Tetrahedron Vol. 41, No. 2, pp. 357 to 364, 1985
Printed in Great Britain.

0040–4020/85 $3.00 + .00

A NOVEL PIMARA-8(9),15-DIENE FROM *LYCOPUS EUROPAEUS*

DRAGOSLAV JEREMIĆ, SLOBODAN MACURA and SLOBODAN MILOSAVLJEVIĆ*

Department of Chemistry and Physical Chemistry, Faculty of Science,
Studentski trg 16, P.O.Box 550, 11001 Belgrade, Yugoslavia

VLATKA VAJS

Institute for Chemistry, Technology and Metallurgy, Belgrade

(*Received in UK* 22 *October* 1984)

Abstract - Identification of a novel trioxygenated $\Delta^{8(9),15}$-pimaric acid methyl ester from plant species *Lycopus europaeus* by means of two-dimensional ^{1}H NMR spectroscopy (COSY and NOESY) is reported.

A silica gel column chromatography (using benzene/diethyl ether as an eluent) of a chloroform extract of the dried *Lycopus europaeus* (the whole plant) afforded a crystalline colourless compound (**1**).

Table 1. MS and IR data of **1**

MS/EI, *m/z* (%)	M (absent), 388.2250 ($C_{23}H_{32}O_5$, M-60, 10), 346.2142 ($C_{21}H_{30}O_4$, M-60-42, 20), 328.2046 ($C_{21}H_{28}O_3$, M-2x60, 7), 305.1756 ($C_{18}H_{25}O_4$, 14), 251.1780 ($C_{19}H_{23}$, 34), 201 (23), 86 (63), 84 (94), 43 (100)
IR, $\nu_{max}^{CCl_4}$, cm^{-1}	*ca.* 3480 (OH), 1725, 1240 (OAc, CO_2Me), 1630 (C=C)

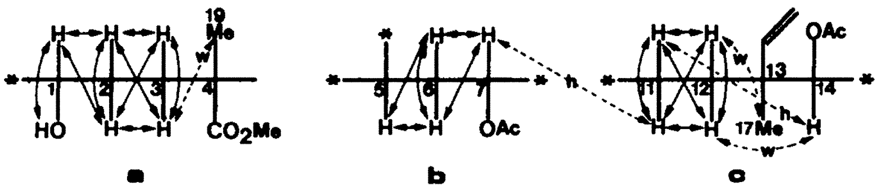
The spectral data of **1** (listed in Tables 1-3) revealed three tertiary methyls, two double bonds (an isolated monosubstituted and a tetrasubstituted one) and a methyl ester group, the structural features typical for tricyclic diterpene belonging to the known $\Delta^{8(9),15}$-pimaric acid group[1] (see Scheme 2). The spectral data also indicated the presence of three oxygen functions, such as two secondary acetoxyls and a secondary hydroxyl. One of the acetoxy groups could be readily attached to C-14, since one of the C<u>H</u>-OAc protons gave rise to a singlet at δ 5.47 in the ^{1}H NMR spectrum (Table 2). In order to obtain an unambiguous proof regarding the position of the oxygen functions and at the same time derive the geometry of this molecule, a 2D ^{1}H NMR study (COSY and NOESY) was undertaken.

Table 2. ^{1}H 500 MHz(C_6D_6) chemical shifts[a] and multiplicity[b] of 1

Proton[c]	δ
H-1β	3.57[d] (1H, unresolved m, $w\frac{h}{2}$ 7.5)
H-2α	*ca.* 1.37[e]
H-2β	*ca.* 1.60[e]
H-3α	2.37 (1H, ddd, 4, 13.5, 13.5)
H-3β	1.35 (1H, ddd, 2, 4, 13.5)
H-5α	2.72 (1H, dd, 2, 13)
H-6α	1.77 (1H, dddd, 1, 2, 2, 14)
H-6β	1.66 (1H, ddd, 4, 13, 14)
H-7β	5.42 (1H, ddd, 2, 2, 4)
H-11α	*ca.* 1.9[e]
H-11β	2.42 (1H, ddd, 4, 6.5, 8)
H-12α	1.43 (1H, dddd, 1, 4, 6.5, 13)
H-12β	2.04 (1H, ddd, 6.5, 9, 13)
H-14α	5.47 (1H, s, $w\frac{h}{2}$ 2.5)
H-15	6.10 (1H, dd, 11, 17)
$=CH_2$(16)	5.18 (1H, dd, 1.5, 11), 5.20 (1H, dd, 1.5, 17)
Me-17	0.91[f] (3H, s)
Me-19	1.23 (3H, s)
Me-20	0.69 (3H, s)
Me-21	3.44 (3H, s)
2xMe (OAc)	2.00, 1.87 (2x3H, s)
OH	1.04

[a] Referenced to TMS as internal standard. [b] Coupling constants (Hz, in parentheses) are obtained by the first-order analysis. [c] The assignments are based on the characteristic chemical shifts, coupling constants and 2-D ^{1}H NMR data (COSY and NOESY); for the notation see structure 1 (Scheme 2). [d] Shifts to *ca.* δ 5.3 upon addition of trichloroacetyl-isocyanate (TAI) to the NMR solution. [e] Overlapped with other signals. [f] Shifts to δ 0.70 upon hydrogenation of 15-double bond (using $H_2/PtO_2/EtOH$, 25°C, atm. pressure).

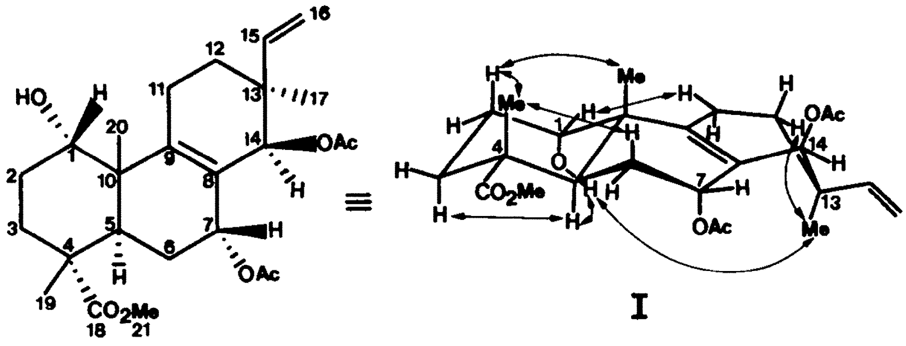
The COSY spectrum (Fig. 1), revealing the interproton coupling network (denoted by double-headed arrows in Scheme 1) and thus a complete spectral assignment, enabled identification of three structural units:



Scheme 1

Notations used: ←——→(geminal and vicinal couplings), ←-w-→(long-range "W" couplings), ←-h-→(long-range homoallylic couplings), * - carbons bearing no protons. [The long-range "W" coupling concerning H-12β and Me-17 was observed in the COSY spectrum (not shown) obtained with higher digital resolution than that shown in Fig. 1].

The substructures a, b and c could be incorporated in A, B and C rings, respectively, of the pimara-8(9),15-diene skeleton (structure 1):



1

I

Scheme 2

Note: The NOEs observed in the NOESY spectrum are denoted by double-headed arrows (in I). The NOEs H-3α,H-5α; H-5α,OH and H-12α,Me-17 were observed in the NOESY contour plot at different contour levels than that shown in Fig. 2.

Relative stereochemistry of 1

The *trans*-A/B fusion (*i.e.* 5α-H geometry), typical for this class of diterpenes, was evident from the observed vicinal couplings between H-5 and C(6)H$_2$ (*i.e.* J_{aa}= 13 and J_{ae}= 2 Hz). At the same time, small vicinal couplings of CH(1)OH (δ 3.57, m, $w_{\frac{h}{2}}$ = 7.5 Hz) and also of CH(7)OAc (δ 5.42, ddd, J_{ea}= 4 and $J_{ee} \approx J_{homoallyl.} \approx$ 2 Hz) are in accordance with (pseudo)axial positions of this oxygen functionality (*i.e.* 1α-hydroxy-7α-acetoxy configuration). The observed ^{13}C NMR chemical shifts (Table 3) of the angular methyls (19 and 20), which are almost identical to those in the previously studied pimara- and isopimaradienes (*e.g.* in 2 and 3)^2, indicated a *cis*-diaxial (β) arrangement of Me-19 and 20. Furthermore, the observance of a long-range "W" coupling between Me-19 and H-3α (see the COSY spectrum, Fig. 1) afforded an additional proof^3 for the proposed β-orientation of Me-19. The comparison of ^{13}C chemical shifts of the remaining A/B carbons to those of the model compounds 2 and 3 (Table 3), taking the known influence of α, β, γ and δ positioned OH and OAc groups into account,^{2,4} also proved the proposed geometry concerning A/B rings.

The NOESY spectrum (Fig. 2), showing the correlation between protons located close to each other (not further than 3.5 Å), revealed the proximity of the following protons: H-2β,Me-20; H-2β,Me-19; H-3α,H-5α; H-5α,OH; H-6β,Me-20; H-6β,Me-19 and H-1β,H-11β, thus yielding an additional evidence for the A/B stereochemistry (as shown in I, Scheme 2).

As far as the relative configuration of C-13 is concerned, *i.e.* whether compound 1 belongs to the pimara (with 13α-Me, as in 2) or isopimara series (with

13β-Me, as in **3**), the conclusive proof was obtained from the NOE observed between 1α-O$\underline{H}$ and 13-C$\underline{H}_3$ (*i.e.* Me-17). Such type of direct dipole-dipole interaction between the structurally remote groups (such as 1-O$\underline{H}$ and 13-C$\underline{H}_3$) could occur only in geometry **I** (Scheme 2). Although this is not obvious from the presentation in Scheme 2, it could be seen on Dreiding models that the distance between the average positions of 1α-O$\underline{H}$ and 13α-CH_3 in **I** (assuming the free rotation around C(1)-O and C(13)-C(17) bonds) is slightly shorter than 3.5 Å. A confirmation of this (pimarane type) geometry was also obtained by the observance of the long-range "W" coupling between Me-17 and H-12β (same as that concerning Me-19 and H-3α, Scheme 1) and also by the similarity of the ^{13}C chemical shift of Me-17 in **1** (δ_C 22.0) to that of the same carbon in **2** (δ_C 21.2).

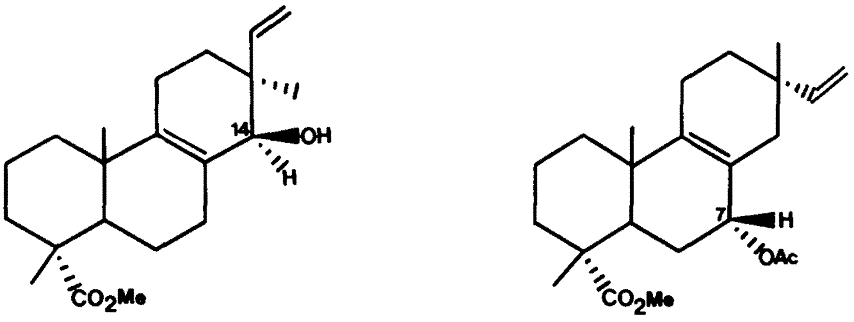
Table 3. ^{13}C 125.7 MHz NMR ($CDCl_3$) chemical shifts[a] (and multiplicity)[b] of **1** and comparison of these to the chemical shifts of **2** and **3**[2]

Carbon[c]	δ_C(**1**)	δ_C(**1**)-δ_C(**2**)	δ_C(**1**)-δ_C(**3**)
1	70.2 (d)	+34.5 $(\alpha)^d$	+35.0 $(\alpha)^d$
2	24.5 (t)	+6.2 $(\beta)^d$	+6.2 $(\beta)^d$
3	29.4 (t)	-7.3 $(\gamma_g)^d$	-6.9 $(\gamma_g)^d$
4	46.6 (s)	-1.2 $(\delta)^d$	-0.7 $(\delta)^d$
5	33.9 (d)	-12.2 $(\gamma_g)^{d,e}$	-7.7 $(\gamma_g)^d$
6	27.6 (t)[f]	*ca.* +5.9 $(\beta)^e$	*ca.* -0.6
7	71.1 (d)	+41.3 $(\alpha)^e$	-0.7
8	125.9 (s)	-1.0	+2.7
9	148.1 (s)	+7.2	+3.8
10	43.5 (s)	+6.4 $(\beta)^d$	+5.7 $(\beta)^d$
11	20.2 (t)	-0.6	-1.2
12	27.5 (t)[f]	*ca.* +1.1	*ca.* -7.0 $(\gamma_g)^e$
13	39.0 (s)	-0.7	+3.9 $(\beta)^e$
14	76.0 (d)	+1.1	+37.5 $(\alpha)^e$
15	143.2 (d)	-2.7	-2.4
16	113.1 (t)	-0.3	+1.5
17	22.0 (q)	+0.8	-6.1
18	177.9 (s)	-1.4	-0.6
19	16.5 (q)	0.0	0.0
20	19.4 (q)	-0.5	+1.0
21	51.9 (q)	0.0	+0.2
2xOAc	21.5 (q)[g] 170.5 (s) 170.3		

---

[a] Referenced to TMS as internal standard. [b] Observed in J-resolved ^{1}H-^{13}C 2D NMR spectrum. [c] The assignments are based on the multiplicity and published data[2,5] regarding the related compounds. [d] The influence of OH (γ_g=γ_{gauche}). [e] The influence of OAc. [f] The assignments may be interchanged. [g] Splits into two lines in C_6D_6, *i.e.* δ_C 21.0 (q) and 21.2 (q).

The NOE observed between Me-17 and H-12α also fit to the proposed pseudoaxial α-geometry of this methyl. It should also be noted that, according to the previous ^{13}C NMR studies[2,5] concerning this class of diterpenes, the chemical shift of C-12 is not much affected by the configuration at C-13. Thus, C-12 absorbs at δ_C 34.0 and δ_C 35.1 in unsubstituted $\Delta^{8(9),15}$-pimaric and isopimaric methyl esters,[2] respectively. The observed upfield shift concerning C-12 in compound **1** (resonating at δ_C 27.5) of *ca*. $\Delta\delta_C$-7, in comparison to that of the analogoues without C-ring substituents (see Table 3), could be explained by the γ *gauche* effect[2,4] of the pseudoaxially 14β-positioned OAc group. The occurrence of the long-range "W" coupling (^4J≈1 Hz) between H-14 and H-12α, typical for the 1,3-positioned equatorial protons, was in a good agreement with pseudoequatorial 14α-H (and also pseudoaxial 14β-OAc) geometry. As it could be seen from the COSY spectrum (Fig.11), an additional small coupling of the homoallylic type concerning H-14α and H-11β was also observed.



2 3

Scheme 3

The twisted-chair conformation of ring C (with pseudoaxial 13α-Me and pseudoequatorial 13β-vinyl) similar to that in **I** (Scheme 2) was also proposed previously (on the basis of ^{13}C NMR data)[5] for the related pimara-8(9),15-diene analogues.

EXPERIMENTAL

Melting point (uncorrected) of compound **1** was taken on Boetius PHMK apparatus. Optical rotation was measured with a Polamat A C. Zeiss apparatus at 23°C in $CHCl_3$. IR spectrum was recorded in CCl_4 solution with a Perkin-Elmer 457 Grating IR spectrophotometer. High and low resolution mass spectra (EI) were measured on a Finnigan MAT 8230 mass spectrometer at 70 eV; the sample was introduced directly into the ion source (150°C) using a direct introducing probe (80°C).
^1H 500 MHz NMR (C_6D_6) and ^{13}C 125.7 MHz NMR ($CDCl_3$ and C_6D_6) spectra (both 1D and 2D) were recorded on a Bruker WM-500 instrument equipped with an Aspect 2000 computer.

^1H-^{13}C J-resolved 2D NMR spectrum was recorded using a gated decoupler method.[6]

Homonuclear correlated 2D NMR spectrum (COSY) was measured using a standard

90°-t_1-90°-t_2 scheme[7a] with quadrature detection in both frequency domains.[7b] Data was processed in usual way[7c] and presented as contour plots of the absolute value spectrum (Fig. 1). A 512 x 2048 data points spectrum was acquired, with 3.1 Hz/Pt resolution in both domains. The time domain data matrix was multiplied with sine function in both domains.

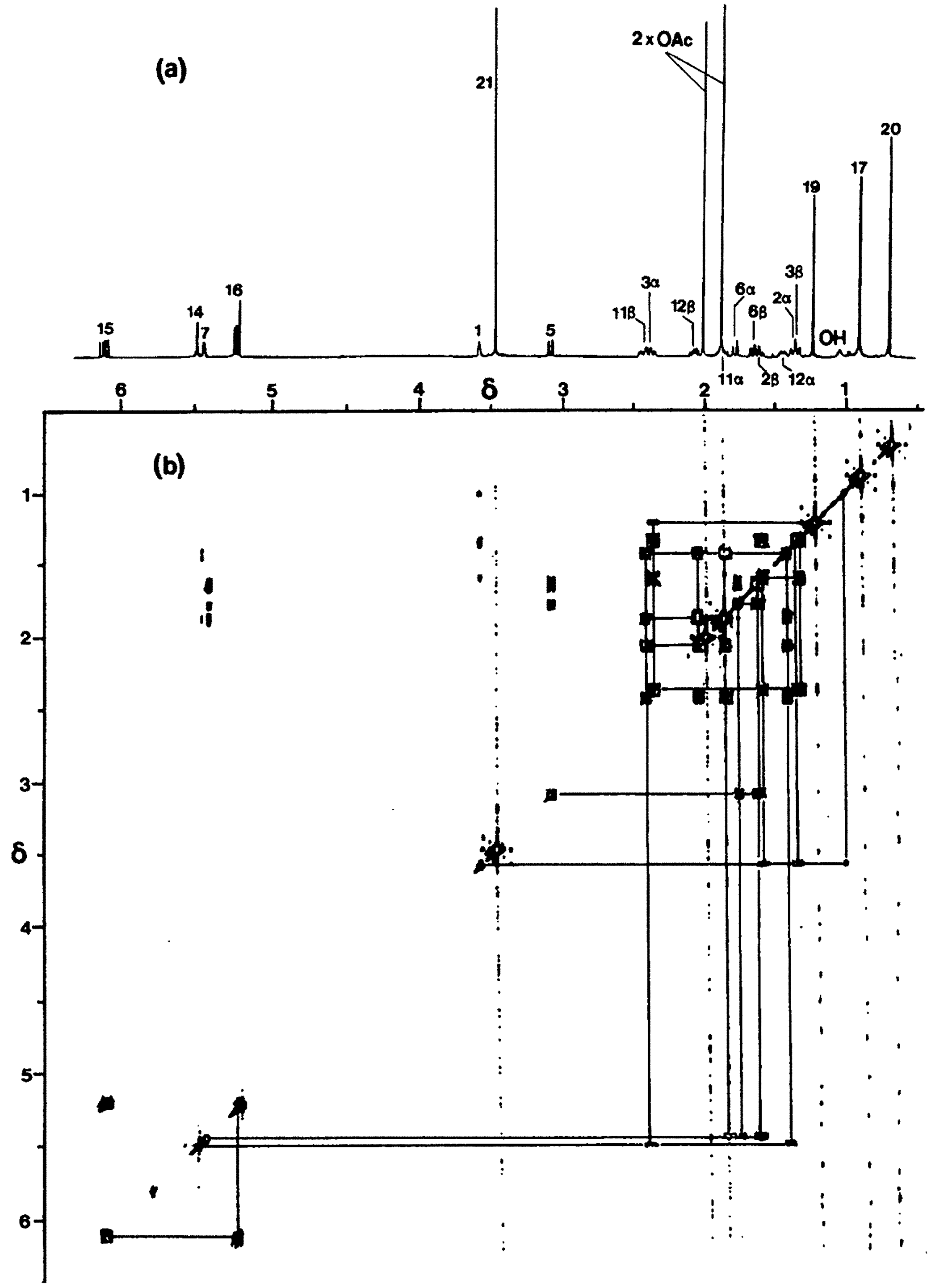


Figure 1. ^{1}H 500 MHz NMR spectra of **1** (20 mg in 0.5 ml of C_6D_6); (a) 1D spectrum; (b) Homonuclear correlated 2D NMR (COSY) spectrum

Cross-relaxation correlated 2D ^{1}H NMR spectrum (NOESY)[8a,b] was recorded with a basic experimental scheme of 2D exchange spectroscopy, *i.e.* $90^\circ-t_1-90^\circ-\tau_m-90^\circ-t_2$,[8c] with quadrature detection in ω_2 and single channel detection in ω_1 domain.[7c] Carrier frequency was set at the left-hand side of the spectrum. Mixing time τ_m was randomly modulated within 10% of its nominal value in order to eliminate effects of coherent magnetization transfer.[8c]

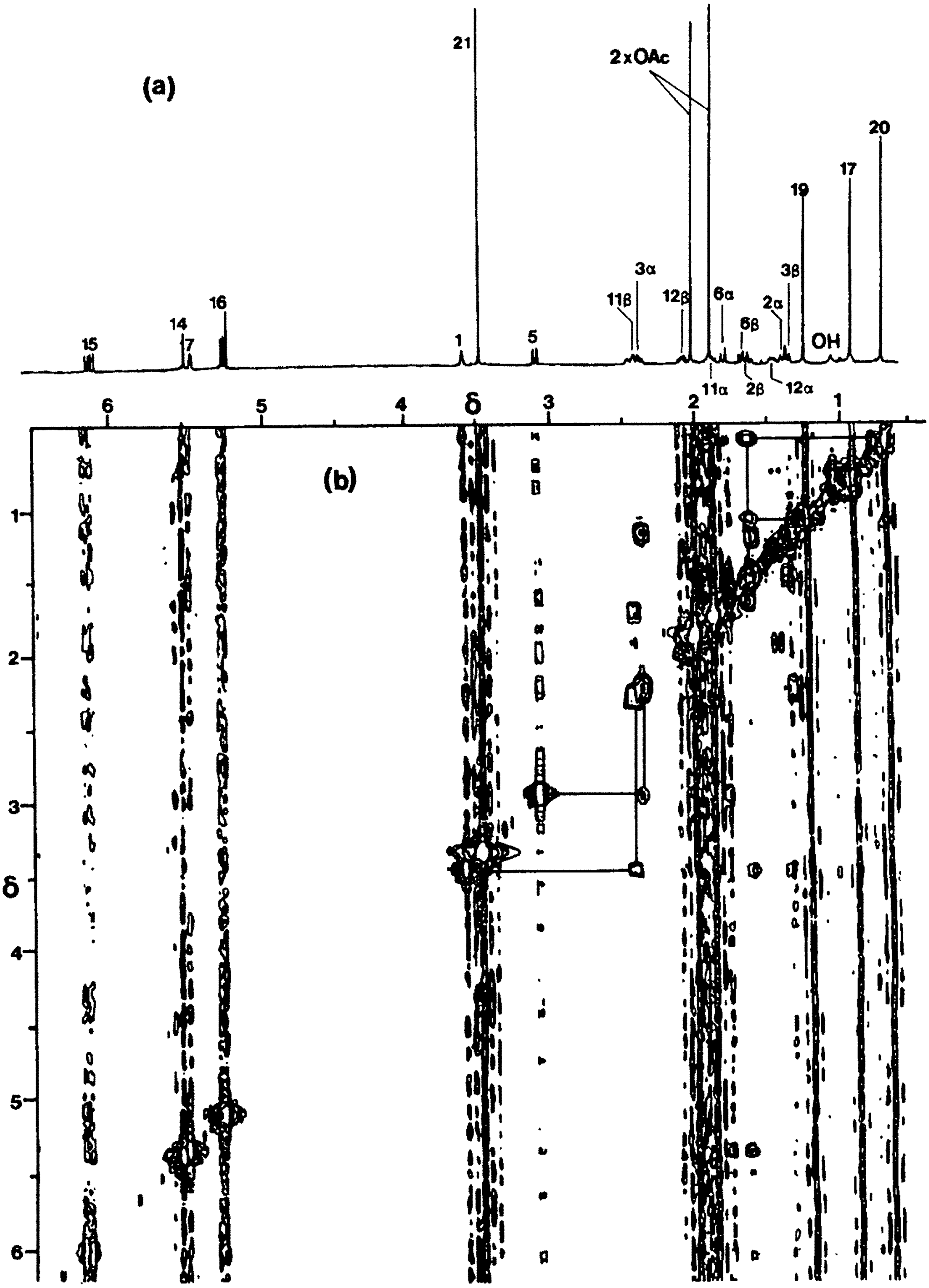


Figure 2. ^1H 500 MHz NMR spectra of 1 (20 mg in 0.5 ml of C_6D_6); (a) 1D NMR spectrum; (b) Cross-relaxation correlated 2D ^1H NMR (NOESY) spectrum. The correlations between the nuclei coupled only by direct dipole-dipole interactions are denoted by straight lines.

Data was processed as usual[7c] and presented as contour plots of absolute value spectrum (Fig. 2). The 128 x 2048 data set was acquired using τ_m of 1.00 s. The time domain data matrix was multiplied with sine function in both domains, giving (after zero-filling and double Fourier transformation) digital resolution of 5.7 Hz/Pt in both domains.

Plant material - *Lycopus europaeus* was identified and collected by Ž. Joksimović (Botanic Garden, Faculty of Science, Belgrade) in Boleč (*ca.* 10 km south-east from Belgrade).

Isolation of diterpene **1** - The chloroform (10 l) extraction (at room temperature for 10 days) of the powdered *Lycopus europaeus* (2.2 kg of the whole plant afforded the crude extract (63 g). After the usual work up,[9] the purified extract (10 g) was chromatographed on a silica gel column, starting elution with benzene. The polarity of the eluent was gradually increased by addition of diethyl ether. The fraction containing diterpene **1** as major component (eluted with 10% Et_2O in C_6H_6) was rechromatographed on a silica gel column (with 10% Et_2O in C_6H_6) to yield the colourless compound **1** (132 mg), m.p. 124°, $[\alpha]_D^{23}$ $(CHCl_3)=-48.1$.

Acknowledgements - This work is supported in part by the Serbian Republic Research Fund. We would like to express our gratitude to Dr. E. Trivelone and Dr. T. Tancredi for using NMR facility at Institute for Chemistry of Molecules of Biological Interest (CNR, Naples, Italy) and also to Dr. U. Rapp (Finnigan MAT GmbH, Bremen, Germany) for running mass spectra.

REFERENCES

[1] a) J. R. Hanson, in *Chemistry of Terpenes and Terpenoids*, edited by A. A. Newman, pp. 155-199, Academic Press, New York (1972); b) K. Yamaguchi, *Spectral Data of Natural Products*, Vol. 1, pp. 278-280, Elsevier, New York (1970)

[2] B. Delmond, M. Taran, J. Valade, M. Petraud and B. Barbe, *Org. Magn. Reson.* **17**, 207 (1981)

[3] L. M. Jackmann and S. Sternhell, *Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, 2nd eddition, p. 337, Pergamon Press, Oxford (1978)

[4] F. W. Wehrli and T. Wirthlin, *Interpretation of Carbon-13 NMR Spectra*, p. 45, Heyden, London (1976)

[5] E. Wenkert and B. L. Buckwalter, *J. Amer. Chem. Soc.* **94**, 4367 (1972)

[6] G. Bodenhausen, R. Freeman, R. Niedermeyer and D. L. Turner, *J. Magn. Reson.* **26**, 133 (1977)

[7] a) W. Aue, E. Bartholdi and R. R. Ernst, *J. Chem. Phys.* **64**, 2229 (1976); b) K. Nagayama, Anil Kumar, K. Wüthrich and R. R. Ernst, *J. Magn. Reson.* **40**, 321 (1980); c) G. Wider, S. Macura, Anil Kumar, R. R. Ernst and K. Wüthrich, *J. Magn. Reson.* **56**, 207 (1984)

[8] a) Anil Kumar, R. R. Ernst and K. Wüthrich, *Biochem. Biophys. Res. Commun.* **95**, 1 (1980); b) S. Macura and R. R. Ernst, *Mol. Phys.* **41**, 95 (1980); c) J. Jeener, B. H. Meier, P. Bachmann and R. R. Ernst, *J. Chem. Phys.* **71**, 4546 (1979); d) S. Macura, Y. Huang, D. Suter and R. R. Ernst, *J. Magn. Reson.* **43**, 259 (1981)

[9] M. Stefanović, A. Jokić and A. Behboud, *Bull. Soc. Chim. Beograd* **37**, 463 (1972)