PERGAMON

Phytochemistry 52 (1999) 1455–1459

PHYTOCHEMISTRY

Diterpenoids from the roots of *Salvia bracteata*

Ayhan Ulubelen[a,*], Sevil Öksüz[a], Ufuk Kolak[a], Nur Tan[a], Candan Bozok-Johansson[b], Cennet Çelik[b], Hans-Jurgen Kohlbau[c], Wolfgang Voelter[c]

[a]*Department of Chemistry, Faculty of Pharmacy, University of Istanbul, 34452 Istanbul, Turkey*
[b]*Department of Microbiology, Faculty of Medicine, University of Marmara, Haydarpaşa, 81326 Istanbul, Turkey*
[c]*Department of Physical Biochemistry, Physiological Institute, University of Tübingen, Hoppe-Seyler Street 4, 7400 Tübingen, Germany*

Received 17 March 1999; received in revised form 2 June 1999; accepted 30 July 1999

Abstract

From the roots of *Salvia bracteata* Banks and Sol. in Russell, Aleppo. two new diterpenoids Salvibracteone (**1**) and bractealine (**2**) have been isolated together with eight known diterpenoids. The structures of the new compounds were established by spectroscopic analysis. The diterpenoids and the crude extract were tested against standard bacterial strains.The crude extract, the new compound bractealine (**1**), and the known compound horminone showed activity against *B. subtilis*, *S. aureus*, and *S. epidermidis*. Ferruginol had slight activity against these strains. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Salvia bracteata*; Lamiaceae; Diterpenoids; Salvibractone; Bractealine

1. Introduction

There are about 90 *Salvia* species growing naturally in Turkey, half of the plants are endemic (Davis, 1982). We have studied the roots of about 40 *Salvia* species since 1968, and obtained mostly abietane and rearranged abietane diterpenoids. Rarely we have isolated pimarane and labdane type skeletons (Ulubelen & Topcu, 1998). Abietane diterpenes in the roots of *Salvia* species are considered as the defining character of the genus (Patudin, Romanova, Sokolov & Pribylova, 1974). In some abietane structures, the C ring is aromatized with one or more phenolic groups and in some others this ring is oxidized to 1,2 or 1,4-benzoquinones. *Salvia* species are used in folk medicine all around the world, they possess antibacterial (Janosik, 1980), antioxidant (Dobrynin, Kolosov, Chernov & Derbentseva, 1976), antidiabetic (Hitokato, Morozumi, Wauke, Saiki & Kurata, 1980) and antitumor (Hanson & Hocking, 1957) activities. In our studies, we have also established antibacterial (Ulubelen, Tan, Sönmez, & Topcu, 1998), antitumor (Topcu, Tan, Kökdil & Ulubelen, 1997), antituberculous (Ulubelen, Topcu & Bozok-Johansson, 1997a) activities. In continuation of our investigations (Ulubelen & Topcu, 1998; Sönmez, Topcu & Ulubelen, 1997; Topcu et al., 1997; Topcu, Ulubelen, Tam & Tao-Che, 1996), we have studied the roots of a perennial herb *S. bracteata* Banks and Sol. in Russel, Aleppo., and isolated 10 diterpenoids of which two were new compounds. After the extraction of the roots, the crude extract was roughly separated on a silica gel column, the combined fractions (A–D) were further separated on a Chromatotron using silica gel rotors. Fractions B and C have yielded the diterperpenoids, lupeol and Δ^7-stigmasterol were obtained from fraction D, only lipids were present in fraction A and they were discarded. The diterpenoids obtained from the Chromatotron were further cleaned on preparative TLC plates. The structures of the known compounds 7-acetylhorminone, horminone (Janot & Potier, 1964), 12-methylhorminone (Ulubelen, Sönmez & Topcu, 1997b), 6,7-dehydroroyleanone (Hensch, Rüedi & Eugster, 1971), ferruginol (Cambie, Madden & Parnell, 1975), 12-

* Corresponding author. Tel.: +90-2125140355; fax: +90-2125193086.

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00453-7

Table 1
NMR data of compounds **1** and **2**

	1			**2**		
	^{13}C	^{1}H	COLOC	^{13}C	^{1}H	COLOC
1α	202.7*s*		H-5, H-11, H-20	31.9*t*	1.56*m*	
1β					3.02*m*	
2α	34.3*t*	2.80*m*		19.8*t*	2.55*m*	
2β		1.85*m*			1.80*m*	
3α	41.1*d*	2.30*m*		41.2*t*	1.60*m*	
3β		1.80*m*			2.25*m*	
4	34.1*s*		H-5, H-7, H-20	30.2*s*		H-1, OMe
5α	45.5*d*	2.65*t*		68.3*s*		H-20, H-3, H-6, H-7
6α	19.2	1.90*m*		18.2*t*	1.58*m*	
6β		1.28*ddd*			1.30*m*	
7α	68.9*d*	4.28*br d*	H-5, H-14, H-20	38.6*t*	2.25*m*	
7β					2.60*m*	
8	128.8*s*			139.2*s*		
9	130.9*s*			147.4*s*		
10	40.1*s*			40.1*s*		
11	120.6*d*	7.09*s*		184.8*s*		
12	140.1*s*		H-11, H-14, H-15	144.2*s*		
13	130.9*s*			139.2*s*		
14	104.7*d*	6.75*s*		183.6*s*		H-16, H-17
15	24.8*d*	3.30sept		148.5*s*		
16	19.2[a]*q*	1.16*d*	H-17	114.2*t*	5.12*br s*	H-17
16′					5.08*br s*	
17	18.6[a]*q*	1.18*d*	H-16	19.8*q*	1.87*s*	H-16
18	31.9*q*	0.84*s*		30.1*q*	1.12*s*	
19	22.7*q*	0.86*s*		22.7*q*	1.08*s*	
20	65.6*t*	4.08*d*		23.9*q*	1.18*s*	
20′		4.16*d*				
OMe	61.5*q*	3.85*s*		59.8*q*	3.58*s*	

[a] These values are exchangeable.

methylferruginol (Brandt & Neubauer, 1939), salvinolone (Lin, Blasco & Cordell, 1989), and sugiol (Chen, Meng, Piantini & Hesse, 1989) were decided by comparing their spectral data to those given in literature and by TLC comparisons with standard samples.

2. Results and discussion

The HRMS of the first new compound salvibractone (**1**) indicated the molecular formula $C_{21}H_{28}O_3$ (*m*/*z* 328.2024, calc. 328.2038) which showed eight degrees of unsaturation as double bond equivalent, of which three were accounted to the tricyclic skeleton, one for the ketone, and three for the double bonds and the remaining one indicated the presence of another ring which formed between C-7 and C-20. The UV spectrum with a maximum at 279 nm indicated a substituted aromatic ring. The IR spectrum exhibited absorbancies at 1605, 1580, 1520 cm^{-1} for the aromatic ring and at 1737 cm^{-1} for a six member ring ketone, the latter signal was correlated by the signal at δ 202.7 in the ^{13}C-NMR spectrum. The ^{1}H-NMR spectrum together with COSY and HETCOR experiments indicated the structure of **1** quite clearly, at δ 7.09 (1H, *s*, H-11), 6.75 (1H, *s*, H-14), 4.28 (1H, *br d*, J = 4 Hz, H-7α), 4.08 (1H, *d*, J = 6.5 Hz) and 4.16 (1H, *d*, J = 6.5 Hz) (oxymethylene protons), 3.85 (3H, *s*, OMe). The signals at δ 3.30 (1H, septet, J = 7 Hz, H-15) and 1.18 (3H, *d*, J = 7 Hz), 1.16 (3H, *d*, J = 7 Hz) (Me-16 and Me-17) showing the presence of isopropyl group, two other methyl groups were observed at δ 0.84 (3H, *s*, Me-18), and 0.86 (3H, *s*, Me-19). The COLOC experiment indicated the placement of the oxymethylene group between C-7 and C-20 (Table 1). The configuration at C-7 was assigned as H-7α was irradiated H-5α (δ 2.65) and H-14 (δ 6.75) enhanced. In the abietane type diterpenoids, H-1β is always observed as a broad doublet at around δ 2.5–3.0, the lack of this signal in compound **1** suggested that the ketone should be placed at C-1. This position for the keto group was correlated by the downfield shift of H-11 (δ 7.09), as well as the slight downfield shift of CH_2-20 protons (δ 4.08 and 4.16). The COLOC experiment showed the position of C-1 keton definitely by giving interaction between C-1 and H-11. Also, C-1

Table 2
Antimicrobial activity of compounds from *S. bracteata*

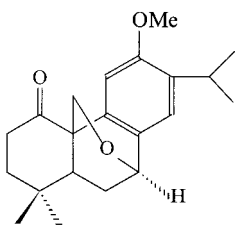
Compounds	Microorganisms		
	B. subtilis	*S. aureus*	*S. epidermidis*
Bractealine (**2**)	32.9[a]	NT[b]	16.80
Horminone	62.5	62.5	31.25
Ferruginol	> 250	> 250	> 250
Crude extract	30.6	61.3	245.5

[a] Values are given as μg/ml.
[b] NT: not tested.

had interactions with H-5 and H-20. The spectral data indicated 1-oxo-12-methoxy-7,20-epoxyabieta-8,11,13-triene structure for salvibractone (**1**).

The second new compound designated as bractealine (**2**), showed ^{1}H-NMR signals for an exomethylene group at δ 5.12 (1H, *br s*) and a methyl signal at δ 1.87 (3H, *br s*). Typical isopropyl group signals were missing (a septet for H-15 and two doublets for Me-16 and Me-17) indicating the presence of an isopropenyl group situated at C-13. The ^{13}C-NMR spectrum suggested the presence of a quinoid structure with signals at δ 184.8 *s* and 183.6 *s* (Table 1). The IR spectrum correlated with the presence of a paraquinoid ring system with the absorbancies at 1676, 1645, 1608 cm^{-1}. The HRMS of **2** indicated a molecular formula $C_{21}H_{28}O_4$ (*m/z* 344.1978, cal. 344.1987) showing eight degrees of unsaturation as double bond equivalents, of which three were accounted to the tricylic ring system, two for carbonyl groups and the remaining three for double bonds. Two of the oxygen atoms in the molecule were accounted for the two carbonyl groups, one for the methoxy group which was observed at δ 3.58 (3H, *s*) in the ^{1}H-NMR spectrum, the remaining one was the hydroxy group situated at C-12. Since there was no proton geminal to methoxy group, it could be placed either at C-5 or C-12, when a methoxy group attached on a double bond or on an aromatic or quinoid system it is usually observed downfield in the ^{1}H-NMR spectrum e.g. δ 3.80–4.10, therefore, the methoxy group was placed at C-5. The HETCOR experiment indicated the correlation between protons and carbons together with the COLOC experiment the structure of **2** was established unambiguously (Table 1). In order to further prove the position of the methoxy group, a NOE experiment was performed; the irradiation of the metyhoxy group (5α-OMe) caused enhancements in H-1α (δ 1.56), H-3α (δ 1.60), C-4α Me (δ 1.12) and H-6α (δ 1.58). From the spectral data the structure of bractealine was deduced as 5-methoxy-12-hydroxy-11,14-dioxo-abieta-8,12,15-triene.

The known and the new diterpenoids, as well as the crude extract of the roots were tested against standard bacterial strains namely *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538 P, *S. epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 8739, *Proteus mirabilis* ATCC 14153, *Klebsiella pneumonia* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231. As seen in Table 2, bractealine (**2**) had good activity (MIC 16.4 μg/ml) against *S. epidermidis*, this activity is comparable with the effects of cephalosporins, amicasin, kanamycin, mezlocillin, the commonly used antibiotics. Following values as the levels of susceptibility can be given (NCCLS, 1998): cefoperazone ≥ 16 μg/ml, amikasin ≥ 16 μg/ml, kanamycin ≥ 16 μg/ml. The crude extract and bractealine against *B. subtilis*; horminone against *S. epidermidis* showed quite good activity. These activities are comparable with those of commonly used antibiotics. Following values as the levels for intermediate susceptibility can be given: cefoperazone ≥ 32 μg/ml, various cephalosporins (cefotaxime, ceftizoxime etc.) ≥ 16–32 μg/ml, nefilmicin ≥ 32 μg/ml. The effect of horminone (MIC ≥ 62.5 μg/ml) against *B. subtilis* and *S. aureus* can also be considered as an intermediate susceptibility level against these bacteria and is compatible with that of nitrofurantoin (MIC ≥ 64 μg/ml). The crude extract against *S. epidermidis* and ferruginol against all three bacteria showed resistance with high MIC (Minimal Inhibitory Concentration) values.



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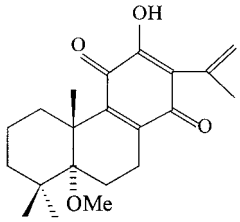

2

3. Materials and methods

3.1. General

UV spectra were recorded on a Shimadzu UV-1601 spectrophotometer; IR spectra on a Perkin–Elmer Model 983 in $CHCl_3$; NMR spectra on a Bruker AC-200 spectometer. HR and EIMS on a VG ZabSpec instrument; optical rotations were determined in an Opt. Act. Ltd. AA-5 polarimeter. Chromatographic separations were carried out on a silica gel (E. Merck) column and on Chromatotron rotors coated with 1 mm thick layers of silica gel 60 PF254 (7749) (E. Merck).

3.2. Plant material

The roots of *S. bracteata* Banks and Sol. in Russell, Aleppo. were collected by one of us (N.T.) from central Turkey, near Ankara in June 1996 and identified by Prof. Dr. Semra Kurucu (Ankara). A voucher specimen is deposited in the Herbarium of the Faculty of Pharmacy, University of Ankara (Ank. 19582).

3.3. Extraction of the crude fractions

Dried and powdered roots of *S. bracteata* (1.2 kg) were exhaustively extracted in a Soxhlet with acetone. The acetone extract, upon evaporation in vacuo yielded a gummy residue (13.6 g). The residue was dissolved in the least amount of CH_2Cl_2 and mixed with Si gel, dried at room temperature and added on top of a Si gel column (4 × 70 cm). The column was eluted with hexane, a gradient of CH_2Cl_2 was added up to 100% followed by EtOH. Similar fractions were combined to yield four main fractions (A–D).

3.4. Purification of fractions

Fractions A–D were applied to Si gel rotors of a Chromatotron, respectively, and eluted with hexane and with gradients of CH_2Cl_2 followed by MeOH. After Chromatotron separation, the compounds were further cleaned on preparative TLC plates. Fraction A yielded only oily compounds and was discarded. Fraction B 7-acetyl-royleanone (80 mg), 12-methylhorminone (25 mg), horminone (16 mg), 12-methylferruginol (10 mg), **1** (22 mg). Fraction C salvinolone (6 mg), **2** (18 mg), sugiol (6 mg), ferruginol (12 mg), 6,7-dehydroroyleanone (12 mg). Fraction D lupeol (16 mg) and Δ^7-stigmasterol (28 mg).

3.5. Salvibractone (*1*)

$[\alpha]_D$ = 0° ($CHCl_3$; *c* 1.0). Amorphous, colorless compound. UV λ^{MeOH} nm (log ε): 279 (3.8), 218 (4.6); IR ν^{CHCl_3} (cm^{-1}): 2924, 2853, 2094, 1737, 1617, 1605, 1580, 1520, 1494, 1368, 1341, 1284, 1163, 1118, 1070, 1026, 988, 918, 850; ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) (Table 1). HRMS *m/z*: 328.2024 $[M]^+$ ($C_{21}H_{28}O_3$). MS *m/z* (rel. int.): 328 $[M]^+$ (25), 298 $[M - OMe + H]^+$ (12), 284 (65), 269 (15), 223 (60), 205 (40), 149 (100), 135 (50), 121 (30), 104 (25), 69 (27), 57 (43).

3.6. Bractealine (*2*)

$[\alpha]_D$ = 0° ($CHCl_3$; *c* 0.8). Amorphous, dark yellow compound. UV λ^{MeOH} nm (log ε): 401 (2.0), 273 (4.2), 222 (4.4); IR ν^{CHCl_3} (cm^{-1}): 3377, 2925, 2854, 2094, 1676, 1645, 1500, 1460, 1392, 1377, 1248, 1153, 1137, 981, 942, 902, 847; ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) (Table 1). HRMS *m/z*: 344.1978 $[M]^+$ ($C_{21}H_{28}O_4$). MS *m/z* (rel. int.): 344 $[M]^+$ (2), 329 $[M - Me]^+$ (20), 313 $[M - OMe]^+$ (55), 262 (100), 245 (25), 203 (18), 137 (16).

3.7. Antimicrobial activity determination

The compounds **1**–**8** and the crude extract were dissolved in 1 : 10 diluted alcohol and further dilutions were made in sterile distilled water. The paper disc diffusion method was used for the preliminary, qualitative evaluation of their antimicrobial effects (NCCLS, 1997b). Overnight cultures of bacteria namely *B. subtilis* ATCC 6633, *S. aureus* ATCC 6538 P, *S. epidermidis* ATCC 12228, *E. faecalis* ATCC 29212, *E. coli* ATCC 8739, *P. mirabilis* ATCC 14153, *K. pneumonia* ATCC 4352, *P. aeruginosa* ATCC 27853, and the yeast *C. albicans* ATCC 10231 were adjusted to approximately 10^6 c.f.u./ml according to McFarland turbidity standards and spread over the appropriate media (Mueller–Hinton agar for bacteria, the same medium enriched with blood for *Enterococcus*, Sabouraud dextrose agar for the yiest *C. albicans*) in Petri dishes. Filter paper discs (⌀ 5 mm) impregnated with the solution (each disc containing 200 μg of compound) were placed on the air dried surface of the media inoculated with respective microorganisms. Discs containing the diluent were used as control. After overnight incubation at 37°C, the zones of inhibition around the discs were measured. The compounds that produced ≥15 mm zones were tested to determine the quantitative antimicrobial effects in respect to the broth media. The Macrodilution method (NCCLS, 1997a) was used and MIC values (μg/ml) were determined. The same test was carried out with 1 : 10 diluted alcohol as control. Diluted alcohol had no effect on the microorganisms.

Acknowledgements

This study was partly supported by JÜLICH-TÜBITAK grant and partly by the Reseach Fund of the University of Istanbul No 451 (given to AU, SÖ).

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