Pergamon

Tetrahedron 55 (1999) 7375–7388

TETRAHEDRON

Diterpenoids from *Lycopus europaeus* and *Nepeta septemcrenata*: Revised Structures and New Isopimarane Derivatives[§]

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Received 26 February 1999; revised 30 March 1999; accepted 15 April 1999

Abstract: Seven isopimarane diterpenoids have been isolated from *Nepeta septemcrenata* (**3**) and *Lycopus europaeus* (**4-9**), both species belonging to the Labiatae family. Compound **3** had been reported as the sole diterpene constituent of *N. septemcrenata*, whereas the physical and spectroscopic data of **4** were identical to those of a compound previously isolated from *L. europaeus* to which structure **1** had been attributed. As a result of exhaustive spectroscopic studies and some chemical correlations between **3** and **4**, we definitely conclude that structure **1** must be amended to **4**. In addition, the structures of five new diterpenoids found in *L. europaeus* (**5-9**) were established by chemical and spectroscopic means, and a *normal* isopimarane absolute configuration for all the isolated compounds, except for **7**, was supported by CD data, application of the Horeau's method and chemical correlations.

Keywords: Terpenes; isopimaranes; stereochemistry; X-ray crystal structure.

Introduction

Previous works on the constituents of *Lycopus europaeus* L. (Labiatae) reported the isolation of two diterpenoids, suggesting a pimarane-type structure (**1** and **2**) for these substances on the basis of ^{1}H and ^{13}C NMR spectroscopic data.[1,2] Recently, Voehler and co-workers[3] isolated from *Nepeta septemcrenata* Ehrenb. (Labiatae) an isopimarane derivative whose structure (**3**, except for the absolute configuration) was rigorously established by spectroscopic studies. These authors indicated[3] that "surprisingly, although the proposed configurations at C-13 and C-14 in **3** and **1** are different, their ^{13}C NMR data are very close"; however, no additional results concerning this point have been published hitherto. In view of these facts, it was obvious that some aspects of the reported structures **1**[1] and **3**[3] needed to be reexamined.

[§] Dedicated to Prof. Dr. Manuel Lora-Tamayo on the occasion of his 95th birthday.

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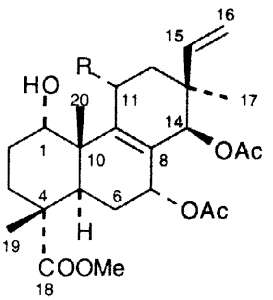
0040-4020/99/$ - see front matter
PII: S0040-4020(99)00361-0

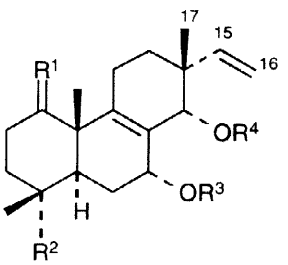
Results and discussion

A reinvestigation of the acetone extract of the aerial parts of *Nepeta septemcrenata* allowed us to isolate **3** as the sole detectable diterpene constituent of this plant, thus confirming previous results.[3] On the contrary, we have found in the acetone extract of *Lycopus europaeus* five new isopimarane derivatives (**5-9**) together with another diterpenoid (**4**), which showed identical ^1H and ^{13}C NMR spectra (Tables 1[4] and 2, respectively) that those reported[1] for **1**. However, attempts at isolating the other pimarane constituent[2] (**2**) from our extract of *L. europaeus* were unsuccessful. The variation observed in the diterpene content of *L. europaeus* could be attributed to the fact that the plant material used for the first study[1,2] came from Central Europe (near Belgrade, Yugoslavia) whereas our material was collected near Madrid, Spain. In the case of *N. septemcrenata*, in which no variation of the diterpene composition was observed, the plant materials extracted in both studies (reference 3 and this work) were collected in the same place (South Sinai, Egypt).



1 R=H

2 R=OAc



	R^1	R^2	R^3	R^4
3	α-OH,β-H	CH_2OAc	Ac	Ac
4	α-OH,β-H	COOMe	Ac	Ac
5	α-OAc,β-H	COOMe	Ac	Ac
6	α-OAc,β-H	COOMe	Ac	H
10	O	CH_2OAc	Ac	Ac
11	α-OH,βH	CH_2OH	H	H
12	α-OAc,β-H	CH_2OAc	Ac	Ac

The ^1H and ^{13}C NMR and NOESY spectra of **3** (Tables 1, 2 and 3, respectively) obtained by us for this work were in complete agreement with previous results[3,5] regarding its isopimarane-type hydrocarbon skeleton. Furthermore, a single-crystal X-ray determination of **3** was undertaken in order to elucidate its structure conclusively. Figure 1 shows the result of the X-ray analysis of **3**, thus confirming the previous deduction on the structure and relative stereochemistry of this compound (see reference 3 and above). Bond lengths and angles of **3** are in good agreement with those found in analogous compounds.[7,8] In the crystalline state, the conformation of ring A is a chair with endocyclic torsion angles between 53° and 57°, whereas ring B possesses a 1,2-diplanar form and ring C has a half-chair conformation, as can be seen by the values of the torsion angles[9] of **3** [C6-C7-C8-C9 -3.2(4)°; C7-C8-C9-C10 -3.3(4)°; C9-C8-C14-C13 28.3(4)°; C14-C8-C9-C11 -5.8(4)°; C8-C9-C11-C12 10.3(4)°].*

The previously unknown[3] absolute stereochemistry of **3** was established by application of the Horeau's method,[10] which defined as *S* the configuration of the C-1 stereogenic centre (see Experimental) and consequently, a *normal* isopimarane absolute configuration for this diterpenoid. This conclusion was also supported[11] by the negative Cotton effect ($\Delta\varepsilon_{303}$ -1.53) shown by the 1-keto derivative **10**, obtained by oxidation of **3**.

*Lists of atomic coordinates, thermal parameters, structure factors, bond lengths, bond angles and torsion angles are deposited as supplementary material at the Cambridge Crystallographic Data Centre.

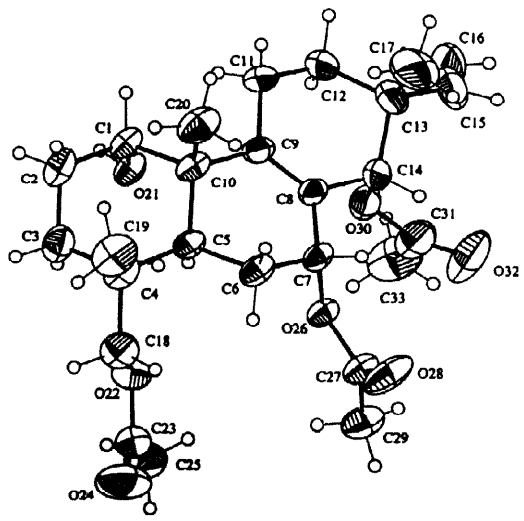


Figure 1. Molecular structure and conformation of **3**. Non-H atoms are represented by displacement ellipsoids at the 30% probability level and H atoms as spheres of an arbitrary radii.[6]

Acetic anhydride-pyridine treatment of diterpenoids **4** and **6**, isolated from *L. europaeus*, yielded the same peracetyl derivative **5**, another constituent of the plant. Combustion analysis and low resolution mass spectrometry established the molecular formula $C_{27}H_{38}O_8$ for **5** and its IR spectrum was devoid of any hydroxyl absorption. The ^1H and ^{13}C NMR spectra of this compound (**5**, Tables 1 and 2) were in agreement[1-3] with an 8,15-pimaradiene or isopimaradiene diterpene structure, possessing acetoxyl groups at the C-1α, C-7α and C-14α or β positions and an 18-carbomethoxyl group. NOE experiments (Table 3) established a *cis* spatial relationship between the H-11β, H-12β, H-14β, Me-17, Me-19 and Me-20 protons of **5**, only compatible with the isopimarane structure depicted in its formula.

Compounds **4** and **6** are the 1-deacetyl and 14-deacetyl derivatives of **5**, respectively, as was supported by their ^1H NMR spectra [Table 1: three acetates in **5**, only two in **4** and **6**; diamagnetic shift of H-1β (Δδ -1.4 ppm) in **4** and of H-14β (Δδ -1.58 ppm) in **6** with respect to **5**]. This conclusion was also in agreement with the HMBC spectra of **4** and **6**, which showed connectivities through three bonds between the carboxyl carbons of the acetates (δ 170.5 and 170.3, and δ 170.6 and 170.3 in **4** and **6**, respectively) and the H-7β and H-14β protons in **4** (δ 5.42 ddd and 5.47 br s), and the H-1β and H-7β protons in **6** (δ 4.91 t and 5.18 ddd, respectively).

Reduction of **5** with $LiAlH_4$ gave the derivative **11**, identical to the compound obtained by alkaline hydrolysis of **3** (see Experimental). Moreover, acetylation of **3** with Ac_2O-pyridine for 48 hours at room temperature gave the tetraacetyl derivative **12**, whereas treatment of the reduction product (**11**) of **5** with the same reagent during 12 hours at room temperature yielded **12** and minor quatities of another substance identical in all respects (mp, mixed mp, TLC, $[\alpha]_D$, ^1H NMR and mass spectra) with the natural diterpenoid (**3**) isolated from *N. septemcrenata*.

From all the above data, it was evident that the diterpenoids **4**, **5** and **6** found in *L. europaeus* possessed a *normal* isopimarane structure like **3**, and we definitely conclude, therefore, that structure **1**, previously assigned[1] to the diterpene isolated from this plant, must be amended to **4**.[12]

Another of the diterpenoids isolated from *L. europaeus* (compound **7**, $C_{25}H_{36}O_6$) showed ^1H and ^{13}C NMR spectra very similar to those of **5** (Tables 1 and 2) and the observed differences were consistent with the absence in **7** of the 1α-acetoxyl substituent of **5**. In particular, the almost identical chemical shifts for the C-4, C-6 - C-8 and C-11 - C-20 carbons in both compounds, as well as the up-field resonance of the C-1, C-2 and C-10 carbons [α- and β-effects, Δδ = δ(**7**) - δ(**5**), -37.4, -4.4 and -3.9 ppm, respectively] and the paramagnetic shifts of the C-3, C-5 and C-9 carbons (γ-effect, Δδ +6.0, +5.4 and +4.1 ppm, respectively) in **7** with respect to **5**,

Table 1. ^{1}H NMR Spectral data of compounds **3-9**[a]

H	3	4	5	6	7	8	9
1α					1.34 ddd	~1.67[b]	~1.67[b]
1β	3.94 m[c]	3.54 m[c]	4.94 t	4.91 t	~1.78[b]	1.88 ddd	1.90 ddd
2α	1.68 dddd	~1.36[b]	~1.87[b]	~1.84[b]	~1.63[b]	~1.65[b]	~1.65[b]
2β	1.93 dddd	1.60 dddd	~1.87[b]	~1.84[b]	~1.63[b]	~1.65[b]	~1.65[b]
3α	1.84 ddd	2.35 ddd	~1.82[b]	~1.80[b]	~1.74[b]	~1.74[b]	~1.74[b]
3β	1.18 dt	1.34 ddd	1.48 ddd	1.51 ddd	1.58 ddd	~1.58[b]	~1.57[b]
5α	2.15 dd	3.06 dd	2.78 dd	2.72 dd	2.34 dd	2.39 dd	2.37 dd
6α	1.76 m	1.78 ddd	1.43 ddd	1.41 ddd	1.41 ddd	1.41 ddd	1.45 ddd
6β	1.76 m	1.64 ddd	1.77 ddd	~1.78[b]	1.81 ddd	1.80 ddd	1.82 ddd
7β	5.15 br dd	5.42 ddd	5.06 ddd	5.18 ddd	5.08 ddd	5.11 dd	5.14 dd
11α	2.48 ddd	2.40 ddd	~1.92[b]	~1.95[b]	2.29 ddd		
11β	2.15 ddd	1.87 dddd	2.04 dddd	~1.85[b]	2.06 dddd	4.27 ddd[d]	4.26 ddd[e]
12α	1.96 ddd	2.05 ddd	1.78 ddd	~1.75[b]	1.90 ddd	2.22 dd	2.26 dd
12β	1.51 ddd	1.43 dddd	~1.47[b]	1.39 ddd	1.47 dddd	1.74 dd	1.56 dd
14β	5.09 br s[f]	5.47 br s[f]	5.16 br s[f]	3.58 br s[f]	5.06 br s[f]	5.21 s	3.63 s
15	5.79 dd	6.11 dd	5.80 dd	5.89 dd	5.78 dd	5.88 dd	6.07 dd
16A	5.00 dd[g]	5.18 dd[g]	5.00 dd[g]	5.05 dd[h]	4.98 dd[g]	5.10 dd[g]	5.16 dd[g]
16B	5.02 dd[h]	5.20 dd[h]	5.04 dd[h]	5.07 dd[g]	4.99 dd[h]	5.18 dd[h]	5.18 dd[h]
Me-17	0.90 s	0.91 s	0.89 s	0.87 s	0.87 s	0.93 s	0.88 s
18A	3.68 d						
18B	3.83 d						
Me-19	0.89 s	1.23 s	1.20 s	1.18 s	1.17 s	1.17 s	1.17 s
Me-20	1.05 s	0.69 s	1.05 s	1.03 s	1.01 s	0.96 s	0.93 s
1α-OH[i]	1.35 d	0.92 br s					
11α-OH[i]						2.54 d	2.62 d
OAc	2.04 s	2.00 s	2.08 s	2.05 s	1.96 s	2.00 s	2.07 s
	1.96 s	1.87 s	2.00 s	2.03 s	1.95 s	1.99 s	
	1.94 s		1.95 s				
COOMe		3.44 s	3.62 s	3.61 s	3.59 s	3.61 s	3.61 s
$J_{H,H}$ (Hz)							
1α,1β					12.2	12.6	12.4
1α,2α					6.0	[b]	[b]
1α,2β					8.3	[b]	[b]
1β,2α	3.2	[b]	2.8	2.8	[b]	3.6	3.7
1β,2β	3.2	2.2	2.8	2.8	[b]	2.4	2.0
2α,2β	13.6	14.9	[b]	[b]	[b]	[b]	[b]
2α,3α	3.2	4.1	[b]	[b]	[b]	[b]	[b]
2α,3β	3.2	2.5	3.0	3.0	3.4	[b]	[b]
2β,3α	12.5	13.5	[b]	[b]	[b]	[b]	[b]
2β,3β	3.2	4.4	2.8	2.9	2.4	[b]	[b]
3α,3β	12.5	13.5	12.8	12.9	12.9	[b]	[b]
5α,6α	2.2	2.1	1.9	2.0	2.0	2.0	1.8
5α,6β	10.0	13.1	13.2	13.2	13.2	13.2	13.2
6α,6β	0	14.5	14.7	14.6	14.5	14.8	14.8
6α,7β	2.8	2.0	2.2	2.3	2.2	1.6	1.7
6β,7β	5.4	4.2	4.4	4.4	4.6	4.5	4.5
11α,11β	18.4	17.4	18.2	[b]	18.8		
11α,12α	6.6	6.5	6.1	[b]	6.8		
11α,12β	4.0	4.5	[b]	3.4	3.2		
11β,12α	12.0	8.8	9.2	[b]	9.3	2.2	1.5
11β,12β	2.1	2.1	2.3	2.1	2.0	6.3	6.3
12α,12β	11.8	13.0	13.3	14.8	13.2	14.9	15.2
15,16A	11.0	11.1	11.0	17.5	10.7	11.0	10.8
15,16B	17.4	17.4	17.7	11.2	17.8	17.7	17.7
16A,16B	1.2	1.5	1.4	1.3	1.2	1.0	1.0
18A,18B	11.1						
7β,11β	<0.5	2.0	2.2	2.3	2.1	0	0
12β,14β	<0.5	1.0	<0.5	<0.5	1.1	0	0
1β,OH[i]	3.1	<0.5					
11β,OH[i]						11.1	3.4

[a]At 400 MHz, except for **3** and **7** (500 MHz), all in $CDCl_3$ solution, except for **4** which was recorded in C_6D_6 solution. Chemical shifts (δ values) are reported with respect to the signal of residual $CHCl_3$ (δ 7.25). All these assignments were in agreement with COSY, TOCSY, HMQC and ROESY spectra. [b]Overlapped signal; approximate δ values were measured from the HMQC spectrum. [c]$W_{1/2}$ = 7.5 Hz. [d]Collapsed into a dd (J = 6.3, 2.2 Hz) after addition of D_2O. [e]Collapsed into a dd (J = 6.3, 1.5 Hz) after addition of D_2O. [f]$W_{1/2}$ = 3 Hz. [g]This is the *cis*-hydrogen with respect to H-15. [h]This is the *trans*-hydrogen with respect to H-15. [i]This signal disappeared after addition of D_2O.

Table 2. ^{13}C NMR Spectral data of compounds **3-9**[a]

C	3	4	5	6	7	8	9
1	70.4 d	70.2 d	72.9 d	72.9 d	35.5 t	34.7 t	34.2 t
2	24.4 t	24.5 t	22.6 t	21.6 t	18.2 t	18.0 t	18.2 t
3	28.2 t	29.4 t	30.1 t	29.9 t	36.1 t	36.3 t	36.3 t
4	35.8 s	46.6 s	46.4 s	46.2 s	47.2 s	47.1 s	47.1 s
5	33.6 d	34.0 d	35.3 d	35.4 d	40.7 d	40.6 d	41.0 d
6	25.5 t	27.6 t	29.2 t	28.2 t	28.2 t	28.0 t	27.9 t
7	71.2 d	71.1 d	70.3 d	72.0 d	72.1 d	69.9 d	71.9 d
8	125.5 s	125.9 s	124.9 s	127.5 s	122.1 s	127.3 s	131.1 s
9	148.6 s	148.1 s	147.2 s	146.3 s	151.3 s	152.0 s	151.9 s
10	43.8 s	43.5 s	42.1 s	41.7 s	38.2 s	38.1 s	37.4 s
11	20.3 t	20.2 t	20.2 t	20.0 t	20.5 t	63.1 d	62.8 d
12	27.5 t	27.5 t	27.5 t	27.5 t	26.9 t	41.6 t	41.1 t
13	39.0 s	39.0 s	39.2 s	39.4 s	38.9 s	40.8 s	42.1 s
14	76.0 d	76.0 d	76.3 d	76.5 d	75.9 d	76.8 d	76.4 d
15	143.4 d	143.2 d	142.6 d	143.5 d	143.7 d	143.4 d	145.2 d
16	113.0 t	113.1 t	113.2 t	113.5 t	112.8 t	114.5 t	113.7 t
17	21.9 q	22.0 q	21.8 q	21.6 q	21.7 q	26.6 q	26.9 q
18	72.3 t	177.9 s	177.8 s	177.8 s	178.2 s	178.2 s	178.2 s
19	17.4 q	16.5 q	16.5 q	16.5 q	16.6 q	16.4 q	16.3 q
20	19.7 q	19.4 q	18.8 q	18.8 q	19.0 q	18.9 q	17.9 q
OAc	170.8 s	170.5 s	170.62 s	170.6 s	170.4 s	170.4 s	170.8 s
	170.5 s	170.3 s	170.57 s	170.3 s	170.3 s	169.6 s	21.4 q
	170.3 s	21.5 q	170.46 s	21.4 q	21.3 q	21.2 q	
	21.2 q	21.4 q	21.21 q	21.2 q	21.2 q	21.1 q	
	21.2 q		21.18 q				
	20.9 q		21.09 q				
COOMe		51.9 q	51.9 q	51.9 q	51.8 q	51.8 q	51.8 q

[a]At 100 MHz, except for **3** and **7** (125.7 MHz), in $CDCl_3$ solution. Chemical shifts (δ values) are reported with respect to the solvent signals (δ 77.00). All these assignments were in agreement with HMQC and HMBC spectra.

Table 3. Significant NOE data for compounds **3**, **5**, **7**, **8** and **14**[a]

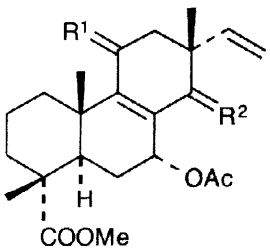
Compound	Observed proton(s)	Observed NOE cross peak with protons
3	H-14β	H-7β, Me-17
	Me-20	H-1β, H-7β, H-11β, Me-19
5	Me-17	H-11β, H-12β, H-14β, H-15, Me-20
	Me-19	H-6β, Me-20
	Me-20	H-1β, H-11β, Me-17
7	Me-17	H-11β, H-12β, H-14β, H-15, Me-20
	Me-19	H-2β, H-6β, Me-20
	Me-20	H-6β, H-7β, H-11β, Me-17, Me-19
8	H-11β	H-1β, H-12α, H-12β, H-14β, Me-17, Me-20
	Me-17	H-12β, H-14β, H-15
	Me-20	H-1β, H-6β, H-7β, H-11β, Me-19
14	H-11α	H-1α, H-1β, H-12α, H-12β, H-15

[a]All these data were obtained from the NOESY spectra and, in some cases (**3**, **5** and **8**), also by 1D NOE experiments.

unambiguously established that the former was the 1-deacetoxy derivative of the latter. The NOESY spectrum of **7** (Table 3) further supported an isopimarane-type structure for this compound.

The absolute configuration of **7** was not ascertained. However, on biogenetic grounds, we suppose that this diterpenoid belongs to the *normal* series, like the other isopimaranes (**4-6**, **8** and **9**) co-occurring in the same plant.

The IR spectrum of diterpenoid **8** ($C_{25}H_{36}O_7$) showed hydroxyl absorption (3530 cm^{-1}) and its ^1H and ^{13}C NMR and NOESY spectra (Tables 1-3, respectively) were consistent with an 8,15-isopimaradiene structure having acetoxyl groups at the C-7α and C-14α positions and an 18-carbomethoxy function, identical to those of **7**. In addition, **8** possessed a secondary hydroxyl group at the C-11 position [geminal proton at δ 4.27 ddd, J = 11.1, 6.3, 2.2 Hz, hydroxyl proton at δ 2.54 d, J = 11.1 Hz (interchangeable with D_2O); δ_C 63.1 d] because it yielded the α,β-unsaturated ketone derivative **13** after oxidation [**13**: λ_{max} 237 nm, log ε 3.91; δ_C 197.5 s (C-11), 147.9 s (C-9), 143.5 s (C-8); C-12 methylene protons at δ 2.89 d and 2.21 dd, J_{gem} = 15.9 Hz, $J_{12\beta,14\beta}$ = 1.2 Hz (Table 4)]. The 11α-configuration of the secondary hydroxyl group was in agreement with NOE experiments (Table 3), which showed NOE enhancement in the signals of the H-14β, Me-17 and Me-20 protons, among others, when the H-11β proton (δ 4.27) was irradiated.



	R1	R2
7	H,H	α-OAc,β-H
8	α-OH,β-H	α-OAc,β-H
9	α-OH,β-H	α-OH,β-H
13	O	α-OAc,β-H
14	α-H,β-OH	α-OAc,β-H
15	α-OAc,β-H	α-OAc,β-H
16	O	O

The positive Cotton effect ($\Delta\varepsilon_{262}$ +1.09) shown by **13** suggested[13] a *normal* isopimarane absolute configuration for **8**, although application of the Horeau's method[10] to this diterpenoid did not provide a reliable result (see Experimental), probably due to strong steric hindrance around the C-11α position. However, when the Horeau's method was carried out with the 11β-epimer of **8** (compound **14**, obtained by treatment of **13** with $NaBH_4$, see Experimental) an 11*S* absolute stereochemistry was established for this asymmetric centre in **14**, and consequently a *normal* isopimarane absolute configuration for **8**.

The 14-deacetyl derivative of **8** was also isolated from the acetone extract of *L. europaeus* (compound **9**, $C_{23}H_{34}O_6$, for its ^1H and ^{13}C NMR spectra see Tables 1 and 2). This compound had its acetoxyl group at the C-7α position, because the HMBC spectrum showed correlation between the carboxyl carbon of the acetate (δ 170.8 s) and the H-7β proton (δ 5.14 dd, J = 4.5 and 1.7 Hz). Acetic anhydride-pyridine treatment of **8** and **9** yielded the same derivative (**15**, $C_{27}H_{38}O_8$). Thus, **9** had the structure depicted in its formula, including the absolute configuration. The presence in **9** of hydroxyl groups at the C-11 and C-14 positions was also supported by oxidation of this diterpenoid to the 11,14-diketo derivative **16**, a compound with typical UV absorptions of an enedione chromophore in the homo-*cisoid* form[14] (λ_{max} 244 and 365 nm, log ε 3.84 and 1.80, respectively).

The amendment of structure **1**[1] for one of the diterpene constituents of *L. europaeus* (correct structure **4**) is interesting from both biogenetic and taxonomic points of view, because pimarane-type diterpenes, excluding **2**,[2,12] have not been found in plants belonging to the Labiatae family.[15]

Experimental section

General experimental procedures.

Mps were determined on a Kofler-type block and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. IR spectra (KBr disk) were obtained on a Perkin-Elmer 681 spectrophotometer. UV spectra were recorded on a Perkin-Elmer Lambda 2 UV/VIS spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Varian INOVA-400 or Varian Unity-500 instrument, at 400 or 500 MHz (^1H) and at 100 or 125.7 MHz (^{13}C), respectively. MS were recorded in the positive EI mode on a Hewlett-Packard HP5989A instrument (70 eV), and no fragments below *m/z* 50 were registered, except for **9**, **12-14** and **16**. Elemental analyses were made with a Carlo Erba EA 1108 apparatus. The purity of the compounds was checked by TLC on precoated plates (Merck, Si gel $60F_{254}$). Merck Si gel No. 7734 (70-230 mesh) deactivated with 10% H_2O, w/v, was used for column chromatography.

Plant materials.

Aerial parts of *Nepeta septemcrenata* Ehrenb. were collected in August 1997 in South Sinai, Egypt, and voucher specimens were deposited in the Herbarium of the Department of Pharmaceutical Sciences, National Research Centre, Cairo, Egypt. *Lycopus europaeus* L. was collected in June 1998, near Navalagamella (Perales river), Madrid province, Spain, and voucher specimens were deposited in the Herbarium of the Royal Botanic Garden, Madrid.

Extraction and isolation of 7α,14α,18-triacetoxy-8,15-isopimaradien-1α-ol (**3**) *from* Nepeta septemcrenata.

Dried and finely powdered aerial parts of *N. septemcrenata* (700 g) were extracted with Me_2CO (5 L x 2) at room temperature for 6 days. The extract (48 g) was chromatographed on a Si gel column (500 g) eluted with petrol (bp 50-70 °C) and then with a petrol-EtOAc gradient from 10 to 100%. Elution with EtOAc-petrol (3:1) gave a fraction (6.8 g) which was rechromatographed [Si gel, 200 g, EtOAc-petrol (1:1) as eluent] yielding **3** (1.65 g, 0.23% on dry plant material): colourless fine needle crystals, mp 154-156 °C (EtOAc - *n*-hexane); $[\alpha]_D^{22}$ -51.2 (*c* 0.654, $CHCl_3$) (lit.[3] mp 148-150 °C). EIMS *m/z* (rel. int.): $[M]^+$ absent, 402 $[M\text{-}AcOH]^+$ (17), 360 (100), 319 (80), 264 (30), 251 (100), 241 (16), 201 (35), 195 (20), 185 (50), 157 (16), 143 (18), 105 (12), 55 (10). (Anal. Found: C, 67.37; H, 8.23%. $C_{26}H_{38}O_7$ requires: C, 67.51; H, 8.28%). IR, ^1H NMR (Table 1) and ^{13}C NMR (Table 2) spectra identical to those previously reported.[3,5]

Extraction and isolation of the diterpenoids from Lycopus europaeus.

Dried and powdered aerial parts of *L. europaeus* (950 g) were extracted with Me_2CO (4 L x 3) at room temperature for 7 days. The extract (43 g) was chromatographed on a Si gel column (800 g) eluted with petrol and a petrol-EtOAc gradient from 10% to 100%. Elution with petrol-EtOAc (9:1) yielded **7** (654 mg, 0.069% on dry plant material, less polar diterpenoid) and a mixture of **5** and **8** (3.6 g). This mixture was easily separated into its constituents by column chromatography over Si gel impregnated with $AgNO_3$ (10%, w/w) and petrol-EtOAc (4:1) as eluent, yielding **5** (2.8 g, 0.29%, less polar constituent of the mixture) and **8** (650 mg, 0.068%). The fractions eluted with petrol-EtOAc (3:1) yielded a residue (7.6 g) which was rechromatographed [Si gel column, 350 g, eluted with C_6H_6-EtOAc (3:1)] giving **6** (2.6 g, 0.27%), **4** (4.4 g, 0.46%) and **9** (200 mg, 0.021%) in order of increasing chromatographic polarity.

Methyl 7α,14α-diacetoxy-1α-hydroxy-8,15-isopimaradien-18-oate (**4**).

Colourless needles, mp 155-156 °C (EtOAc - *n*-hexane); $[\alpha]_D^{24}$ -53.2 (*c* 0.423, $CHCl_3$). IR, ^1H NMR (Table 1[4]), ^{13}C NMR (Table 2) and mass spectra identical to those reported for the diterpenoid previously isolated from *L. europaeus*[1] [lit.[1] mp 124 °C; $[\alpha]_D^{23}$ -48.1 ($CHCl_3$)].

Methyl 1α,7α,14α-triacetoxy-8,15-isopimaradien-18-oate (**5**).

Colourless needles, mp 129-131 °C (EtOAc - *n*-hexane); $[\alpha]_D^{24}$ -70.9 (*c* 1.746, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 3080, 1630, 910 (vinyl), 1730, 1250 (OAc), 1720 (COOMe), 1650 (tetrasubstituted olefin), 3000, 2940, 2870, 1450, 1370, 1110, 1060, 1020, 965, 940, 870, 850. ^1H NMR: Table 1. ^{13}C NMR: Table 2. EIMS *m/z* (rel. int.): $[M]^+$ absent, 430 $[M\text{-}AcOH]^+$ (4), 388 (9), 370 (5), 347 (9), 328 (5), 268 (10), 251 (100), 250 (40), 235 (28), 227 (12), 210 (11), 200 (10), 195 (27), 185 (13), 169 (11), 167 (17), 157 (10), 143 (13), 131 (8), 105 (8), 91 (6), 81 (5), 55 (8). (Anal. Found: C, 66.40; H, 7.89%. $C_{27}H_{38}O_8$ requires: C, 66.10; H, 7.81%).

Methyl 1α,7α-diacetoxy-14α-hydroxy-8,15-isopimaradien-18-oate (**6**).

Colourless flakes, mp 168-170 °C (petrol); $[\alpha]_D^{24}$ +18.9 (*c* 0.722, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 3580, 3460 (OH), 3080, 1630, 910 (vinyl), 1720 (OAc and COOMe), 1660 (tetrasubstituted olefin), 1240 (OAc), 3000, 2920, 1450, 1425, 1370, 1110, 1050, 1025, 1010, 940, 920, 850. ^1H NMR: Table 1. ^{13}C NMR: Table 2. EIMS *m/z* (rel. int.): $[M]^+$ absent, 403 $[M\text{-}H_2O\text{-}C_2H_3]^+$ (14), 388 $[M\text{-}AcOH]^+$ (3), 386 (8), 371 (7), 326 (15), 311 (23), 269 (24), 267 (34), 258 (24), 251 (76), 238 (27), 235 (28), 217 (70), 216 (75), 201 (100), 200 (28), 185 (36), 171 (36), 157 (25), 143 (26), 105 (27), 91 (21), 55 (22). (Anal. Found: C, 66.70; H, 8.28%. $C_{25}H_{36}O_7$ requires: C, 66.94; H, 8.09%).

Methyl 7α,14α-diacetoxy-8,15-isopimaradien-18-oate (**7**).

Colourless prisms, mp 158-160 °C (MeOH); $[\alpha]_D^{25}$ -106.5 (*c* 1.622, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 3080, 1630, 910 (vinyl), 1740, 1250 (OAc), 1720 (COOMe), 1650 (tetrasubstituted olefin), 3000, 2930, 2860, 1450, 1425, 1370, 1330, 1185, 1160, 1140, 1110, 1040, 1015, 960, 940, 850, 820, 780, 750, 735, 680, 660. ^1H NMR: Table 1. ^{13}C NMR: Table 2. EIMS *m/z* (rel. int.): $[M]^+$ absent, 372 $[M\text{-}AcOH]^+$ (30), 330 (98), 315 (25), 312 (22), 289 (89), 262 (28), 253 (32), 247 (100), 237 (91), 229 (30), 187 (23), 183 (23), 159 (24), 144 (56), 131 (42), 105 (22), 91 (20), 81 (16), 79 (13), 67 (10), 55 (19). (Anal. Found: C, 69.18; H, 8.27%. $C_{25}H_{36}O_6$ requires: C, 69.42; H, 8.39%).

Methyl 7α,14α-diacetoxy-11α-hydroxy-8,15-isopimaradien-18-oate (**8**).

Colourless needles, mp 159-161 °C (EtOAc - *n*-hexane); $[\alpha]_D^{24}$ -23.3 (*c* 0.381, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 3530 (OH), 3090, 1635, 930 (vinyl), 1720 br (OAc and COOMe), 1650 (tetrasubstituted olefin), 1250 (OAc), 3000, 2920, 2860, 1430, 1370, 1200, 1160, 1140, 1110, 1060, 1040, 1030, 980, 940, 820, 720. ^1H NMR: Table 1. ^{13}C NMR: Table 2. EIMS *m/z* (rel. int.): $[M]^+$ absent, 388 $[M\text{-}AcOH]^+$ (2), 347 (6), 328 (100), 313 (50), 285 (18), 278 (44), 269 (34), 260 (37), 253 (40), 251 (41), 235 (24), 225 (54), 201 (28), 200 (27), 160 (39), 145 (31), 131 (30), 121 (30), 105 (35), 91 (27), 81 (21), 55 (29). (Anal. Found: C, 67.10; H, 8.20%. $C_{25}H_{36}O_7$ requires: C, 66.94; H, 8.09%).

Methyl 7α-acetoxy-11α,14α-dihydroxy-8,15-isopimaradien-18-oate (**9**).

Colourless needles, mp 120-121 °C (EtOAc - *n*-hexane); $[\alpha]_D^{18}$ +49.3 (*c* 0.667, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 3350 (OH), 3080, 1635, 920 (vinyl), 1735, 1240 (OAc), 1715 (COOMe), 1665 (tetrasubstituted olefin), 2950, 1450, 1375, 1210, 1160, 1110, 1045, 1030, 1010, 960, 950, 850, 735. ^{1}H NMR: Table 1. ^{13}C NMR: Table 2. EIMS *m/z* (rel. int.): $[M]^+$ absent, 346 $[M-AcOH]^+$ (0.5), 331 (1), 330 (1), 328 (2), 287 (2), 278 (100), 203 (28), 185 (26), 159 (22), 145 (21), 131 (23), 121 (23), 105 (30), 91 (33), 81 (20), 79 (20), 67 (22), 55 (31), 43 (46), 41 (32). (Anal. Found: C, 68.02; H, 8.33%. $C_{23}H_{34}O_6$ requires: C, 67.95; H, 8.43%).

X-Ray crystallographic analysis of **3**.

A colourless prism of **3** (0.50 x 0.33 x 0.20 mm) was used for X-ray analysis. Crystal data: $C_{26}H_{38}O_7$, 462.56 $g.mol^{-1}$; orthorhombic, space group $P2_12_12_1$; cell constants 21.880(2), 12.687(1), 9.518(1) Å, from 85 reflections with θ up to 35°, Z = 4, volume 2642.1(4) $Å^3$, calculated density 1.163 $Mg.m^{-3}$. Data collection: Seifert XRD 3000S diffractometer; 4105 independent reflection intensities were collected, up to 66° in θ, in the ω/2θ scan mode, with CuK_α monochromated radiation (λ = 1.15418 Å). No decay was observed in two reference reflections measured every 90 min and 3353 reflections were considered at the 2σ(I) level.

The structure was solved by direct methods (SIR92)[16] and difference Fourier techniques; no absorption correction was applied (μ = 0.680 mm^{-1}). The structure was refined using full matrix least-squares on F^2. All non-H atoms were refined with anisotropic thermal parameters. The H-atoms were found in the difference Fourier map and treated using appropriate riding models. The refinement converged to R = 0.052. All calculations were done with the program SHELX93[17] on a VAX 6410 computer. All the geometric calculations were performed with the program PARST,[18] and scattering factors and anomalous dispersion were taken from the *International Tables for X-Ray Crystallography*.[19]

Application of Horeau's method to compound **3**.

This was performed in the usual manner.[10] Compound **3** (55.84 mg, 0.12086 mmol) and (±)-α-phenylbutyric anhydride (91.08 mg, 0.2938 mmol) in pyridine solution (2.00 mL) for 18 h at 20 °C: α_1 = -3.147, α_2 = -2.790; $\alpha_1 - 1.1\alpha_2$ = -0.078; configuration 1*S*.

Preparation of 7α,14α,18-triacetoxy-8,15-isopimaradien-1-one (**10**) *from compound* **3**.

To a solution of **3** (75 mg, 0.16 mmol) in Me_2CO (10 mL) was added an excess of Jones' reagent[20](0.5 mL) at 18 °C with stirring. After 15 min, the excess of Jones' reagent was destroyed by addition of EtOH and then the reaction mixture was diluted with H_2O (30 mL). Extraction with CH_2Cl_2 (4 x 10 mL) and work-up as usual gave a residue (70 mg) from which pure **10** (65 mg, 0.14 mmol, 87%) was obtained after chromatography [Si gel column, petrol-EtOAc (3:1) as eluent]: colourless needles, mp 199-200 °C (EtOAc - *n*-hexane); $[\alpha]_D^{19}$ -97.1 (*c* 0.573, $CHCl_3$); CD nm (Δε): 341 (0), 303 (-1.53), 250 (0), 227 (+1.14), 222 (0) (*c* 0.061, MeOH). IR (KBr) ν_{max} cm^{-1}: 3100, 1645, 910 (vinyl), 1750 br, 1730, 1710 (OAc and ketone), 1665 (tetrasubstituted olefin), 1250, 1240 (OAc), 2980, 2940, 2870, 1450, 1375, 1080, 1040, 950, 875. ^{1}H NMR: Table 4. ^{13}C NMR: Table 5. EIMS *m/z* (rel. int.): $[M]^+$ absent, 400 $[M-AcOH]^+$ (2), 341 (25), 340 (29), 307 (7), 280 (8), 267 (31), 262 (42), 249 (100), 211 (20), 209 (18), 208 (20), 181 (26), 169 (25), 105 (12), 91 (8), 55 (11). (Anal. Found: C, 67.61; H, 7.86%. $C_{26}H_{36}O_7$ requires: C, 67.80; H, 7.88%).

Table 4. ^{1}H NMR Spectral data of compounds **10-16**[a]

H	10	11	12	13	14	15	16
1α				1.12 ddd	1.43 ddd	~1.56[b]	~1.19[b]
1β		3.90 br s[c]	4.95 t	2.71 ddd	2.19 ddd	1.45 ddd	2.60 ddd
2α	2.60 ddd	~1.65[b]	~1.60[b]	~1.60[b]	~1.62[b]	~1.58[b]	~1.63[b]
2β	2.51 ddd	1.85 dddd	~1.85[b]	~1.65[b]	~1.62[b]	~1.58[b]	~1.72[b]
3α	1.72 ddd	~2.00[b]	~1.85[b]	~1.70[b]	~1.72[b]	~1.73[b]	~1.73[b]
3β	1.98 ddd	0.90 ddd	1.22 ddd	~1.60[b]	~1.58[b]	~1.59[b]	~1.62[b]
5α	2.28 dd	2.25 dd	2.21 t	2.20 dd	2.24 dd	2.50 dd	2.24 dd
6α	~1.81[b]	1.65 ddd	1.74 m	1.41 ddd	1.37 ddd	1.39 ddd	1.52 ddd
6β	~1.82[b]	1.72 ddd	1.74 m	1.76 ddd	~1.70[b]	1.81 ddd	1.69 ddd
7β	5.13 ddd	4.07 br dd	5.14 br dd	5.24 dd	5.16 dd	5.09 ddd	5.78 dd
11α	2.87 ddd	2.36 ddd	~2.00[b]		4.61 dd		
11β	2.13 dddd	~1.97[b]	~1.85[b]			5.42 td	
12α	~1.93[b]	~2.00[b]	~1.90[b]	2.89 d	2.27 dd	1.93 d	3.00 d
12β	1.46 ddd	1.40 ddd	1.49 ddd	2.21 dd	1.58 ddd	1.93 d	2.58 d
14β	5.09 br s[d]	3.55 br s[d]	5.16 br s[d]	5.35 d	5.17 br s[d]	5.27 s	
15	5.79 dd	6.06 dd	5.79 dd	5.75 dd	5.76 dd	6.00 dd	5.95 dd
16A	4.99 dd[e]	5.04 dd[f]	4.99 dd[e]	4.96 dd[f]	4.99 dd[f]	4.94 dd[f]	5.05 d[f]
16B	5.00 dd[f]	5.07 dd[e]	5.03 dd[f]	5.08 dd[e]	5.00 dd[e]	4.99 dd[e]	5.16 d[e]
Me-17	0.89 s	0.85 s	0.90 s	1.02 s	1.07 s	0.93 s	1.23 s
18A	3.68 d	2.89 d	3.71 d				
18B	3.93 d	3.53 d	3.85 d				
Me-19	1.02 s	0.70 s	0.89 s	1.19 s	1.20 s	1.16 s	1.19 s
Me-20	1.37 s	0.96 s	1.06 s	1.19 s	1.24 s	1.01 s	1.24 s
OAc	2.04 s		2.047 s	2.03 s	1.95 s	2.01 s	2.00 s
	1.95 s		2.045 s	2.00 s	1.94 s	2.00 s	
	1.92 s		2.038 s			1.97 s	
			2.037 s				
COOMe				3.60 s	3.59 s	3.61 s	3.60 s
$J_{H,H}$ (Hz)							
1α,1β				12.8	12.7	12.6	12.0
1α,2α				3.3	4.0	[b]	[b]
1α,2β				12.8	12.3	[b]	[b]
1β,2α		[b]	2.8	5.0	3.5	3.7	2.9
1β,2β		1.9	2.8	2.9	2.5	2.3	2.0
2α,2β	13.8	14.2	[b]	[b]	[b]	[b]	[b]
2α,3α	4.8	[b]	[b]	[b]	[b]	[b]	[b]
2α,3β	7.7	4.1	3.7	[b]	[b]	[b]	[b]
2β,3α	10.1	14.2	[b]	[b]	[b]	[b]	[b]
2β,3β	5.1	2.7	3.2	[b]	[b]	[b]	[b]
3α,3β	13.8	13.3	13.5	[b]	[b]	[b]	[b]
5α,6α	5.5	3.6	7.8	1.7	2.0	2.0	1.8
5α,6β	9.9	12.1	7.8	13.2	13.2	13.2	12.8
6α,6β	[b]	14.2	[b]	14.9	14.8	14.7	14.9
6α,7β	2.4	2.6	2.6	1.7	1.6	1.9	1.5
6β,7β	5.0	4.9	4.3	4.5	4.4	4.5	4.4
11α,11β	18.8	17.8	[b]				
11α,12α	6.1	6.1	[b]		6.1		
11α,12β	4.1	3.7	4.2		3.1		
11β,12α	11.0	[b]	[b]			5.0	
11β,12β	6.3	5.3	6.0			5.0	
12α,12β	12.9	12.9	13.2	15.9	14.2	0	15.4
15,16A	10.7	17.6	10.9	17.6	17.4	17.6	17.4
15,16B	17.8	11.0	17.6	10.9	11.1	11.0	10.6
16A,16B	1.2	1.5	1.3	0.7	1.1	1.2	0
18A,18B	11.2	11.8	11.0				
7β,11β	1.7	<0.5	<0.5			1.1	
12β,14β	<0.5	<0.5	<0.5	1.2	1.1	0	

[a]At 400 MHz, except for **10**, **13** and **16** (500 MHz), in $CDCl_3$ solution. Chemical shifts (δ values) are reported with respect to the signal of residual $CHCl_3$ (δ 7.25). All these assignments were in agreement with COSY, TOCSY, HMQC and ROESY spectra.[b]Overlapped signal; approximate δ values were measured from the HMQC spectrum. [c]$W_{1/2}$ = 8 Hz. [d]$W_{1/2}$ = 3 Hz. [e]This is the *cis*-hydrogen with respect to H-15. [f]This is the *trans*-hydrogen with respect to H-15.

Preparation of 8,15-isopimaradien-1α,7α,14α,18-tetraol (**11**) *from compound* **3**.

To a solution of **3** (80 mg, 0.17 mmol) in EtOH (10 mL) was added an ethanolic solution of KOH (10%, w/v, 5 mL) and the reaction mixture was left at room temperature for 10 h. Then H_2O (50 mL) was added and the reaction mixture was extracted with $CHCl_3$ (4 x 30 mL). The organic extract was dried over Na_2SO_4, filtered and evaporated to dryness giving pure **11** (55 mg, 0.16 mmol, 94%): amorphous white solid, mp 80-90 °C; $[\alpha]_D^{20}$ +30.7 (*c* 0.215, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 3380 br (OH), 3090, 1640, 910 (vinyl), 1650 (tetrasubstituted olefin), 2930, 2880, 1440, 1415, 1385, 1375, 1130, 1035, 1010, 950, 875, 840, 795. ^1H NMR: Table 4. ^{13}C NMR: Table 5. EIMS *m/z* (rel. int.): $[M]^+$ absent, 318 $[M-H_2O]^+$ (9), 300 (13), 272 (11), 269 (28), 251 (18), 214 (24), 201 (100), 185 (40), 145 (14), 143 (14), 119 (14), 105 (15), 91 (16), 55 (11). (Anal. Found: C, 71.12; H, 9.31%. $C_{20}H_{32}O_4$ requires: C, 71.39; H, 9.59%).

Table 5. ^{13}C NMR Spectral data of compounds **10-16**[a]

C	10	11	12	13	14	15	16
1	213.3 s	71.1 d	73.3 d	34.4 t	35.0 t	34.0 t	34.2 t
2	37.1 t	24.2 t	21.5 t	18.1 t	18.0 t	18.1 t	18.0 t
3	34.1 t	27.5 t	29.1 t	36.2 t	36.3 t	36.2 t	36.0 t
4	35.2 s	37.1 s	35.7 s	47.2 s	47.2 s	47.1 s	47.2 s
5	39.2 d	30.0 d	34.9 d	41.3 d	41.2 d	39.9 d	40.8 d
6	26.0 t	26.6 t	25.7 t	27.6 t	28.6 t	28.2 t	26.8 t
7	70.9 d	70.4 d	70.2 d	70.4 d	71.2 d	69.0 d	64.5 d
8	124.8 s	130.9 s	124.6 s	143.5 s	125.5 s	130.2 s	140.0 s
9	146.8 s	142.7 s	147.6 s	147.9 s	150.0 s	145.6 s	157.6 s
10	52.5 s	43.2 s	42.2 s	38.1 s	38.2 s	38.9 s	38.7 s
11	22.7 t	19.7 t	20.3 t	197.5 s	65.0 d	65.4 d	197.0 s
12	28.0 t	26.6 t	28.7 t	46.5 t	39.2 t	38.7 t	50.1 t
13	38.7 s	38.9 s	39.2 s	43.3 s	39.1 s	39.9 s	50.9 s
14	76.4 d	76.8 d	76.3 d	74.7 d	75.4 d	77.0 d	198.9 s
15	143.3 d	145.6 d	142.6 d	141.3 d	143.1 d	142.6 d	140.4 d
16	113.1 t	112.2 t	113.2 t	114.3 t	113.1 t	112.7 t	115.2 t
17	21.9 q	20.9 q	22.6 q	23.2 q	23.8 q	23.8 q	23.7 q
18	71.0 t	69.8 t	72.4 t	178.2 s	178.3 s	178.2 s	177.9 s
19	19.9 q	18.2 q	17.3 q	16.6 q	16.8 q	16.5 q	16.5 q
20	20.1 q	19.8 q	18.9 q	18.3 q	20.5 q	19.8 q	18.5 q
OAc	170.7 s		170.8 s	170.1 s	170.4 s	170.8 s	169.5 s
	170.4 s		170.6 s	170.0 s	170.3 s	170.5 s	20.9 q
	170.2 s		170.5 s	21.0 q	21.2 q	170.3 s	
	21.2 q		170.4 s	20.9 q	21.1 q	21.5 q	
	21.1 q		21.3 q			21.2 q	
	20.9 q		21.1 q			21.0 q	
			21.0 q				
			20.9 q				
COOMe				51.8 q	51.8 q	51.9 q	52.0 q

[a]At 100 MHz, except for **10**, **13** and **16** (125.7 MHz), in $CDCl_3$ solution. Chemical shifts (δ values) are reported with respect to the solvent signals (δ 77.00). All these assignments were in agreement with HMQC and HMBC spectra.

Preparation of 1α,7α,14α,18-tetraacetoxy-8,15-isopimaradiene (**12**) *from compound* **3**.

Treatment of **3** (130 mg, 0.28 mmol) with Ac_2O-pyridine (1:1, 10 mL) at room temperature for 48 h and work-up in the usual manner yielded **12** (116 mg, 0.23 mmol, 82%, after crystallisation from MeOH): colourless needles, mp 118-119 °C; $[\alpha]_D^{20}$ -54.3 (*c* 0.608, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 3080, 1635, 925 (vinyl), 1745,

1735, 1245 (OAc), 1640 (tetrasubstituted olefin), 2970, 2940, 1470, 1435, 1380, 1375, 1030, 1020, 965, 880. ^{1}H NMR: Table 4. ^{13}C NMR: Table 5. EIMS *m/z* (rel. int.): $[M]^+$ absent, 444 $[M\text{-}AcOH]^+$ (4), 402 (18), 384 (7), 361 (12), 264 (28), 251 (100), 249 (31), 201 (36), 195 (15), 185 (15), 143 (16), 55 (9), 43 (71). (Anal. Found: C, 66.43; H, 8.06%. $C_{28}H_{40}O_8$ requires: C, 66.64; H, 7.99%).

Acetylation of compounds **4** *and* **6** *to give compound* **5**.

Ac_2O-pyridine (1:1, 5 mL) treatment of **4** (50 mg) and **6** (40 mg) for 48 h at room temperature and work-up in the usual manner yielded the same derivative (46 mg and 32 mg, respectively), which was identical in all (mp, $[\alpha]_D$, IR, ^{1}H NMR and mass spectra) to **5** (see above).

Preparation of compound **11** *from compound* **5**.

To a solution of **5** (500 mg, 1.02 mmol) in anhydrous THF (25 mL) was added a solution of $LiAlH_4$ (400 mg, 10.5 mmol) in THF (50 mL). The mixture was stirred at room temperature for 24 h under Ar. Then, the reaction mixture was cooled at -20 °C and a saturated aqueous solution of Na_2SO_4 was added dropwise to the mixture. The white precipitate was filtered through a pad of Celite and the solids washed with abundant CH_2Cl_2-MeOH mixture (3:1). The residue obtained by evaporation of the solvents (320 mg) was subjected to column chromatography [Si gel, EtOAc-MeOH (32:1) as eluent] giving 297 mg (0.88 mmol, 86%) of an amorphous white solid ($[\alpha]_D^{22}$ +30.4, *c* 0.287, $CHCl_3$) whose ^{1}H and ^{13}C NMR spectra were identical to those of **11** (see above). Comparison (TLC, several eluents) with an authentic sample confirmed the identity.

Acetylation of compound **11** *to give compounds* **3** *and* **12**.

A sample of **11** (obtained from **5**, 200 mg, 0.59 mmol) was treated with Ac_2O-pyridine (1:1, 10 mL) at room temperature for 12 h. Work-up in the usual manner yielded a crude product (280 mg) which showed two spots on TLC, both less polar than **11**. Column chromatography of this mixture [Si gel, petrol-EtOAc (3:1) as eluent] allowed the isolation of **12** [less polar constituent, 182 mg, 0.36 mmol, 61%; mp 118-120 °C (MeOH); $[\alpha]_D^{20}$ -53.2 (*c* 0.261, $CHCl_3$), identical to the product obtained by acetylation of **3** (see above)] and another substance [89 mg, 0.19 mmol, 32%; mp 156-157 °C (EtOAc - *n*-hexane); $[\alpha]_D^{20}$ -52.8 (*c* 0.403, $CHCl_3$)] identical in all respects (mp, mixed mp, $[\alpha]_D$, ^{1}H NMR, MS and TLC) with the natural diterpenoid (**3**) isolated from *Nepeta septemcrenata* (see above and reference 3).

Application of Horeau's method[10] *to compound* **8**.

Compound **8** (58.10 mg, 0.12969 mmol) and (±)-α-phenylbutyric anhydride (97.37 mg, 0.3141 mmol) in pyridine solution (2.00 mL) for 24 h at 20 °C: α_1 = -0.470, α_2 = -0.426; α_1 - $1.1\alpha_2$ = -0.002; insufficient resolution for establishing the absolute configuration at C-11.[10]

Preparation of methyl 7α,14α-diacetoxy-11-oxo-8,15-isopimaradien-18-oate (**13**) *from compound* **8**.

Treatment of **8** (225 mg, 0.50 mmol) with Jones' reagent[20] at 0 °C for 10 min, and work-up as described above for **10**, gave **13** (203 mg, 0.455 mmol, 91%, after crystallisation from MeOH): colourless prisms, mp 136-138 °C; $[\alpha]_D^{20}$ -38.3 (*c* 0.783, $CHCl_3$); CD nm (Δε): 426 (0), 342 (-0.46), 279 (0), 262 (+1.09) (*c* 0.115, MeOH). UV (MeOH) λ_{max} nm (log ε): 237 (3.91). IR (KBr) ν_{max} cm^{-1}: 3090, 1635, 940 (vinyl), 1745 br, 1240

(OAc), 1720 (COOMe), 1685, 1615 (α,β-unsaturated ketone), 2970, 1455, 1430, 1375, 1190, 1165, 1110, 1040, 1020, 970, 915, 820. ^{1}H NMR: Table 4. ^{13}C NMR: Table 5. EIMS *m/z* (rel. int.): 446 $[M]^+$ (0.5), 404 (1.5), 386 (6), 344 (36), 276 (37), 216 (68), 201 (20), 188 (17), 187 (16), 173 (18), 159 (19), 105 (18), 91 (19), 67 (11), 55 (13), 43 (100), 41 (15). (Anal. Found: 67.31; H, 7.66%. $C_{25}H_{34}O_7$ requires: C, 67.24; H, 7.68%).

Sodium borohydride reduction of **13**: *compounds* **8** *and* **14** *(methyl 7α,14α-diacetoxy-11β-hydroxy-8,15-isopimaradien-18-oate).*

Compound **13** (170 mg, 0.38 mmol) in MeOH solution (15 mL) was treated with $NaBH_4$ (45 mg, 1.2 mmol) at room temperature for 3 h. Work-up in the usual manner gave a mixture of two compounds, both most polar than the starting material. This mixture was subjected to column chromatography [Si gel, petrol-EtOAc (4:1) as eluent] yielding **14** (less polar constituent, 118 mg, 0.26 mmol, 68%) and **8** [36 mg, 0.08 mmol, 21%; identical in all respects (mp, mixed mp, ^{1}H NMR and TLC) with the natural diterpenoid (**8**)].

Compound **14**: colourless needles, mp 130-133 °C (EtOAc - *n*-hexane); $[\alpha]_D^{20}$ -96.4 (*c* 0.275, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 3500 (OH), 3080, 1640, 935 (vinyl), 1730, 1720, 1715 (OAc and COOMe), 1260 (OAc), 2950, 1450, 1430, 1380, 1190, 1135, 1065, 1045, 1020, 1010, 910, 850, 820, 735. ^{1}H NMR: Table 4. ^{13}C NMR: Table 5. EIMS *m/z* (rel. int.): $[M]^+$ absent, 388 $[M\text{-}AcOH]^+$ (2), 346 (8), 328 (38), 313 (11), 278 (19), 260 (26), 253 (33), 200 (25), 159 (27), 133 (28), 105 (29), 91 (29), 81 (18), 79 (17), 69 (13), 67 (15), 55 (20), 43 (100), 41 (18). (Anal. Found: C, 66.81; H, 8.19%. $C_{25}H_{36}O_7$ requires: C, 66.94; H, 8.09%). For some NOE data see Table 3.

Application of Horeau's[10] *method to compound* **14**.

Compound **14** (29.76 mg, 0.066 mmol) and (±)-α-phenylbutyric anhydride (72 mg, 0.232 mmol) in pyridine solution (2.00 mL) for 18 h at 20 °C: α_1 = -1.370, α_2 = -1.205, α_1 - 1.1α_2 = -0.044; configuration 11*S*.

Acetylation of compounds **8** *and* **9** *to give methyl 7α,11α,14α-triacetoxy-8,15-isopimaradien-18-oate* (**15**).

Ac_2O-pyridine (1:1, 6 mL) treatment of **8** (30 mg, 0.067 mmol) and **9** (20 mg, 0.049 mmol) for 72 h at room temperature, followed by work-up in the usual manner, quantitatively yielded the same derivative **15**: colourless needles, mp 132-134 °C (*n*-hexane); $[\alpha]_D^{18}$ -7.9 (*c* 0.253, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 3080, 1640, 940 (vinyl), 1735 br (OAc and COOMe), 1240 (OAc), 2950, 1455, 1375, 1160, 1120, 1045, 1020, 960, 910. ^{1}H NMR: Table 4. ^{13}C NMR: Table 5. EIMS *m/z* (rel. int.): $[M]^+$ absent, 431 $[M\text{-}COOMe]^+$ (0.3), 371 $[M\text{-}AcOH\text{-}COOMe]^+$ (0.6), 370 $[M\text{-}AcOH]^+$ (0.8), 328 (56), 313 (25), 278 (14), 260 (12), 253 (19), 251 (18), 235 (15), 209 (10), 201 (10), 200 (18), 185 (16), 181 (16), 171 (15), 160 (39), 145 (21), 131 (18), 105 (18), 91 (15), 81 (10), 55 (14), 43 (100), 41 (11). (Anal. Found: C, 65.87; H, 7.73%. $C_{27}H_{38}O_8$ requires: C, 66.10; H, 7.81%).

Preparation of methyl 7α-acetoxy-11,14-dioxo-8,15-isopimaradien-18-oate (**16**) *from compound* **9**.

Treatment of **9** (110 mg, 0.27 mmol) with Jones' reagent[20] at room temperature for 30 min and work-up in the usual manner gave **16** (106 mg, 0.26 mmol, 96%): yellowish prisms, mp 156-158 °C (EtOAc - *n*-hexane); $[\alpha]_D^{18}$ +81.8 (*c* 0.582, $CHCl_3$). UV (MeOH) λ_{max} nm (log ε): 244 (3.84), 365 (1.80). IR (KBr) ν_{max} cm^{-1}: 3080,

1635, 940 (vinyl), 1740, 1240 (OAc), 1725 (COOMe), 1690 br, 1600 (enedione), 2960, 2860, 1455, 1440, 1370, 1205, 1130, 1110, 1065, 1025, 945, 905, 860, 820, 760, 700, 665. ^{1}H NMR: Table 4. ^{13}C NMR: Table 5. EIMS *m/z* (rel. int.): 402 $[M]^+$ (0.5), 359 (21), 342 (36), 292 (63), 282 (23), 267 (26), 217 (40), 199 (29), 187 (21), 174 (27), 159 (27), 129 (22), 128 (21), 105 (22), 91 (31), 67 (22), 59 (20), 55 (19), 43 (100), 41 (26). (Anal. Found: C, 68.73; H, 7.49%. $C_{23}H_{30}O_6$ requires: C, 68.63; H, 7.51%).

Acknowledgement

We thank Prof. F. M. Hammouda and Dr. H. A. Hussiney, Department of Pharmaceutical Sciences, National Research Centre, Cairo, Egypt, for the facilities given for the collection of *N. septemcrenata*, and Prof A. Martínez Pozo, Department of Biochemistry and Molecular Biology I, Faculty of Chemical Sciences, Complutense University, Madrid, Spain, for CD measurements. One of us (A. A. H.) thanks the "Agencia Española de Cooperación Internacional" for a studentship. This work was supported by funds from the "Comisión Asesora de Investigación Científica y Técnica" (grant AGF98-0805 and project PB96-0001-C03-02) and the "Consejería de Educación y Cultura de la Comunidad de Madrid" (project 06G/001/96).

References and notes

1. Jeremic, D.; Macura, S.; Milosavljevic, S.; Vajs, V. *Tetrahedron* **1985**, *41*, 357-364.
2. Milosavljevic, S.; Jeremic, D.; Macura, S.; Vajs, V. *Croat. Chem. Acta* **1985**, *58*, 399-405.
3. Khalil, A. T.; Gedara, S. R.; Lahloub, M. F.; Halim, A. F.; Voehler, M. *Phytochemistry* **1997**, *44*, 475-478.
4. The ^{1}H NMR data of **4** reported in Table 1 include the reassignments for the H-11α, H-11β, H-12α and H-12β protons with respect to those shown in reference 1. Moreover, the H-5α proton of **4** resonates at δ 3.06 (Table 1 in this work and Figure 1 in reference 1) and not at δ 2.72, as it is erroneously reported in Table 2 of the paper of Milosavljevic and co-workers.[1]
5. There is an error in the chemical shift of the H-14β proton of **3** reported in reference 3 (Table 1,[3] δ 4.09). The correct value is δ 5.09 (Table 1 in this work and discussion of results in reference 3).
6. Hall, S. R.; Flack, H. D.; Stewart, J. M. *XTAL 3.2 Reference Manual*, University of Western Australia: Lamb, Perth, 1994.
7. Corazza, E. *Acta Cryst.* **1975**, *B31*, 1445-1450.
8. Kuzakov, E. V.; Shmidt, E. N.; Bragyanskaya, I. Y.; Rybalova, T. V.; Gatilov, Y. V.; Barkhash, V. A. *Zh. Org. Khim.* **1995**, *31*, 196-203.
9. Toromanoff, E. *Tetrahedron* **1980**, *36*, 2809-2931.
10. Horeau, A.; Nouaille, A. *Tetrahedron Lett.* **1971**, *12*, 1939-1942.
11. Crabbé, P. *Applications de la Dispersion Rotatoire Optique et du Dichroïsme Circulaire Optique en Chimie Organique*, Gauthier-Villars: Paris, 1968; pp 193-198.
12. Unfortunately, the other pimarane diterpenoid (**2**) previously found in *L. europaeus* by the Yugoslav authors[2] was not isolated in our study of this plant (see Discussion), thus precluding a reexamination of its structure. However, in view of the present evidence about structures **1** and **4**, and on biogenetic grounds, it is highly probable that compound **2**[2] could also be an isopimarane derivative.
13. See reference 11; pp 438-443, and references cited therein.
14. Scott, A. I. *Interpretation of the Ultraviolet Spectra of Natural Products*, Pergamon Press: London, 1964; pp 61-68.
15. Connolly, J. D.; Hill, R. A. *Dictionary of Terpenoids*, Volume 2, Chapman & Hall: Cambridge, U. K., 1991; pp 857-864, 870-882.
16. Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Burla, M. C.; Polidori, G.; Camalli, M. *SIR92*, Universities of Bari and Perugia, and Ist. Strutt. Chimica CNR: Rome, Italy, 1992.
17. Sheldrick, G. M. *SHELX93, Program for the Refinement of Crystal Structures*, Univertity of Götingen: Götingen, Germany, 1993.
18. Nardelli, M. PARST, *Comput. Chem.* **1983**, *7*, 95-98.
19. *International Tables for X-Ray Crystallography*, Volume C, Kluver Academic Publishers: Dordrescht, Germany, 1992.
20. Bowers, A.; Halsall, T. G.; Jones, E. R. H.; Lemin, A. J. *J. Chem. Soc.* **1953**, 2548-2560.