Pergamon

PII: S0031-9422(97)00310-5

Phytochemistry, Vol. 46, No. 3, pp. 531–534, 1997

Printed in Great Britain
0031-9422/97 $17.00+0.00

ABIETANE AND ICETEXANE DITERPENOIDS FROM *SALVIA BALLOTAEFLORA* AND *SALVIA AXILLARIS*

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(*Received* 5 *December* 1996)

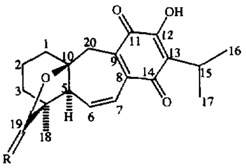
Key Word Index—*Salvia ballotaeflora*; *Salvia axillaris*; Labiatae; icetexane and abietane diterpenoids; conacytone; anastomosine; 19-deoxyicetexone; 19-deoxyisoicetexone; 7,20-dihydroanastomosine; cryptotanshinone.

Abstract—From the aereal parts of *Salvia ballotaeflora* three new icetexane diterpenoids were isolated besides anastomosine and conacytone. The structure of these compounds were established as 19-deoxyicetexone, 19-deoxyisoicetexone and 7,20-dihydroanastomosine by spectroscopic means. Cryptotanshinone was the sole diterpenoid isolated from both aerial parts and roots of *S. axillaris*. © 1997 Elsevier Science Ltd

INTRODUCTION

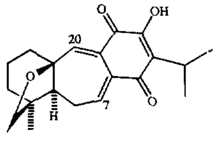
The phytochemical studies of the aerial parts and roots of European and Asiatic *Salvia* spp. led to the isolation of a number of diterpenoids with an abietane skeleton in almost 100% of the species studied [1]. The same chemical profile has been found for some Californian salvias (subgenus Audibertia) [2–5]. On the other hand, the phytochemical study of the aerial parts of several species of American salvias (subgenus Calosphace), led to the isolation of several diterpenes mainly of the neo-clerodane-type, in the major part of the sections studied, although some abietane and icetexane type diterpenoids have been isolated from few spp., belonging to Sections Erythrostachys, Conzatianna and Tomentellae [1]. Some abietane and icetexane diterpenoids isolated from *Salvia* spp. have been shown to posses an interesting, *in vitro*, antitumoural activity against colon, lung and breast carcinoma [6].

In a previous work on a population of *Salvia ballotaeflora* Benth (Section Tomentellae) collected near to Monterrey (Nuevo León State, México) three abietane-derived diterpenoids named icetexone, romulogarzone and conacytone were isolated [7]. In continuation of our studies on Mexican *Salvia* spp. looking for abietane-derived diterpenoid with potential antitumour properties, we have studied a different population of *S. ballotaeflora*. From the aerial parts of a population of *S. ballotaeflora* collected near to San Luis Potosi (México) we isolated three new icetexane-type diterpenoids (**1**–**3**) besides anastomosine and conacytone. The structures of the new compounds were established by spectroscopic means. From the aerial parts and roots of *Salvia axillaris* Moc. et Sessé (Section Axillares), the 20-*nor*-abietane cryptotanshinone (**5**) was the sole diterpenoid isolated.



1. R = H,H

4. R = O



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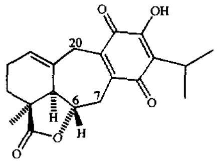

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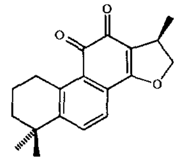

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RESULTS AND DISCUSSION

The aerial parts of *Salvia ballotaeflora* afforded, besides oleanolic acid and the previously known abietane derived diterpenoids icetexone (**4**) and anastomosine, three new icetexane diterpenoids whose structures were established as 19-deoxyicetexone (**1**), 19-deoxyisoicetexone (**2**) and 7,20-dihydro-

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anastomosine (**3**). From the aerial parts and roots of *Salvia axillaris* the 20-*nor*-abietane cryptotanshinone (**5**) was isolated. The structures of the new compounds were established from the following considerations.

Compound **1** was isolated as an orange crystalline solid which showed in the IR spectrum bands due to hydroxy (3348 cm^{-1}), quinone carbonyl groups (1649 and 1640 cm^{-1}) and conjugated double bonds (1601 cm^{-1}). The UV spectrum showed bands at 211, 290 and 318 nm, indicating the presence of an *ortho*-hydroxy-*p*-benzoquinone system in **1** [8, 9]. The ^1H NMR spectrum (Table 1) of **1** showed an AB system at δ 2.58 ($J = 13.8$ Hz) and δ 2.98 characteristic of the C-20 methylene protons of an icetexane skeleton [1]. An ABX system was observed at δ 6.5 (*dd*, $J = 12$, 4.8 Hz), 6.8 (*dd*, $J = 12$, 1.8 Hz) and 2.3 (*dd*), which was ascribed to the H-6, H-7 and H-5 protons, respectively. The characteristic signals of an isopropyl group bonded to a quinone system were also observed (H-15, δ 3.21, *sept*, $J = 6.9$ Hz and Me-16 and Me-17, δ 1.23 *d*) [8, 9]. These data, besides the ^{13}C NMR spectrum suggested that **1** possesses identical B and C rings to that present in icetexone (**4**), an icetexane-type diterpenoid previously isolated from a different population of *S. ballotaeflora* [7]. Other relevant signals in the ^1H NMR spectrum of **1** (Table 1) are those ascribed to the C-19 oxymethylene protons at δ 3.69 (*d*, $J = 7.8$ Hz, H-19 pro-*S*) and δ 3.49 (*dd*, $J = 7.8$ and 1.8 Hz, H-19 pro-*R*). The H-19 pro-*R* diastereotopic proton also shows a long-range coupling with H-3α as indicated by COSY experiments. A singlet at δ 92.9 and a triplet at δ 77.6 in the ^{13}C NMR spectrum of **1** supported the presence of an ethereal linkage between the C-10 and C-19 carbon atoms. These data suggest that compound **1** is the 19-deoxy derivative of icetexone (**4**). ^{13}C NMR spectrum of **1** supports the above discussion. Compound **1** must be named 19-deoxyicetexone.

Table 1. ^1H NMR data for compounds **1**–**3** ($CDCl_3$, TMS)

H	**1***	**2†**	**3‡**
1	—	—	5.68 *br d* (4.0)
5	2.30 *dd* (1.8, 4.8)	1.57 *br d* (9.6)	2.36 *br d* (10.5)
6α	6.50 *dd* (4.8, 12)	1.98 *m*	
6β		2.64 *dd* (9.6, 13.7)	3.81 *dt* (2, 10.5)
7α	6.80 *dd* (1.8, 12)	7.42 *br dd* (5.4, 9.6)	2.46 *ddd* (2, 10.5, 15)
7β	—	—	4.11 *dd* (2, 15)
20α	2.58 *d* (13.8)	7.27 *br s* —	2.73 *br d* (15)
20β	2.98 *d* (13.8)	— —	4.10 *d* (15)
15	3.21 *sept* (6.9)	3.37 *sept* (7.0)	3.23 *sept* (7)
3H-16	1.23 *d* (6.9)	1.25 *d* (7.0)	1.23 *d* (7)
3H-17	1.23 *d* (6.9)	1.26 *d* (7.0)	1.23 *d* (7)
3H-18	1.03 *s*	1.11 *s*	1.26 *s*
H-19 *pro-R*	3.49 *dd* (1.8, 7.8)	3.53 *dd* (2, 8)	— —
H-19 *pro-S*	3.69 *d* (7.8)	3.79 *d* (8)	
—OH	7.17 *s*	7.63 *br s*	7.06 *s*

Coupling constants in Hz are in parentheses. Chemical shifts are in δ values;

Run at *300, †200 and ‡500 MHz. Assignments confirmed by COSY experiments.

The mass spectrum of compound **2** indicated an isomeric molecular formula ($C_{20}H_{24}O_4$) concerning **1** (see Experimental). The ^1H NMR spectrum of **2** revealed the presence of a C-19 : C-10 ethereal linkage similar to that present in **1** and an isopropyl group bonded to a quinone system (Table 1). Besides these signals, the ^1H NMR data indicated that **2** is devoid of the AB-system due to the C-20 methylene protons of the icetexane skeleton. A broad singlet observed at δ 7.27 was ascribed to the olefinic H-20. The chemical shift of this proton indicated that C-20 is part of a conjugated system involving the quinone system. A broad *dd* at δ 7.42 was assigned to H-7, which also is part of the delocalized system of **2** (COSY experiments). The above findings indicated that **2** possesses identical B/C-rings to those present in the *nor*-icetexane diterpenoids fruticulin A and demethylfruticulin A, previously isolated from *Salvia fruticulosa* (Section Tomentellae) [10]. A similar functionality is also present in anastomosine [11] from *Salvia anastomosans* a species recently classified in Section Tomentellae [12]. UV spectrum of **2** supports the above discussion. Consequently, compound **2** must be named 19-deoxyisoicetexone.

Compound **3** was isolated as a yellow crystalline solid. The mass spectrum indicated a $C_{20}H_{22}O_5$ molecular formula and the UV spectrum was consistent with the presence of an *ortho*-hydroxy-*p*-benzoquinone system, which was also supported by the IR spectrum (see Experimental). Besides the quinone bands, the IR spectrum also showed the presence of a saturated γ-lactone (1770 cm^{-1}) in compound **3**. A doublet triplet at δ 3.81, in the ^1H NMR spectrum of **3** (Table 1), was ascribed to the germinal proton of this moiety. COSY experiments indicated that this proton was coupled with a broad doublet at δ 2.36 ($J = 10.5$ Hz) and with the protons of a methylene group at δ 2.46 (*ddd*, $J = 10.5$ and 15 Hz) and δ 4.11 (*dd*, $J = 2$ and 15 Hz). These signals were ascribed to H-5 and to the C-7 methylene protons H-7α and β, respectively. HMQC spectrum supported these assignments. The chemical shift observed for H-7β was due to the spatial proximity of this atom to the deshielding zone of the C-14 carbonyl group (Dreiding models). The coupling constants between these protons were consistent with an α-axial orientation for H-5 and a *β*-orientation for H-6. The *γ*-lactone ring present in **3** was, therefore, similar to that present in anastomosine [11]. Inspection of a Dreiding model of **3** indicated that the $\Delta^{1(10)}$ and Δ^{8}-double bonds exert a protective effect on H-6*β*, accounting for the chemical shift observed for this proton. The lack of the $\Delta^{1(10)}$-double bond produced a downfield shift (*δ* 4.3) of this proton, as observed in the tetrahydroanastomosine [11].

Other relevant signals observed in the ^{1}H NMR spectrum of **3**, were those due to the olefinic proton at C-1 (*br d*, *δ* 5.68) and the C-20 methylene protons at *δ* 2.73 (*br d*, H-20*α*) and *δ* 4.10 (*d*, H-20*β*) (Table 1). The chemical shift observed for H-20*β* was due to the proximity of the C-11 carbonyl group. COSY experiments indicated that H-20*α* weakly coupled with H-1 and H-7*α*. The ^{13}C NMR spectrum (Table 2) supported the previous discussion. Thus, compound **3** was related to anastomosine and must be named 7,20-dihydroanastomosine.

From the chromatographic purification of the acetone extract from the aerial parts and roots of *Salvia axillaris*, only the 20-nor-abietane cryptotanshinone (**5**) was isolated. The identity of this compound was confirmed by comparison of the physical properties obtained with those described in the literature [13].

The presence of an anastomosine derivative in this population of *Salvia ballotaeflora* supports the recent inclusion of *S. anastomosans* in Section Tomentellae which is one of the older of Calosphace subgenus [14]. On the other hand *Salvia axillaris* belongs to the most primitive Section of subgenus Calosphace (Section Axillares) and is considered a paleoendemic species [15]. It is noteworthy that cryptotanshinone, originally isolated from the Chinese species *Salvia miltiorrhiza* Bunge [13], has been recently isolated from several species belonging to Subgenus Audibertia, from Baja California (México), such as *S. mellifera*, *S. munzii* and *S. apiana* [3–5]. In this context it is interesting that Epling considered that *S. axillaris* is related to the species of subgenus Audibertia [16]. These findings are of phytogeographical significance and indicate a close chemical relation between some Chinese and American salvias. Although some botanical similarities between some areas of vegetation in Mexico and Asia (China) are well documented [17], this is, to the best of our knowledge, the first time that this relationship is chemically supported for the *Salvia* genus. The same chemical relationship has been found in some Mexican *Scutellaria* spp., such as *Scutellaria drummondii* [18], *S. caerulea*, *S. seleriana* and *S. guatemalensis* (B. Esquivel *et al.* unpublished results). These findings also suggest that some elements of the Mexican Labiatae could have migrated in the past from the Asiatic continent through North America to Mexico. Recently, the isolation of ferulic acid depsides, commonly found in Chinese species [19, 20], from the Mexican endemic species *Salvia adenophora* [6] reinforce this chemical relationship. The evaluation of the antitumoural activity of compounds **1**–**5** is in progress.

Table 2. ^{13}C NMR data of compounds **1**–**3** ($CDCl_3$, TMS)*

C	**1**†	**2**†	**3**‡
1	39.8 *t*	38.7 *t*	123.7 *d*
2	20.2 *t*	20.0 *t*	21.3 *t*
3	39.1 *t*	38.0 *t*	24.4 *t*
4	43.9 *s*	46.0 *s*	41.8 *s*
5	59.3 *d*	55.2 *d*	57.4 *d*
6	141.8 *d*	25.3 *t*	78.5 *d*
7	124.1 *d*	145.1 *d*	30.8 *t*
8	135.5 *s*	126.0 *s*	139.7 *s*
9	138.6 *s*	132.0 *s*	142.4 *s*
10	92.9 *s*	86.0 *s*	125.6 *s*
11	183.3 *s*	182.6 *s*	183.3 *s*
12	150.7 *s*	154.7 *s*	150.0 *s*
13	124.4 *s*	126.0 *s*	128.2 *s*
14	186.5 *s*	184.5 *s*	185.1 *s*
15	24.3 *d*	25.0 *d*	24.7 *d*
16	19.9 *q*	19.4 *q*	19.8 *q*
17	19.9 *q*	19.4 *q*	19.8 *q*
18	19.5 *q*	19.5 *q*	20.1 *q*
19	77.6 *t*	73.8 *t*	180.2 *s*
20	33.2 *t*	143.8 *d*	33.0 *t*

*Multiplicities were determined by the DEPT pulse sequence. † Run at 75 MHz. ‡ Run at 125 MHz. Assignments were confirmed by the HETCOR, HMQC and HMBC spectra.

EXPERIMENTAL

General. Mps: uncorr.; EIMS were obtained at 70 eV by direct inlet; UV MeOH; ^{1}H NMR: 200, 300 and 500 MHz, $CDCl_3$; ^{13}C NMR: 50 and 75 MHz $CDCl_3$, TMS as int. standard. HMBC and HMQC experiments were performed in a Varian Unity Plus Spectrometer (500 MHz). *Salvia ballotaeflora* was collected nearby San Luis Potosí (state of San Luis Potosí, México) and *S. axillaris* was collected near to Tamazulapan (state of Oaxaca, México). Voucher specimens (MEXU BER550 and MEXU 702364, respectively) were deposited at the herbarium of the Instituto de Biología UNAM.

Extraction, fractionation and isolation of the diterpenoids from Salvia ballotaeflora. Dried and powdered aerial parts of *S. ballotaeflora* (1250 g) were extracted with Me_2CO (2 × 10 l) for 4 days at room temp. The solvent was removed *in vacuo* to yield 100.2 g of a gummy residue. 45 g of this extract was subjected to vacuum chromatography over silica gel. Mixts of petrol–EtOAc of increasing polarity were used as eluents. Some frs eluted with petrol–EtOAc (9 : 1) were combined (5.53 g) and rechromatographed using the same solvents as eluents to afford compounds **1** (717 mg), **2** (518 mg) and **3** (27 mg). Other frs eluted with petrol–EtOAc (9 : 1) of the original chromatography were combined (6.24 g) and subjected to further chromatographic purification affording 10 mg of conacytone (**4**) and 28 mg of anastomosine. From the frs eluted with petrol–EtOAc (8:2), oleanolic acid (2 g) was isolated as an amorphous powder.

19-*Deoxyicetexone* (**1**). Orange crystalline solid, mp 228–230° (hexane–Me_2CO); $[\alpha]_D = +95$ (*c* 0.2, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3348, 1649, 1640, 1601; UV λ_{max}^{MeOH} nm (log ε): 211, (4.2), 290 (3.8), 318 (3.9); ^1H NMR: Table 1; ^{13}C NMR: Table 2; MS *m/z* (rel. int.): 330 (8), 329 (20), 328 (100), 313 (20), 300 (18), 299 (17), 317 (12), 285 (25), 270 (5), 257 (15), 256 (8), 71 (12), 57 (15), 43 (15). $C_{20}H_{24}O_4$ requires M^+ at *m/z* 328.

19-*Deoxyisoicetexone* (**2**). Yellow crystalline solid, mp 231–234° (hexane–Me_2CO); $[\alpha]_D = +38.6$ (*c* 0.13, MeOH); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3446, 1694, 1600; UV λ_{max}^{MeOH} nm (log ε): 206 (4.1), 238 (4.0), 276 (4.1), 314 (4.1); ^1H NMR: Table 1; ^{13}C NMR: Table 2; MS *m/z* (rel. int): 330 (10), 329 (30), 328 (100), 313 (25), 302 (10), 301 (40), 300 (70), 285 (95), 261 (70), 257 (80), 244 (20), 243 (30), 217 (30), 215 (25), 91 (15), 83 (15), 54 (30), 43 (40), 41 (30). $C_{20}H_{24}O_4$ requires M^+ at *m/z* 328.

7,20-*Dihydroanastomosine* (**3**). Yellow crystalline solid, mp 217–220° (hexane–Me_2CO); $[\alpha]_D = +55$ (*c* 0.1, CH_2Cl_2) IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3410, 1770, 1639, 1640, 1608; UV λ_{max}^{MeOH} nm (log ε): ^1H NMR: Table 1; ^{13}C NMR: Table 2; MS *m/z* (rel.int.) 344 (5), 343 (20), 342 (100), 327 (5), 314 (25), 299 (10), 286 (15), 271 (15), 243 (10), 225 (5), 197 (5), 179 (5), 165 (4), 141 (5), 129 (5), 105 (5), 83 (5), 81 (4), 41 (7). $C_{20}H_{22}O_5$ requires M^+ at *m/z* 342.

Extraction and isolation of cryptotanshinone from Salvia axillaris. Aerial parts of *S. axillaris* (310 g) were extracted with Me_2CO (3 × 3.5 l) for 3 days at room temp. The solvent of the combined extracts was removed to yield 8.4 g of a gummy residue which was subjected to CC over silica gel. Mixts of petrol–EtOAc of increasing polarity were used as eluents. From the frs eluted with petrol–EtOAc (9:1) and (8:2) cryptotanshinone (**5**) (30 mg) was obtained as an orange crystalline solid. Compound (**5**) (18 mg) was also obtained after the chromatographic sepn (as indicated above) of the acetone extract (4 × 400 ml, 2 days) obtained from the roots (11.7 g) of the same species.

Acknowledgements—The authors thanks María del Rosario García Peña (Instituto de Biología UNAM) for the botanical classification of plant material. We also thank to Drs Francisco González Medrano, Fernando Chiang and Miguel Angel Martínez (Instituto de Biología UNAM) for their helpful commentaries on the biogeography of Mexican plants. We are indebted to Dr F. del Rio and I. Chávez, B. Quiroz, R. Patiño, L. Velasco and F. Pérez for technical assistance. This work was supported in part by DGAPA-UNAM (Grant PAPIID IN 212594).

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