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A DITERPENE FROM *NEPETA SEPTEMCRENATA*

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Key Word Index—*Nepeta septemcrenata*; Lamiaceae; diterpene; isopimarane; flavone; structure elucidation; relative stereochemistry; antimicrobial activity.

Abstract—A new isopimarane-type diterpene was isolated from the ethanol extract of the aerial parts of *Nepeta septemcrenata*. Its structure was established as 1α-hydroxy-7α,14α,18-triacetoxy-isopimara-8,15-diene using different spectroscopic techniques (UV, IR, MS and 1 and 2D NMR). In addition, 7-*O*-methylapigenin was also isolated and identified. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Nepeta septemcrenata Ehrenb. is a perennial herb that grows wild in South Sinai [1]. A few members of the genus *Nepeta* are reported to possess biological activities especially reduction of serum lipids and anti-inflammatory effects [2, 3]. Several diterpenes have been isolated from the genus *Nepeta* [4–8]. This paper describes the characterization of a new isopimarane derivative from the ethanol extract of the aerial parts of *N. septemcrenata*.

RESULTS AND DISCUSSION

Column chromatography of the chloroform-soluble fraction of the ethanol extract afforded two pure compounds, **1** and **2**. The latter was identified as 7-*O*-methylapigenin based on its mp, MS, IR and UV data [9].

The mass spectrum of **1** (determined by LC-MS) displayed a quasimolecular ion peak at m/z 480 $[M + NH_4]^+$ suggesting a molecular formula $C_{26}H_{38}O_7$. The presence of three acetoxyl functions was evident from the IR bands at 1740 and 1250 cm^{-1} and NMR three singlets at δ_H 1.95, 1.97 and 2.05, each integrating for three protons and correlated with the carbon signals at δ_C 21.26, 21.26 and 21.00, respectively, assigned to three acetate methyls by the HETCOR experiment. In addition, the three acetate carbonyl signals appeared at δ_C 170.43, 170.54 and 170.88. The vinyl function was represented in the IR spectrum by bands at 1660 and 930 cm^{-1} as well as by signals of an ABX system at δ 5.79 (*dd*, $J = 17.4$, 11.2 Hz), 5.02 (*dd*, $J = 17.4$, 1.3 Hz) and 5.00 (*dd*, $J = 11.2$, 1.3 Hz) [10]. Other functional groups were represented by a hydroxyl, as revealed by an IR band at 3500 cm^{-1}, and a tetrasubstituted double bond as indicated by ^{13}C NMR signals at δ 125.4 and 148.7 (Table 1).

Based on a total unsaturation number of 8, the presence of three acetate carbonyls and two double bonds; **1** should have a tricyclic structure. The presence of a vinyl group and three methyl singlets at δ_H 1.05, 0.90 and 0.89 proved that **1** had an isopimarane skeleton substituted at C-18. The tetrasubstituted double bond could only be situated between C-8 and C-9. One acetoxyl function was attached to the C-14 methine, since this is the only position to afford a one proton singlet at δ 5.09; correlated with a carbon signal at δ_C 75.95. Another acetoxyl function was attached to the C-18 methylene as revealed by an AB system at δ_H 3.69 ($J = 11.1$ Hz) and 3.82 ($J = 11.1$ Hz) correlated with the carbon signal at δ_C 72.22. These and further assignments were unambiguously confirmed through HMQC and HMBC experiments (Table 1). Thus the third acetoxyl function was assigned to C-7 and the free hydroxyl to C-1. In addition, the proton and carbon chemical shifts of each acetoxyl group could be assigned (Table 1).

The relative stereochemistry of **1** was determined on the bases of the coupling constants of the proton signals, the chemical shifts of the carbon signals as well as the result of phase sensitive 2D-NOESY experiments (Fig. 1) and the computer-generated model (Fig. 2). The hydroxyl group at C-1 and the acetoxyl group at C-7 were axial, judging from the poorly resolved signal of H-1 (δ 3.95, *br s*) and the small coupling constant of H-7 (δ 5.15, *dd*, $J = 4.2$, 2.7 Hz). Their additive γ-shielding effect, together with the C-18 acetoxyl, on C-5 (*ca* −21 ppm) compared with the reported value in Δ^8-isopimarane [11] confirmed their α, axial orientation. The NOESY spectrum further confirmed the stereochemistry at C-1, C-5 and C-10 by revealing cross peaks between the C-1 hydroxyl proton (δ 1.35) and H-5α (δ 2.15) on one hand the H-1β (δ 3.95) and H-20 (δ 1.05) on the other hand and thus proving the

Table 1. ^{1}H- and ^{13}C-NMR spectral data (500 and 125 Mz, $CDCl_3$) of compound **1***

Position	δ_H (J in Hz)	δ_C	^{1}H long range coupled†	
			$^3J_{CH}$	$^2J_{CH}$
1β	3.95 *br s*	70.43	3, 5, 9, 20	10
2α	1.68 *m*	24.41	4, 10	1, 3
2β	1.90 *m*		4	3
3α	1.85 *m*	28.12	1, 18, 19	2, 4
3β	1.18 *m*		1, 5, 19	2, 4
4	—	35.83		
5α	2.15 *t*(7.7)	33.50	3, 7	4, 6, 10
6(α + β)	1.76 *m*	25.50		
7β	5.15 *br dd*(4.2, 2.7)	71.18	5, 9, 14, 21	8
8	—	125.40		
9	—	148.62		
10	—	43.74		
11α	2.49 *ddd*(18.4, 6.4, 3.9)	20.30	8, 13	9, 12
11β	2.15 *m*		8, 10	9
12α	1.96 *m*	27.34	9, 14, 17	11, 13
12β	1.51 *m*		9, 14, 15	11, 13
13	—	39.02		
14β	4.09 *s*	75.95	7, 9, 12, 15, 17, 25	8, 13
15	5.79 *dd*(17.4, 11.2)	143.36	12, 14, 17	13
16 *cis*	5.00 *dd*(11.2, 1.3)	113.03	13	15
16 *trans*	5.02 *dd*(17.4, 1.3)		13	15
17	0.90 *s*	21.88	12, 15	13
18 a	3.69 *d*(11.1)	72.22	3, 5, 19, 23	4
18 b	3.82 *d*(11.1)		3, 5, 19, 23	4
19	0.89 *s*	17.42	3, 5, 18	4
20	1.05 *s*	19.71	1, 5, 9	10
21	—	170.43		
22	1.95 *s*	21.26		21
23	—	170.88		
24	2.05 *s*	21.00		23
25	—	170.54		
26	1.97 *s*	21.26		25
OH	1.35 *d*	—	2, 10	1

* Signal assignments are based on the anlysis of 1D (^{1}H, ^{13}C, APT, DEPT) and 2D (COSY, HETCOR, HMQC, HMBC, NOESY) NMR spectra (δ in ppm from TMS).

† $^3J_{CH}$ and $^2J_{CH}$ indicate the carbons long range coupled with each proton through three and two bonds, respectively, as observed in the HMBC spectra.

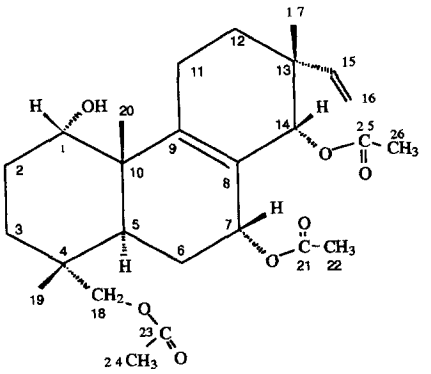
trans fusion of rings A and B. The NOESY correlations (Fig. 1) clearly covered all spatial interactions confirming that all the methyls at C-4, C-10 and C-13 and the downfield protons at C-1, C-7 and C-14 were on the same face of the molecule (β-face) while all the oxygenated functions at C-1, C-7 and C-14 as well as C-18 and the vinyl group were on the other face (α-face). Based on these results, the structure of this new diterpene was established as 1α-hydroxy-7α,14α,18-triacetoxy-isopimara-8,15-diene. Reviewing the current literature, a Δ^8-pimaric acid methyl ester derivative with the same functionality at C-1, C-7 and C-14 as **1** was isolated from the lamiaceous plant *Lycopus europaeus* [12]. Surprisingly, although the proposed configurations at C-13 and C-14 in this compound and in **1** are different, their ^{13}C NMR data are very close. However, the low energy conformational structure of **1** (Fig. 2) was found to be consistent with the phase sensitive NOESY data (Fig. 1) pertaining to ring C.

Investigation of the antimicrobial activities of **1** [13] revealed that it possessed a moderate activity against the Gram-positive bacteria (*Staphylococcus aureus*) but no activity was observed against the following organisms: *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Saccharomyces cereviseae*.

EXPERIMENTAL

General. Mps: uncorr.; LCMS: Vestec 201 Thermospray MS; NMR: 300 MHz for ^{1}H and 75 MHz for ^{13}C while HMBC and HMQC were performed on a Bruker AMX-500 Spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. NOESYPH was performed on a Bruker AM-400 operating at 400 MHz. The molecular modelling was conducted using Molecular Mechanics Energy minimization Program MM2-plus marketed as 'Chem-3D Plus, Version 3.0' by Cambridge Scientific Computing, Inc. 875 Massachusetts Ave, Cambridge, MA.

Plant material. The fresh plant material of *Nepeta septemcrenata* was collected from South Sinai, Egypt,



1

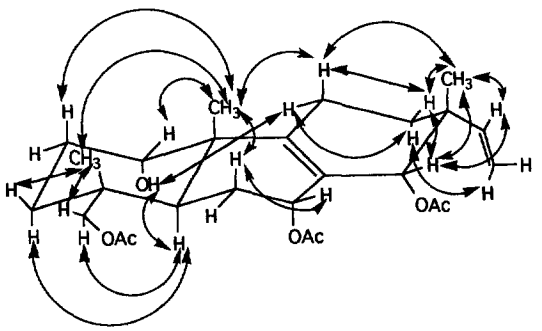


Fig. 1. Important NOESYPH correlations at compound **1**.

in June 1992 during the flowering season. The plant identity was verified by Dr N. El-Hadidy, Faculty of Science, Cairo University. A voucher specimen is deposited at the Pharmacognosy Department, Faculty of Pharmacy, Mansoura University.

Extraction and isolation. The aerial part (350 g) was reduced into a fine powder and defatted with petrol (60–80°) and then extracted exhaustively with EtOH. The alcohol extract was concentrated to a small vol. *in vacuo* then diluted with H_2O and extracted with $CHCl_3$. The dried $CHCl_3$ extract (17.8 g) was applied onto the top of a glass column packed with silica gel (400 g) and eluted with $CHCl_3$ then with a $CHCl_3$/MeOH gradient. The fractionation procedure was monitored by TLC (precoated silica gel plates; $CHCl_3$–MeOH; vanillin–H_2SO_4 as a spray reagent). The frs eluted with $CHCl_3$ were further purified using silica gel CC and petrol–$CHCl_3$ for elution and the major compound (R_f 0.87 in $CHCl_3$–MeOH 49:1) was crystallized from Et_2O to afford **1**. The frs eluted with 5% MeOH in $CHCl_3$ were further purified using prep. TLC (silica gel GF_{254}, $CHCl_3$–MeOH 19:1). The plates were visualized under UV light and the major band (R_f 0.82) were scraped, eluted with $CHCl_3$–MeOH (4:1), and concd *in vacuo* to afford **2**.

1α-Hydroxy-7α,14α,18-triacetoxy-isopimara-8,15-diene (**1**). Fine needle crystals (115 mg), mp 148–150°; LC-MS, $[M+NH_4]^+ = 480$, $C_{26}H_{38}O_7$; IR ν_{max}^{KBr} cm^{-1}: 3500, 3005, 2900, 1740, 1660, 1390, 1250, 1130, 1035, 930, 820; ^1H NMR and ^{13}C NMR: Table 1.

Antimicrobial activity of **1**. Nutrient agar plates were

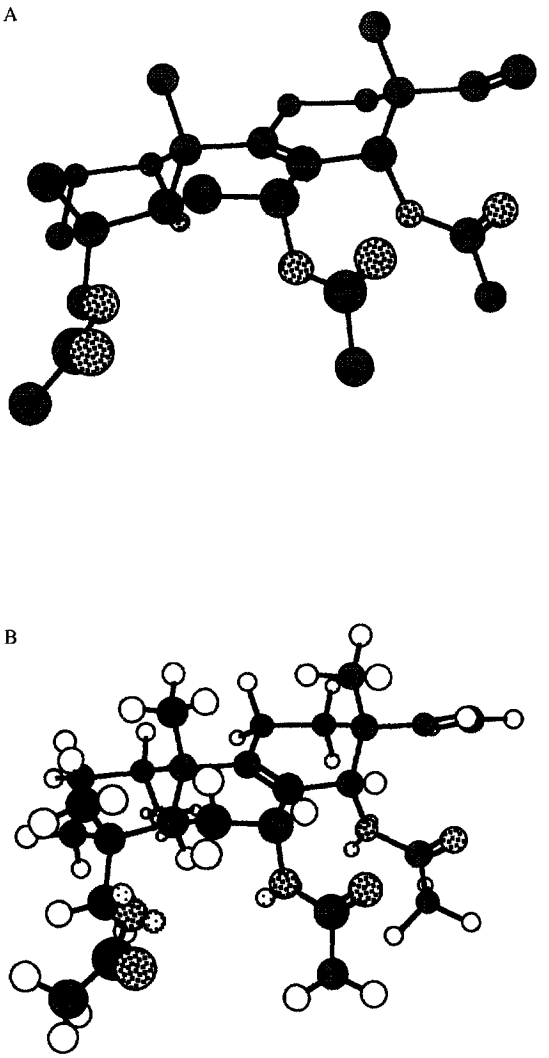


Fig. 2. A computer-generated model of **1** (56.73 kcal mol^{-1}, hiding hydrogens and lone pairs of electrons in A).

seeded using 0.1 ml of the diluted organisms. Cylindrical plugs were removed from the agar plate using a sterile cork borer. Compound **1** (100 μl) 1 mg ml^{-1} DMSO) or 100 μl of solvent (blank) were added to each well such that each assay was triplicated. Plates of *S. aureus*, *E. coli* and *P. aeruginosa* were incubated at 37°, while those of *C. albicans* and *S. cerviseae* were incubated at 30°. Results were taken after 24 hr incubation and were recorded as average diameter of the inhibition zone in mm.

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