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A Novel Tetraoxygenated $\Delta^{8(9),15}$ -Pimaric Acid Methyl Ester From *Lycopus Europaeus* — II**

Slobodan Milosavljević, Dragoslav Jeremić and Slobodan Macura

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

and

Vlatka Vajs

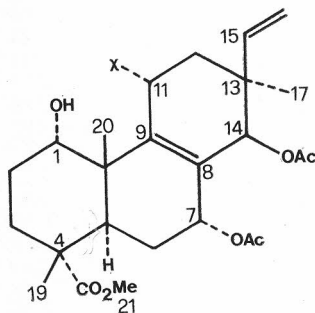
Institute for Chemistry, Technology and Metallurgy, Belgrade

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Identification of a novel diterpene (1 α -hydroxy-7 α ,11 α ,14 β -triacetoxy- $\Delta^{8(9),15}$ -pimaric acid methyl ester) from plant species *Lycopus Europaeus* by means of two-dimensional ^1H homonuclear correlated NMR spectroscopy (COSY) is reported.

INTRODUCTION

In the course of our studies concerning the constituents of Yugoslav plant species, a crystalline diterpene exhibiting a pimara-8(9),15-diene structure (I, Scheme 1) was isolated from the chloroform extract of dried *Lycopus Europaeus* (the whole plant) by means of silica gel column chromatography.



I : X = H

II : X = OAc

Scheme 1

* Dedicated to Professor Mihailo Lj. Mihailović on the occasion of his 60th birthday.

** For part I see ref. 1.

Its structure elucidation, using 1D NMR (^1H 500 MHz and ^{13}C 125.7 MHz) and 2D NMR (COSY, NOESY and ^1H - ^{13}C J-resolved) spectroscopy, was reported elsewhere.¹ As a continuation of this work, an additional crystalline colourless compound (II) was isolated from the fraction eluted before that containing I. In order to determine the structure of II, the spectroscopic study (including IR, MS, 1D ^1H NMR and 2D/COSY NMR) of this molecule was undertaken.

TABLE I
MS^a and IR Data of II

MS/EI, m/z (%)	M (absent), 386 ($\text{C}_{23}\text{H}_{30}\text{O}_5$, M-2 \times 60, 19), 378 (M-60—68 ^b , 15), 344 (M-2 \times 60—42, 48), 326 (M-3 \times 60 or 344—18, 32), 311 (326—15, 23.5), 276 (344—68 ^b , 62), 267 (326—59, 37), 183 (48), 43 (100)
IR, $\nu_{\text{max}}^{\text{KBr}}$, cm^{-1}	ca. 3530 (OH), 1750, 1720, 1240 (OAc, CO_2Me)

^a The origin of ions (in parentheses) is not verified (by metastables or precise mass measurement).

^b 68 mass units correspond to $\text{CH}_2=\text{C}(13)-(\text{CH}_3)\text{CH}=\text{CH}_2$ moiety.

TABLE II
 ^1H 500 MHz NMR (CDCl_3) Chemical Shifts^a and Multiplicity^b of II and I

Proton ^c	(II)	(I) ^d
H-1 β	3.87 (m, w h/2 ~7.5)	3.97 (m, w h/2 ~7.5)
H-2 α	ca. 1.66 ^e	1.75 (dddd, 15, 3.5, 3.5, 3)
H-2 β	ca. 1.95 ^e	ca. 1.95 ^e
H-3 α	2.26 (ddd, 13.5, 13.5, 3.5)	2.24 (ddd, 13.5, 13.5, 3.5)
H-3 β	ca. 1.42	1.43 (ddd, 13.5, 3.5, 3.5)
H-5 α	2.67 (dd, 13, 2)	2.73 (dd, 13, 2)
H-6 α	ca. 1.46 ^e (fd, 13)	ca. 1.46 ^e (fd, 13)
H-6 β	ca. 2.0 ^e	1.83 (ddd, 13, 13, 4)
H-7 β	5.20 (fd, ~4)	5.09 (fm, w h/2 ~8.5)
H-11 α		2.17 (ddd, 18, ~8, ~8)
H-11 β	6.07 (dd, 6, 2.5)	2.49 (ddd, 18, 6, 4)
H-12 α	ca. 1.61 ^e	ca. 1.54 ^e
H-12 β	2.33 (dd; 15, 6)	ca. 1.97 ^e
H-14 α	5.24 (s, w h/2 ~3.5)	5.12 (s, w h/2 ~3.5)
H-15	5.77 (dd, 17, 10)	5.82 (dd, 17, 10)
=CH ₂ (16)	{5.02 (dd, 10, 2) 4.99 (dd, 17, 2)}	{5.03 (dd, 10, 2) 5.04 (dd, 17, 2)}
Me-17	1.07 (s)	0.92 (s)
Me-19	1.22(s)	1.21 (s)
Me-20	1.15 (s)	1.06 (s)
Me-21	3.64 (s)	3.63 (s)
Me (OAc)	1.98, 1.99, 2.07 (3 \times s)	1.98, 1.99 (2 \times s)

^a Refer to TMS as internal standard. ^b Couplings (Hz) are obtained by the first-order analysis. ^c Assignments are based on the characteristic chemical shifts, couplings and 2D ^1H NMR data (COSY). ^d Comparison of the ^1H NMR (CDCl_3) data of I to those previously measured in C_6D_6 (see ref. 1) revealed pronounced solvent shifts for the majority of protons. ^e Overlapping with other signals.

Abbreviations used: s — singlet, d — doublet, fd — doublet with fine structure and m — multiplet.

RESULTS AND DISCUSSION

The spectral data of *II* (listed in Tables I and II), revealing the same main structural features as in *I* (i. e. three tertiary methyls, a methyl ester group, an endocyclic tetrasubstituted and a monosubstituted isolated double bond), indicate that both compounds are in the same diterpene group.

While compound *I* exhibited three oxygen functions, i. e. 1α -hydroxy- $7\alpha,14\beta$ -diacetoxyl substitution pattern, the ^1H NMR spectrum of *II* revealed three secondary acetoxy groups and a secondary hydroxyl (see Table II). The acetoxy group associated with one-proton singlet (δ 5.24) in the ^1H NMR spectrum of *II* could be assigned to C(14)HOAc (as in *I*).

The COSY spectrum of *II* (Figure 1), showing the complete interproton coupling network, enabled spectral assignment and identification of three independent structural units (as far as the couplings are concerned). These subunits (*a*, *b* and *c*), together with the corresponding fragments previously identified (using the same method)¹ in *I*, are shown in Scheme 2:

The subunits *a*, *b* and *c* fit very well into the rings A, B and C, respectively, of the pimara-8(9),15-diene skeleton. As evident from Scheme 2, the COSY spectra clearly indicate the same substitution pattern of A/B rings in both compounds. This is also supported by the similarity of the ^1H NMR chemical shift and multiplicity data referring to A/B rings in these compounds (see Table II), e. g. one-proton doublets of doublets ($J_{ac} \sim 2$ and $J_{aa} \sim 13$ Hz) at δ ca. 2.7 in both compounds (typical of the axial H-5 α in the *trans*-fused A/B rings) and an unresolved multiplet ($wh/2 \sim 7.5$ Hz) at δ 3.97 and 3.87 in *I* and *II*, respectively, (assigned to equatorial H-1 β). At the same time, small (vicinal) coupling of C(7)HOAc in *II* (δ 5.20, fd, $J \leq 4$ Hz), typical of J_{ca} and J_{cc} , are in a good agreement with the pseudoaxial position (i. e. 7 α) of this acetoxy (the same as in *I*). The signal of the same proton in the NMR spectrum of *I* (δ 5.09, fm, $wh/2 \sim 8.5$ Hz) exhibited a more complex pattern, due to an additional small coupling assigned (according to the COSY spectrum)* as $^5J_{7\beta,11\alpha}$ (homoallylic). The absence of this coupling in *II* indicates that in this compound the remaining acetoxy group was attached to 11 α -position (as shown in Scheme 1). The structural and stereochemical analogy of the A/B rings in *II* to those in *I* is also verified by the occurrence of a long-range »W« coupling (typical of *trans*-1,2-diaxial Me, H arrangement)² relating to Me-19 and H-3 α in both compounds, thus indicating the same relative configuration at C(4) (i. e. axial 4 β -Me) in *I* and *II*.

The most striking difference between the structures of *I* and *II* was shown (according to the COSY spectra*, see Figure 1 and Scheme 2) to be in ring C, which contains four and three strongly coupled protons (in *I* and *II*, respectively). While in *I* this group of protons absorbs within a relatively narrow highfield portion of the spectrum (i. e. δ 2.5—1.5), in compound *II* the resonances concerning only a pair of protons from ring C (assigned as geminal, according to strong mutual coupling of ca. |15 Hz|) could be observed in this spectral region. One of these protons (resonating at δ 2.33) was assigned as H-12 β on the basis of its long-range »W« coupling to Me-17 (similar to that relating to Me-19, H-3 α). According to the same evidence, the pseudoaxial 13 α -geometry (corresponding to the pimara series)** was assigned to Me-17.

* For the COSY spectrum of *I* see ref. 1.

** 13 β -Me,13 α -vinyl: isopimara series.

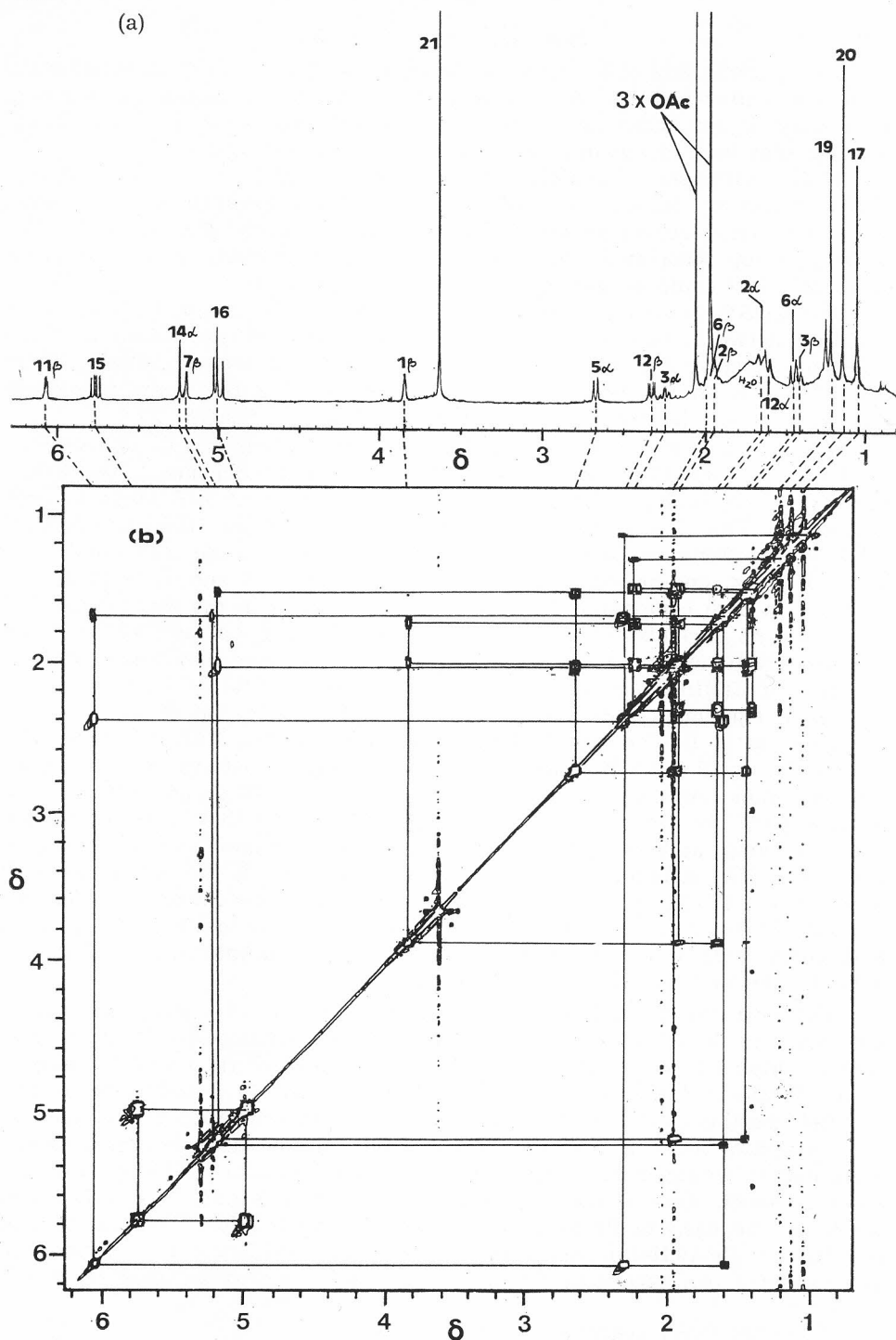
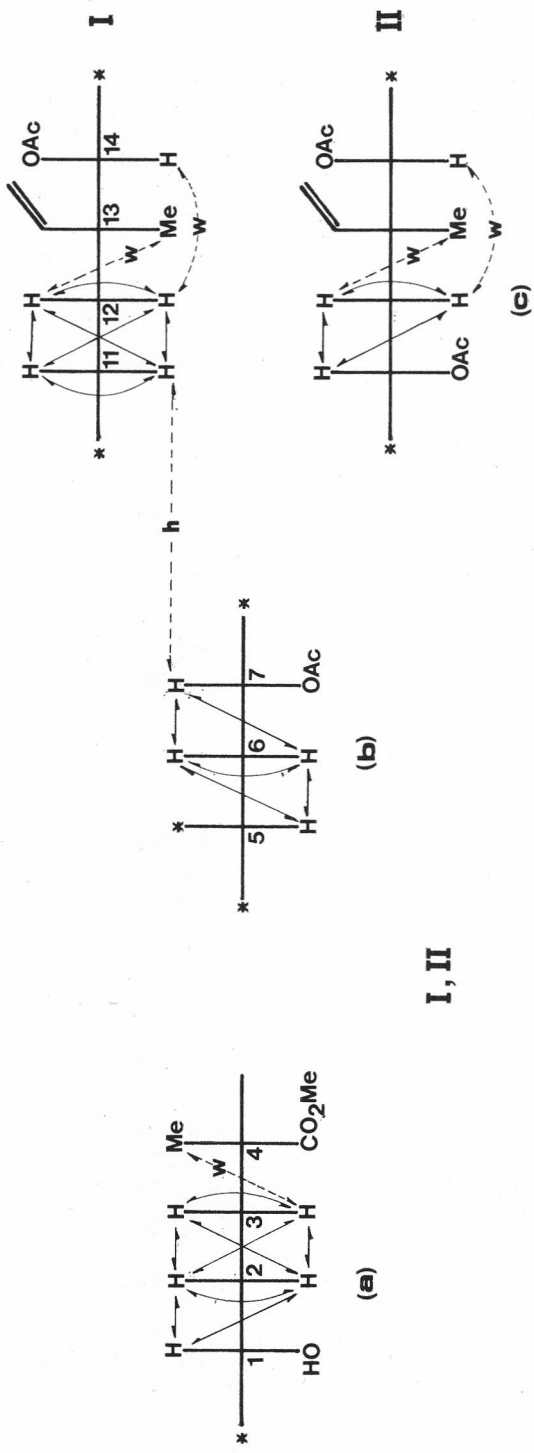
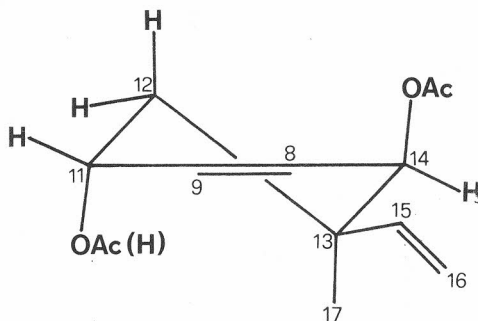


Figure 1. ¹H 500 MHz NMR spectra of *II* (4 mg in 0.5 ml of CDCl₃); (a) 1D NMR spectrum; (b) Homonuclear correlated 2D NMR (COSY) spectrum



Scheme 2

The identification of the remaining signal (δ 1.61) originating from this methylene group, as pseudoequatorial H-12 α , was evident from the long-range »W« coupling of this proton to C(14)HOAc, which is characteristic of the 1,3-positioned diequatorial hydrogens.² This also proved the pseudoaxial 14 β -OAc geometry. The same »W« couplings (i. e. H-12 β ,Me-17 and H-12 α ,H-14 α) were also observed previously in compound *I*, in which 13 α -Me,14 β -OAc configuration was also proved by means of ¹³C NMR and 2D NOE NMR data.¹ Finally, the low-field doublet of doublets from proton α -to the OAc group (δ 6.07) also belonging (according to the COSY spectrum of *II*) to ring C, could be assigned, according to vicinal couplings ($J_1 \sim 2.5$ and $J_2 \sim 6.0$ Hz) to H-11 β . This (11 α -OAc) geometry is also supported by the evidence from the COSY spectrum (quoted above) regarding the absence of homoallylic coupling of H-7 β in *II*, contrary to *I* in which this type of coupling was shown to occur between H-7 β and H-11 α .



Scheme 3

The proposed twisted-chair conformation of ring C (with pseudoaxial 13 α -Me) similar to that in *I* and *II* (Scheme 3) was also proposed previously (on the basis of ¹³C NMR data)³ for the related pimara-8(9),15-dienes.

EXPERIMENTAL

Melting point (uncorrected) of compound *II* was taken on a Boetius PHMK apparatus. IR spectrum of the solid sample was recorded in a KBr disc with a Perkin-Elmer 457 Grating IR spectrophotometer. Low-resolution mass spectrum (EI) was measured on a Varian MAT CH-5 spectrometer at 70 eV; the solid sample was introduced directly into the ion source (150°) using a direct introducing probe (140°). ¹H 500 MHz NMR (CDCl₃) spectra (1D and 2D) of *I* and *II* were recorded on a Bruker WM-500 instrument equipped with an Aspect 2000 computer.

Homonuclear correlated 2D NMR spectrum (COSY) was measured using a standard 90°—t₁—90°—t₂ scheme⁴ with a quadrature detection in both frequency domains.⁵ Data were processed in the usual way⁶ and presented as contour plots of the absolute value spectrum (Figure 1). A 512 × 2048 data points spectrum were acquired with 3.1 Hz/Pt resolution 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 of Belgrade).

Isolation of dierpene *II* — Chloroform extraction of *Lycopus Europaeus* (2.2 kg of dried whole plant) and silica gel column chromatography (eluting with benzene/diethyl ether) of the concentrated extract (worked up in the usual manner)⁷ was reported in the preceding paper¹ dealing with isolation of *I* (132 mg).

Compound II (6 mg of colourless crystals, m. p. 121°) was isolated as ether insoluble portion of the fraction eluted (with 10% Et₂O in C₆H₆) just before that containing the major compound I.

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IZVOD

Novi diterpen iz *Lycopus Europaeus*

S. Milosavljević, D. Jeremić, S. Macura i V. Vajs

Opisana je identifikacija novog diterpena (metil estra 1 α -hidroksi-7 α ,11 α ,14 β -triacetoksi $\Delta^{8(9),15}$ -pimarinske kiseline) iz biljke *Lycopus Europaeus* pomoću dvodimenzionalne ¹H homonuklearne korelisane NMR (COSY) spektroskopije.