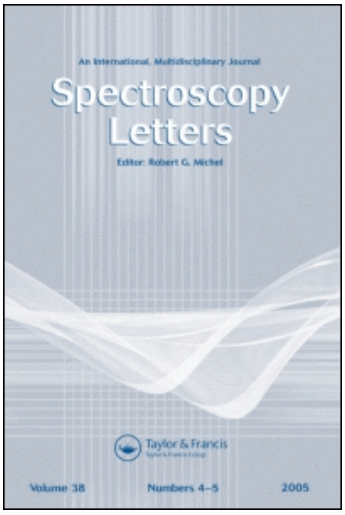


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# 2D NMR STUDY ON 4-O-TETRAHYDROPYRANYL-EPIISOPICROPODOPHYLLIN

**Keyword:** 2D NMR, podophyllotoxin derivative, 4-O-THP-epiisopicropodophyllin

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## ABSTRACT

A complete assignment of the two dimensional $^{1}H$ and $^{13}C$ NMR spectra of the title compound, which is a key intermediate for preparation of podophyllotoxin derivatives, is presented. The proton signals have been assigned from DQF- COSY,TOCSY,$^{1}H$-$^{1}H$ coupling patterns,and by the comparisons of chemical shifs with those of similar podophyllum lignans. Complete $^{13}C$ NMR assignments have been made from HMQC,HMBC and DEPT spectra. Further information on the stereochemistry of the molecule was obtained from 2D NOESY and NOE-difference techniques.

## INTRODUCTION

In 1975, Aiyar and Chang[1] published the $^{1}H$ NMR spectral data of epiisopicropodophyllin (EIPP) in DMSO. Later, Rodrigo et al.[2] published the NMR data of the 4-O-methyl ether (MEIPP) in $CDCl_3$. On the other hand, 4-O-tetrahydropyranylpodophyllotoxin (THPP) was first obtained by Gensler[3] but no NMR data for the structure of this ether was given. In this work, a detailed NMR analysis, including $^{1}H$, $^{13}C$, DEPT spectra, 2D $^{1}H$-$^{1}H$ correlation (DQFCOSY and TOCSY), 2D $^{1}H$-$^{13}C$ correlation (HMQC and HMBC ), 2D NOE spectrum correlation (NOESY) and NOE-difference experiments was used for the structure elucidation of 4-O-tetrahydropyranylepiisopicropodophyllin (THPEIPP).

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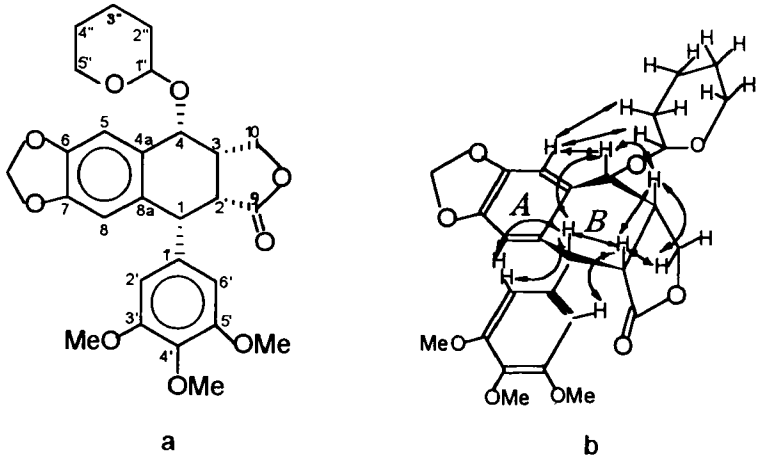


Fig.1. The structure of THPEIPP



## RESULTS AND DISCUSSION

The $^1$H NMR spectrum of THPEIPP was assigned on the basis of chemical shifts, 2D COSY spectrum, $^1$H-$^1$H coupling patterns, relative peak areas and by the comparisons of the $^1$H NMR data with those of MEIPP.

There are five signals at $\delta$ 6.835(1H),6.454(1H),6.425(2H),3.836(3H) and 3.811 (6H) which were readily assigned to H-5, H-8, H-2'6', 4'-OMe and 3'5'-OMe respectively on the basis of the relative intensity of the signals in the 1D spectrum and the lack of cross peaks in the 2D COSY(Fig.2) and TOCSY(Fig.3)spectra.For H-5 and H-8,they can be differentiated by the appearance of two cross peaks for H5-C4a and H8-C8a in the HMBC spectrum (listed in Table 2). Furthermore, the NOE observed from NOESY(Fig.5) and NOE difference spectrum (Fig.6) between the signals of H-5 and H-4 and between H-8 and H-1 gave full proof due to the proton pairs closing to each other in the space. This conclusion is consistent with other derivatives of podophyllotoxin[4].The assignment of $OCH_2O$ was quite straightforward, for it provided an easily recognizable ABq system at $\delta$ 5.946ppm with no cross peaks in 2D COSY and TOCSY spectra.The H-1 and H-4 were readily identified as doublets at 4.159 and 4.588ppm, because both of the protons presented strong cross peaks, as shown in Fig.2,caused by coupling with H-2(3.303ppm) and H-3 ( 2.970ppm) respectively.The two quartets at $\delta$ 4.497 ppm and 4.416 ppm were assigned to the unequal protons of

TABLE 1
$^1H$ and $^{13}C$ NMR Chemical Shifts and C-H Correlations of THPEIPP, and Comparison of the Major $^1H$ NMR Data with EIPP and MEIPP[a]

| $^1H$ NMR,δ(J) | | position | THPEIPP (in $CDCl_3$) | | |
|---|---|---|---|---|---|
| EIPP (in DMSO) | MEIPP (in $CDCl_3$) | | $^1H$ NMR δ(J) | $^{13}C$ NMR δ | DEPT -135 |
| 4.66d | 4.07d(3.3) | 1 | 4.159d(5.07) | 44.740 | CH |
| | 3.5m | 2 | 3.303dd(9.70,5.16) | 44.869 | CH |
| | 3.5m | 3 | 2.970m | 41.073 | CH |
| | 4.38d(5.8) | 4 | 4.588d(6.90) | 74.508 | CH |
| | | 4a | - | 131.509 | C |
| 7.14s | 7.03s | 5 | 6.835s | 107.196 | CH |
| | | 6 | - | 147.492 | C |
| | | 7 | - | 153.555 | C |
| 6.53s | 6.54s | 8 | 6.454s | 109.824 | CH |
| | | 8a | - | 146.741 | C |
| | | 9 | - | 178.316 | C |
| | | 10α | 4.497dd(9.05,5.83) | 70.211 | $CH_2$ |
| | | 10β | 4.416dd(9.41,7.14) | 70.211 | $CH_2$ |
| | | 1' | - | 138.786 | C |
| 6.36s | 6.90s | 2'/6' | 6.425s | 105.721 | CH |
| | | 3'/5' | - | 153.233 | C |
| | | 4' | - | 136.724 | C |
| | | 1" | 4.520dd(6.47,2.44) | 99.216 | CH |
| | | 2" | 1.45m?1.69-1.92m? | 30.357 | $CH_2$ |
| | | 3" | 1.76-1.92m? | 20.740 | $CH_2$ |
| | | 4" | 1.54m? | 25.221 | $CH_2$ |
| | | 5" | 3.970m,3.530m | 64.337 | $CH_2$ |
| 6.10 | 5.93ABq(1.2) | $OCH_2O$ | 5.946ABq(1.37) | 101.177 | $CH_2$ |
| 3.63 | 3.89s | 4'-OMe | 3.836s | 60.934 | $CH_3$ |
| 3.63 | 3.86s | 3'/5'-OMe | 3.811s | 56.131 | $CH_3$ |
| 1 | 2 | Ref. | this | work | |

[a] THPEIPP = 4-O-tetrahydropyranylepiisopicropodophyllin
MEIPP = 4-O-methylepiisopicropodophyllin
EIPP = epiisopicropodophyllin



H-10α and H-10β, both the signals gave two strong cross peaks in the COSY spectrum due to coupling with H-3(2.970ppm) and H-10β or H-10α respectively. A quartet at 3.303 ppm corresponded to H-2, which gave two strong cross peaks in the COSY, caused by coupling with H-1 and H-3. A multiplet at ca. 2.970 ppm can be assigned to H-3, which presented four strong cross peaks in the COSY, by coupling with H-2 and H-4 along with H-10.The remaining quartet at 4.520 ppm with 1 proton intensity, partially overlapped with that of H-10α,and was very difficult to interpret. Fortunately, a strong cross peak appeared in the COSY, at 4.520 ppm and ca.1.45 ppm. The upfield signal (1.45 ppm, might be due to H-2") is the resonance region of the pyranyl ring protons.Therefore, the quartet at 4.520 ppm can be assigned to H-1" on the pyranyl ring, which is connected with two oxygen atoms.The full $^1H$ assignments are summerized in Table 1 and the coupling constants are included in

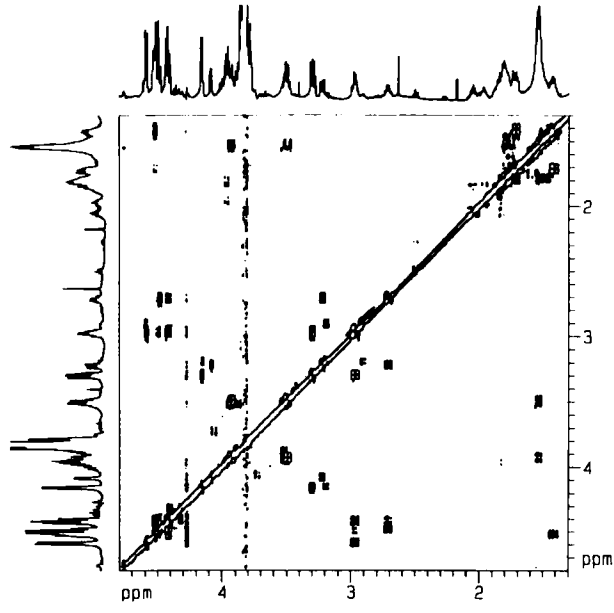


Fig. 2. 2D DQFCOSY spectrum

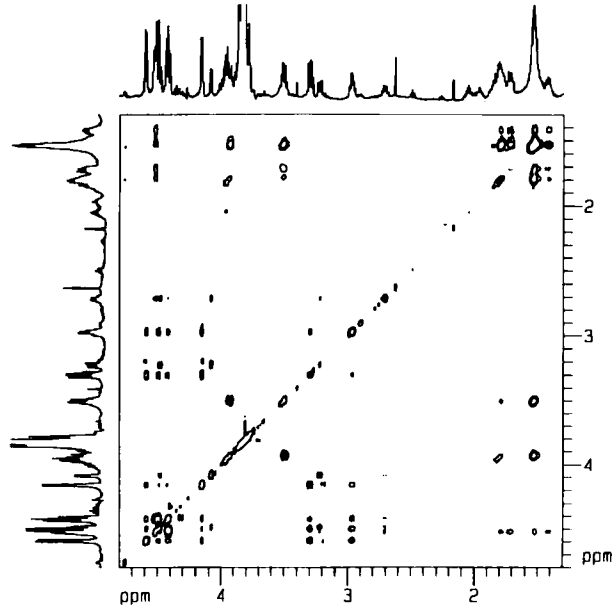


Fig. 3. 2D TOCSY spectrum

TABLE 2
Summary of Correlation for THPEIPP from Cross Peaks of 2D DQFCOSY,TOCSY,HMQC,HMBC and NOESY Spectra

| DQFCOSY & TOCSY[a] | | HMQC & HMBC[b] | NOESY |
|---|---|---|---|
| H1-H2 | *H1-H10α | H1-C1,C1',C2,C3,C2'/6',C8,C4 | H1-H2,H4,H8,H2'/6' |
| H2-H3 | *H2-H4 | H2-C2,C1', C4 | H2-H3,H10β,H2'/6' |
| H3-H4 | *H2-H10α | H3-C3,C4 | H3-H4,H10β |
| H3-H10β | *H2-H10β | H4-C4,C3, C2, C4a, C5, C10 | H4-H5 |
| H10α-H10β,α | *H4-H10α | H5-C5,C4, C4a, C6, C7 | H5-H1",H2" |
| H1"-H2" | *H4-H10β | H8-C8,C6, C7, C8a | H8-H2'/6' |
| H2"-H2" | *H1"-H3" | H10α,β-C10,C4 | H1"-H2",H4" |
| H4"-H5" | *H1"-H4" | $OCH_2O$-$OCH_2O$ | H2"-H4" |
| H5"-H5" | *H2"-H5" | H2'/6'-C2'/6',C1, C1', C3'/5',C4' | H3"-H4" |
| *H1-H3 | *H3"-H5" | H1"-C1" | H5"-H1",H4",H5" |
| *H1-H4 | *H1-H10β | H2"-C2" | |

[a] The cross peaks labled * only appear in TOCSY
[b] The cross peaks labled __ only appear in HMBC

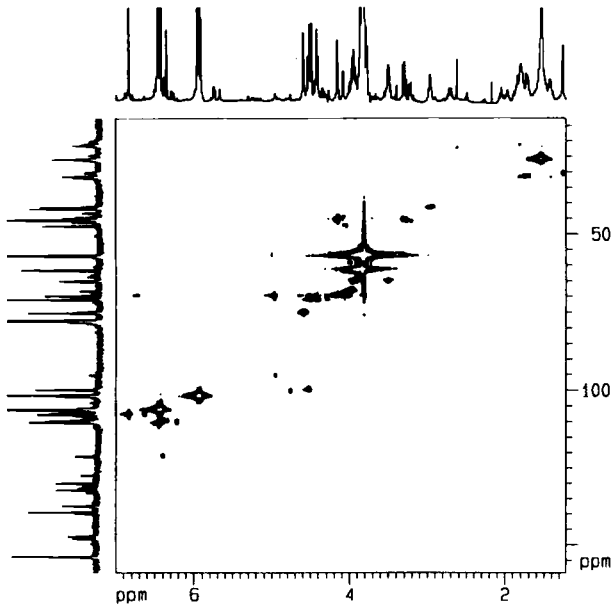


Fig. 4. 2D HMQC spectrum

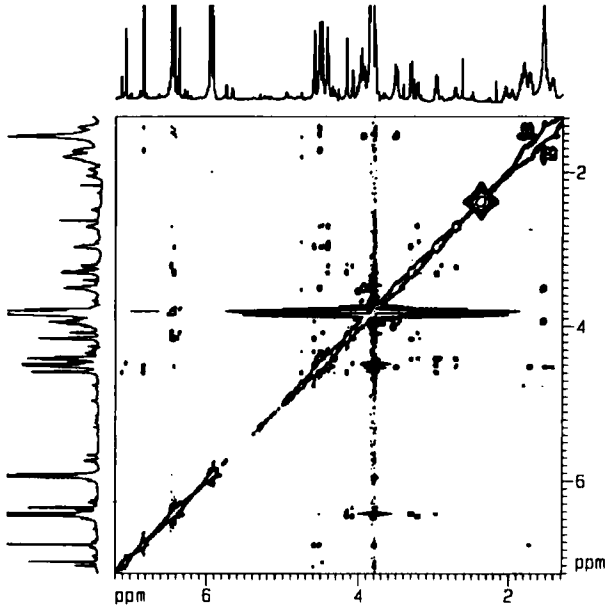


Fig. 5. 2D NOESY spectrum

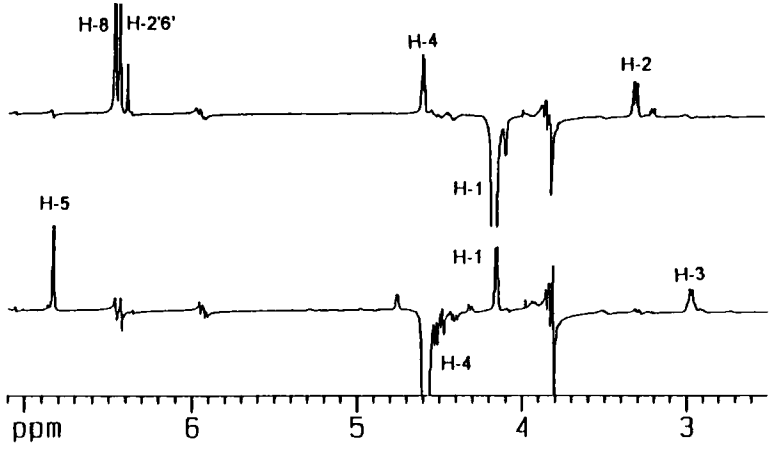


Fig. 6. NOE-DIF spectrum, irradiated at H-1(up) and H-4(down)

parentheses.However, a complete analysis for the protons on the pyranyl ring is not possible for the very similar coupling and close signals. A comparison of the major $^1H$ NMR data of three similar componds is also included in the Table.

The above assignment for proton resonances can be used to assign the $^{13}C$ resonances of the compound via heteronuclear correlation 2D experiments. The C-H correlations in Table 1 were obtained according to the HMQC (Fig.4) and DEPT-135 spectra, and by the comparisons of chemical shifts with those of podophyllotoxin stereoisomers or derivatives from the previous work[4]. The quaternary carbons could also be identified by the heteronuclear multiple bond correlation HMBC experiment shown in Table 2.

A further elucidation of the stereostructure of THPEIPP was obtained through a study of the 2D NOESY, that was used for the assignments of the proton pairs which are close to each other in the space, especially those connected with C-1, C-2, C-3 and C-4, which are related to the configuration and linked with biological activity.

There are twenty cross peaks in the NOESY spectrum as shown in Fig.5.The correlations of all the cross peaks are listed in Table 2 and some of them are shown in the structural formula (Fig.1 b) by the arrows. The correlations of H1-H2, H2-H3, H3-H4 and H3-H10b indicate that all the relations between C1 and C2, C2 and C3, and C3 and C4 are cis configurations. This conclusion was supported by their coupling constants ( Table 1 ) and confirmed by the NOE-difference spectrum shown in Fig.6, as did NOE enhancements of H-2 and H-4 when H-1 was irradiated,and did of H-1 and H-3 when H-4 irradiated. Note that the cross peaks of H4-H5 and H1-H2'6' in NOESY (Fig.5) and the NOE observed between the signals of H-1 and H-4 in NOE-DIF spectrum(Fig.6) imply that the major conformation of the reduced ring B of the compound is in the boat form.

The THP group cannot rotate freely about the C-O bond and the plane of the ring is perpendiculer to that of the ring B as shown in Fig.1(b), because of the appearance of the cross peaks of both H5-H1" and H5-H2", and no cross peaks of H10-H1" or H10-H2" in NOESY spectrum.The THP ring can exist in both chair and boat conform-ations with the EIPP substituent in an equatorial orientation, but the latter is dominant as the appearance of cross peaks of H1"-H4" and H3"-H4" in the NOESY spectra.

## EXPERIMENTAL

All the NMR spectra were recorded for a 32 mg/ml $CDCl_3$ solution on a Bruker DMX 500 spectrometer with $^1H$-$^{13}C$-$^{15}N$ triple resonance probe at 300K.The chemical shifts of the solvent was used as an internal reference and set at 7.270 ppm for $^1H$ and 77.053 ppm for $^{13}C$ relative to TMS.

1D $^{1}H$ and $^{13}C$ NMR spectra were acquired into 64K and 45K data points with spectral width of 3561.25 and 22727.27 Hz respectively. 20K scans were used for $^{13}C$ NMR. 2D DQFCOSY, NOESY and TOCSY spectra were observed in phase sensitive mode. For DQFCOSY, 512 $t_1$ increasments were accumulated into 2Kdata points with 32 scans for each. NOESY spectra were taken with mixing time of 600 ms, 512 $t_1$ increamints,1K data points and 24 scans. 2K data points, 256 $t_1$ increaments and 160 scans were used for acquisition of the TOCSY spectra with spinlock time of 61.5 ms.

The proton detected HMQC and HMBC were acquired with gradient pulses for selection. The spectral widths were 3561.25 Hz and 22637.97 Hz for proton and carbon respectively. Sine-squared window function, 90° shifted were applied over 1024 complex data points in the F2 dimension and over 256 complex data points in the F1 dimension. The HMBC experiment was set for multibond($^{1}H$-$^{13}C$) coupling of around 8Hz(long range coupling evolution time 50 ms) with a low-pass J-filter to suppress one-bond couplings of around 145 Hz.

The synthesis of the sample started from podophyllotoxin, which was reacted with dihydropyran to afford a 4-O-THP-podophyllotoxin. The latter was saponified to yield a hydroxy carboxylate, followed by acidification to give the all-cis(1α,2α,3α,4α) product with epimerisation at C-3.



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