Structure and Antibacterial Activity of a New Labdane Diterpenoid from *Salvia leriaefolia*

Zohreh Habibi,[†] Fereshteh Eftekhar,[‡] Keivandokht Samiee,[‡] and Abdolhossein Rustaiyan*[,§]

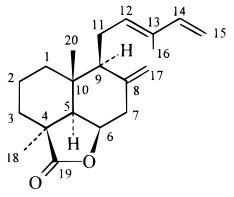
Department of Chemistry, Shahid Beheshti University, Tehran, Iran, Department of Biology, Shahid Beheshti University, Tehran, Iran, and School of Pharmacy, Shahid Beheshti University of Medical Sciences, P.O. Box 14155-6153, Tehran, Iran

Received June 11, 1999

Phytochemical investigation of the choloroform extract of *Salvia leriaefolia* afforded 8(17),12*E*,14-labdatrien-6,19-olide (**1**), and its structure was determined by a combination of spectral methods. Compound **1** was found to possess antibacterial activity against *Staphylococcus aureus.*

The genus *Salvia* (Lamiaceae) comprises about 700 herbs and shrubs, growing in the temperate and warmer zones of the world.[1] Some of these species are used as medicinal, aromatic, and ornamental plants. *Salvia officinalis* L. is one of the most widespread species and, since ancient times, has been used in the treatment of various disorders, such as tuberculosis,[2] psoriasis,[3] and seborrheic eczema.[3] It shows strong antibacterial and antifungal activities.[4] The rhizomes of *S. miltiorrhiza* Bunge. have been used widely to treat coronary heart diseases, particularly angina pectoris and myocardial infarction.[5]

Previous chemical investigations on different species of *Salvia* have shown the presence of flavonoids,[6] diterpenoids,[7] sesterterpenes,[8−11] and essential oils.[12,13] Except for a report on the essential oil obtained from aerial parts of *Salvia leriaefolia* Benth.[14] no other studies have been reported on this species. As part of our continuing studies on Iranian plants, we have examined *S. leriaefolia* and isolated a new labdane diterpene (**1**). In this paper, we report the structure of **1** and its antibacterial activity.



1

The molecular formula of **1** was suggested as $C_{20}H_{28}O_2$ by HREIMS. The IR spectrum showed bands demonstrative of carbonyl γ-lactone (1770 cm^{-1}) and $CH{=}CH_2$ (3080, 1645, 990, 895 cm^{-1}) groups. The ^{13}C NMR spectrum of this compound contained resonances for 20 carbons, including six olefinic carbons and a carbonyl carbon (approximately 183 ppm, Table 1). More detailed analysis was performed by ^1H NMR measurements. The typical lowfield signals at δ 6.35 (dd, J = 11, 17 Hz, H-14), 4.93 (d, J = 11 Hz, H-15c), 5.08 (d, J = 17 Hz, H-15t), and 5.47 (t-like, J = 7 Hz, H-12), together with an olefinic methyl signal at δ 1.73 (br s, Me-16) in the ^1H NMR spectrum, suggested the presence of a (12,13*E*)-isoprene group. In the HMBC spectrum, the isolated methylene proton (H_2-11) resonances at δ 2.35 and 2.20 gave long-range correlations with the tetrasubstituted olefinic carbon signal at δ 134.68 (C-13). Two olefinic proton signals at δ 4.73 and 4.95 were assigned to an exomethylene group. The ^1H NMR spectrum of **1** (Table 1) was very similar to that of (12,13*E*)-biformen,[15] and the observed differences resulted from the presence in **1** of a γ-lactone (6,19-olide) group [δ 4.79 (ddd, J = 4, 8.5, 4 Hz, H-6); 1.68 (d, J = 4 Hz, H-5); 1.28 (Me-18)]. This conclusion was also supported by the ^{13}C NMR and IR spectral signals typical of a lactone group (δ_C 183.07; IR 1770 cm^{-1}).

Table 1. NMR Data of Compound **1**[a]

position	δ_C	δ_H	HMBC
1	35.79 (t)[b]		
2	20.37 (t)		
3	43.37 (t)		
4	28.78 (s)		
5	52.48 (d)	1.68 d (4)	C-9, C-19
6	76.75 (d)	4.79 ddd (4, 4, 8.5)	C-4, C-8, C-10
7	35.48 (t)	2.90 dd (16, 4)(α)	
		2.66 dd (8.5, 16)(β)	
8	144.04 (s)		
9	53.15 (d)	1.92 dd (3.5, 9)	C-8, C-10
10	36.02 (s)		
11	26.54 (t)	2.35 ddd (15, 7, 3.5)	C-8, C-10, C-13
		2.20 ddd (9, 15, 7)	
12	132.87 (d)	5.47 t-like (7)	
13	134.68 (s)		
14	141.76 (d)	6.35 dd (17, 11)	
15	113.60 (t)	4.93 d (11) (cis)	
		5.08 d (17) (trans)	
16	12.30 (q)	1.73 br s	
17	111.05 (t)	4.95 s	
		4.73 s	
18	18.43 (q)	1.28 s	C-3, C-4, C-5, C-19
19	183.07 (s)		
20	24.49 (q)	0.92 s	C-1, C-9, C-10

[a] Chemical shifts were determined at 400 (^1H) and 100 (^{13}C) MHz in $CDCl_3$. ^1H and ^{13}C NMR chemical shifts refer to $CHCl_3$ at 7.26 ppm and $CDCl_3$ at 77.0 ppm, respectively. J values in Hertz are in parentheses. [b] Multiplicities were established by DEPT experiments.

The relative stereochemistry of **1** was established by NOE difference experiments. Strong NOEs were observed between the CH_3-18 protons and H-5, H-5 and H-6, and H-6 and H-9, respectively. Thus, the configuration of the Decalin system was established as trans and the side chain at C-9 as equatorial. From these NOE data, together with analysis of coupling constant values, all ^1H NMR signals could be assigned as shown in Table 1. Accordingly, compound **1** was assigned as 8(17),12*E*,14-labdatrien-6,19-olide. The results showed that compound **1** was active

* To whom correspondence should be addressed. Tel.: +98 21 2716370. Fax: +98 21 8795008. E-mail: Pharmacy@Kanoon.net.
[†] Department of Chemistry.
[‡] Department of Biology.
[§] School of Pharmacy.

10.1021/np990287h CCC: $19.00
Published on Web 01/20/2000

against the Gram-positive bacterium species *Staphylococcus aureus* at a concentration level of 0.4 mM.

Experimental Section

General Experimental Procedures. The optical rotation was measured on a polarimeter using a sodium lamp (589 nm). The IR spectrum was recorded on a Shimadzu IR-470 spectrometer. NMR experiments was recorded on a Varian VNMR-400 MHz spectrometer at 400 and 100 MHz for ^{1}H and ^{13}C NMR, respectively. LREIMS were recorded on a Hewlett-Packard 5995A spectrometer and HRMS (EI, 70 eV) were obtained on a MAT 95 SC mass spectrometer. Si gel 60 F_{254} was used for TLC, while Si gel (60−120 mesh) was used for column chromatography.

Plant Material. *Salvia leriaefolia* was collected in July 1998, from near Sabzewar, Khorassan Province, in northeast Iran at an altitude of 1400 m. Voucher specimens (A.R., no. 112) are deposited at the Herbarium of the Department of Botany, Shahid Beheshti University, Tehran, Iran.

Extraction and Isolation. Air-dried and powdered aerial parts of the plant (750 g) were extracted with $CHCl_3$ (6 L) at room temperature for 24 h. The extract was concentrated in vacuo, weighed, and suspended in hot MeOH (10 mL^{-1} of extract) and then cooled to −15 °C. After standing for 4 h at −15 °C, the waxy precipitate was removed by filtration, and the filtrate evaporated in vacuo to afford a syrupy residue (25 g). The residue was submitted to column chromatography on Si gel, eluted with light petroleum and increasing amounts of diethyl ether until a 50:50 ratio was maintained. Fractions were collected (70 mL each), and similar fractions were combined and chromatographed on preparative Si gel 60 F_{254} plates, using petroleum ether−Et_2O (4:1), to give a light yellow residue (80 mg), which was purified by repeated preparative TLC on Si gel using petroleum ether−EtOAc (4:1) to afford **1** (60 mg).

8(17),12*E*,14-Labdatrien-6,19 olide (1): obtained as a colorless oil: $[\alpha]_D^{25}$, +18° (*c* 0.8, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3080, 1770, 1645, 990, 895 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 300 $[M]^+$ (20), 285 (25), 173 (30), 81 (100), 41 (75); HREIMS *m*/*z* 300.2410 (calcd for $C_{20}H_{28}O_2$, 300.2413).

Antibacterial Activity. The antibacterial activity of **1** was determined by the standardized disk method of Kirby and Bauer.[16] Laboratory standard ATCC strains (*Staphylococcus aureus* ATCC # 25923, *Enterococcus faecalis* ATCC # 29212, *Escherichia coli* ATCC # 25922, and *Pseudomonas aeruginosa* ATCC # 85327) were used as test bacteria. The results were reported as the diameter of the zone of inhibitation around each disk (in mm). The results showed that compound **1** was active against the Gram-positive bacterium *S. aureus* at a concentration level of 0.4 mM (20-mm zone diameter) but had only moderate inhibitory activity against the Gram-negative bacteria *E. coli* (13-mm zone diameter) and *P. aeruginosa* (12-mm zone diameter).

Acknowledgment. The financial support of the Research Council of the Shahid Beheshti University is gratefully acknowledged.

References and Notes

(1) Chadefaud, M.; Emberger, L. *Traite de Botanique* (*Systematique*); Masson et Compagnie Editeurs: Paris, 1960; p 839.
(2) Dobrynin, V. N.; Kolosov, M. N.; Chernov, B. K.; Derbentseva, N. A. *Khim. Prir. Soedin.* **1976**, *5*, 686−688.
(3) Janosik, I. Czechoslovakian Patent 185, 262, Sep. 15, 1980; *Chem. Abstr.* **1981**, *95*, 68027f.
(4) Hitokoto, H.; Morozumi, S.; Wauke, T.; Sakai, S.; Kurata, H. *Appl. Environ. Microbiol.* **1980**, *39*, 818−822.
(5) Chang, H. M.; But, P. P. *Pharmacology and Applications of Chinese Materia Medica*; World Science: Singapore, 1986; Vol. 1, p 255.
(6) Ulubelen, A.; Evren, N.; Tuzlaci, E.; Johansson, C. *J. Nat. Prod.* **1988**, *51*, 1178−1183.
(7) Rodriguez-Hahn, L.; Esquival, B.; Cardenas, J.; Ramamoorthy, T. P. In *Advances in Labiatae Science*; Harley, R. M., Reynolds, T., Eds.; Royal Botanic Gardens: Kew, Richmond, UK, 1992; pp 335−347.
(8) Rustaiyan, A.; Niknejad, A.; Nazarians, L.; Jakupovic, J.; Bohlmann, F. *Phytochemistry* **1982**, *21*, 1812−1813.
(9) Rustaiyan, A.; Koussari, S. *Phytochemistry* **1988**, *27*, 1767−1769.
(10) Rustaiyan, A.; Sadjadi, A. *Phytochemistry* **1987**, *26*, 3078−3079.
(11) Gonzalez, M. S.; Sansegundo, J. M.; Grande, M. C.; Medarde, M.; Bellido, I. S. *Tetrahedron* **1989**, *45*, 3575−3576.
(12) Rustaiyan, A.; Masoudi, S.; Jassbi, A. *J. Essent. Oil Res.* **1997**, *9*, 599−600.
(13) Rustaiyan, A.; Komeilizadeh, H.; Masoudi, S.; Jassbi, A. *J. Essent. Oil Res.***1997**, *9*, 713−714.
(14) Rustaiyan, A.; Masoudi, S.; Rabbani, M.; Motiefar, R.; Larijani, K. *J. Essent. Oil Res.* In press.
(15) Bohlmann, F.; Czerson, H. *Phytochemistry* **1979**, *18*, 115−118.
(16) Bauer, A. W.; Kirby, W. M. M.; Sherris, J. C.; Turck, M. *Am. J. Clin Pathol.* **1966**, *45*, 493−496.

NP990287H