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ABIETANE DITERPENOIDS OF *SALVIA ANASTOMOSANS**

CRISTINA SÁNCHEZ, JORGE CÁRDENAS, LYDIA RODRÍGUEZ-HAHN† and T. P. RAMAMOORTHY§

Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, México, D. F.; §Instituto de Biología, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, México, D. F.

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Key Word Index—*Salvia anastomosans*, Labiatae; anastomosine; 9(10→20)-*abeo*-abietane

Abstract—The aerial parts of *Salvia anastomosans* afforded, in addition to oleanolic and ursolic acids, three abietane diterpenoids: conacytone and icetexone, of known structures, and anastomosine, a new 9(10→20)-*abeo*-abietane diterpenoid whose structure was elucidated by spectroscopic and chemical means.

INTRODUCTION

The genus *Salvia* L., is represented in Mexico by over 300 species classified in the subgenus *Calosphace* [1, 2]. Our systematic study of the Mexican *Salvia* species has shown that the diterpenoid content of plants studied depends on the Section to which it belongs [3]. Most of the diterpenoids isolated from *Salvia* species, subgenus *Calosphace*, are *neo*-clerodane diterpenoids or can be derived biogenetically from a clerodane precursor [3–5]. The presence of abietane diterpenoids in the species of the Section *Erytrostachys* [6] and 9(10→20)-*abeo*-abietance diterpenoids in the species of the Section *Tomentellae* studied up to now [7, 8], could have a phytogeographical significance.

In continuation of our systematic studies of Mexican *Salvia* spp., we have analysed the aerial parts of *S. anastomosans* Ramamoorthy, a perennial shrub recently classified in the Section Tomentellae (*Salvia*, subgenus *Calosphace*)[9].

RESULTS AND DISCUSSION

Extraction of the aerial parts of a population of *S. anastomosans* collected near Tamazulapan (Oaxaca) afforded, after extensive chromatography, oleanolic and ursolic acids and three abietane diterpenes, conacytone (**1**) and icetexone (**2**) previously isolated from *S. ballotaeflora*, and anastomosine to which we assigned structure **3**.

Anastomosine (**3**) has the molecular formula $C_{20}H_{20}O_5$. Its IR spectrum exhibited the characteristic absorptions due to a chelated hydroxyl group (3361 cm^{-1}), *p*-quinoid carbonyls (1656 cm^{-1}), double bonds (1602 cm^{-1}) and a saturated γ-lactone function (1778 cm^{-1}).

The ^1H NMR spectrum (Table 1) showed the signals due to an isopropyl group attached to a *p*-quinone ring (δ3.38, 1H, *septet*, $J=7$ Hz, H-15; δ1.25, 6H, *d*, $J=7$ Hz, Me-16 and Me-17) and a singlet (3H) at δ1.34 assigned to the tertiary Me-18. A singlet at δ7.74 (2H, one exchangeable with D_2O) was ascribed to the chelated phenolic hydroxyl group and to H-20. The signal centred at δ7.5 (1H, *dd*, $J=3$ and 0.4 Hz) was attributed to H-7, the 0.4 Hz coupling constant indicated a long range coupling with H-20. The proton geminal to the lactone closure was responsible for a double doublet observed at δ4.73 (1H, $J=11$ and 3 Hz), it was ascribed to H-6 as it was shown to be coupled to H-7 and H-5 by double resonance experiments. Irradiation at δ4.73 (H-6) transformed the doublet at δ7.5 (H-7) into a broad singlet and a doublet ($J=11$ Hz) at δ2.6 (H-5) into a singlet. The coupling constant of 11 Hz indicated a *trans*-diaxial relationship between H-6 and H-5; H-5 is α-axially oriented on biogenetic considerations [7], therefore H-6 must be β-axial. A signal at δ6.65 (*br t*, $W_{\frac{1}{2}}=10$ Hz) was ascribed to the vinylic H-1. Irradiation at δ2.5 (H-5 and H-2) transformed the signal at δ6.65 into a singlet and the double doublet at δ4.73 (H-6) into a doublet ($J=3$ Hz). It also simplified a double triplet observed at δ1.85 (1H, $J=13$ and 3 Hz) to a doublet ($J=13$ Hz) which could be ascribed to the 3β proton.

The ^{13}C NMR spectrum of anastomosine (Table 2) is in agreement with the structure (**3**) proposed for it, the assignments were made by comparison with the data of similar structures [7, 8]. C-6 was observed as a doublet at δ78.60.

Catalytic hydrogenation of **3** followed by air treatment, gave the tetrahydro-derivative **4**, in which hydrogenation of the 1, 10 double bond occured from the less hindered α face of the molecule. In the ^1H NMR of **4** (Table 1), H-6 is observed as a triple doublet at δ4.3 ($J=10$ and 4 Hz) due to the coupling of this proton with the C-7 methylene (H-7β, δ3.8, *dd*, $J=16$ and 4 Hz; H-7α, δ2.57, *dd*, $J=16$ and 10 Hz) and H-5. The paramagnetic displacement observed for H-7β (δ3.8) can be attributed to the deshielding effect exerted by the C-14 carbonyl group (molecular models). These data support the assignment of stereochemistry for C-5 and C-6 proposed for anastomosine (**3**). The β proton at C-20 is also deshielded by the C-11 carbonyl group and appears at δ3.05 as a double doublet ($J=14$ and 6 Hz), the 6 Hz coupling constant is adequate

*Contribution No 951 from Instituto de Química, UNAM.
†Author to whom correspondence should be addressed.

Table 1 ^{1}H NMR data of compounds 3–7($CDCl_3$, TMS as int stand.)

H	3	4	5	6	7
1	6 65 *t* (4)				
3	1 85 *dt* (13 3)				
5	2 60 *d* (11)	2 08 *m*	2 07 *m*	2 75 *m*	2 05 *m*
6	4 73 *dd* (11,3)	4.30 *td* (10,4)	4 29 *td* (10, 4)	6 13 *dd* (11 5, 5)	4 28 *td* (10, 3)
		α 2 57 *dd*	2 55 *dd*		2.78 *dd*
7	7 50 *dd* (3, 0.4)	(16, 10)	(15, 10)	6 58 *dd* (11 5, 2)	(14, 10)
		β 3 80 *dd*	3.72 *dd*		3.64 *dd*
		(16, 4)	(15, 4)		(14, 3)
15	3 38 *sept* (7)	3 23 *sept* (7)	3 25 *sept* (7)	3 24 *sept* (7)	3 40 *sept* (7)
Me-16	1 25 *d* (7)	1 23 *d* (7)	1 21 *d* (7)	1 20 *d* (7)	1 33*d* (7)
Me-17				1 22 *d* (7)	
Me-18	1.34 *s*	1 30 *s*	1 31 *s*	1 27 *s*	1 30 *s*
		α 2.50 *br d* (14)	2 44 *br d* (14)	2.42 *m*	2 57 *dd* (12, 3)
20	7 74 *s*	β 3 05 *dd* (14, 6)	3 02 *dd* (14, 6)	3 01 *dd* (12, 6)	3 14 *dd* (12, 6)
OH	7 74 *s*	7 03	—		
					3.75 *s*
OMe	—	—	3 96 *s*	3.93 *s*	3 84 *s*
					3 64 *s*
CO_2Me	—	—	—	3 64 *s*	—

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

for a dihedral angle of H-20β–C-20–C-10–H-10– of ≃ 50°, therefore H-10 must be α axially oriented.

Reductive methylation of anastomosine (**3**) yielded three main products which were characterized by spectral means. One of them was proved to be the methyl ether (**5**) of the tetrahydroderivative **4**. The less polar product was characterized as the methyl ester derivative **6** It showed in the IR spectrum a band at 1722 cm^{-1} due to the ester moiety. In the ^{1}H NMR spectrum (Table 1) a singlet (3H) at δ3.64 was assigned to this function. It also showed the methyl ether bound to C-12 as a singlet (3H) at δ3.93. The AB portion of an ABX system was observed at δ6.58 (*dd*, J = 11.5 and 2 Hz) and 6.13 (*dd*, J = 11.5 and 5 Hz) These signals were assigned to H-7 and H-6, respectively The coupling constant of 11.5 Hz indicated a *cis* double bond in a seven-membered ring[10]. The shape and coupling constants of H-6 and H-7 are equivalent to the proton reasonance signals due to H-6 and H-7 in the spectrum of icetexone (**2**). The formation of product **6** supports the γ-lactone closure at C-6 and the relative configuration proposed for C-5 in anastomosine (**3**).

The third product obtained proved to have structure **7** in which the C-ring has been aromatised. The IR spectrum of **7** showed a γ-lactone band at 1764 cm^{-1} and a band at 1602 cm^{-1} due to the aromatic *C* ring. In the ^{1}H NMR spectrum of **7** (Table 1), three singlets (3H each) were ascribed to the methyl ether groups The proton geminal to the lactone closure, H-6, was observed as a triple doublet at δ4.28 (J = 10 and 3 Hz). The assignment of the signals (Table 1) was based on double resonance experiments in a $CDCl_3/C_6D_6$ (1 : 1) solution

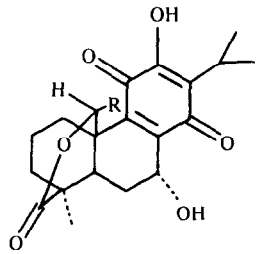
The formation of products **4**–**7** under reductive conditions is in accordance with structure **3** proposed for anastomosine.

Anastomosine (**3**) can be derived biogenetically from a 20-substituted icetexone derivative as shown in Scheme 1. The isolation of icetexone (**2**) and anastomosine from the same source makes this biogenetical relationship more plausible.

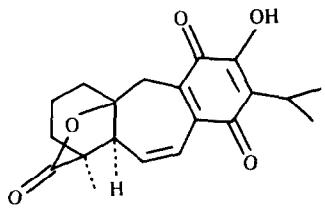
The presence of icetexone and anastomosine in *S anastomosans* provides chemical support for its classification in Section *Tomentellae(salvia,* subgenus *Calosphace)*, as this type of rearranged abietane diterpenoids have been found in all of the species of *Salvia* of this Section studied up to now [7, 8] We propose the name of *icetexane* for the 9(10→20)-*abeo*-abietane skeleton

EXPERIMENTAL

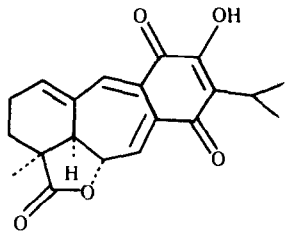
Mps uncorr MS were obtained at 70 eV by direct inlet ^{1}H and ^{13}C NMR were performed at 80 and 20 MHz, respectively, using TMS as int standard Plant material was collected at 5 km from Tamazulapan on the way to Chilapa (Oaxaca, México) and a voucher specimen (MEXU 4773) is deposited at the Herbarium of the Instituto de Biología, UNAM



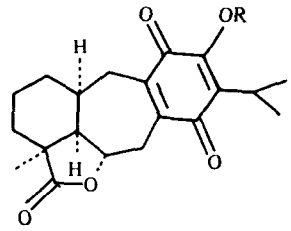
1 R=OH



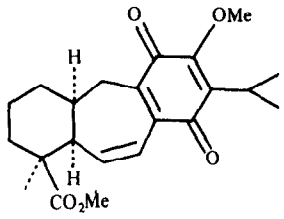
2



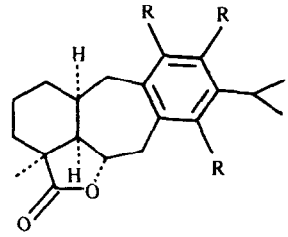
3



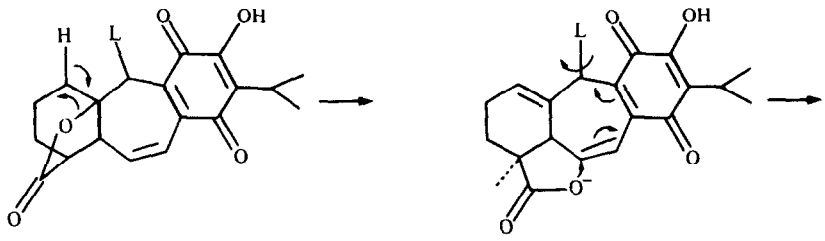
4 R=H
5 R=Me



6



7 R=OMe



Scheme. 1.

Isolation of the constituents from Salvia anastomosans. Dried aerial parts of *S. anastomosans* Ramamoorthy, (2.6 kg) were extracted with Me_2CO (20 l) at room temp. for one week. The solvent was removed under red. pres. and the gummy residue obtained (300 g) was chromatographed over silica gel (1 kg deactivated with 10% H_2O) Mixtures of petrol–EtOAc of increasing polarity were used as eluents. From the fractions eluted with petrol–EtOAc (19:1) conacytone (**1**, 3.5 g) and icetexone (**2**, 558 mg) were isolated and identified by comparison with authentic samples. From the fractions eluted with petrol–EtOAc (9:1), a mixture of oleanolic and ursolic acids was obtained, which were identified as their methyl esters

Repeated chromatography of the non-crystalline fractions eluted with petrol–EtOAc (9:1) yielded 345 mg of anastomosine

Table 2 ^{13}C NMR chemical shifts of compound **3** (20 HMz, $CDCl_3$, TMS as int standard)

C	
1	141 57 *d*[a]
2	23 08 *t*[b]
3	25 03 *t*[b]
4	41 53 *s*
5	47.72 *d*
6	78 60 *d*
7	143 06 *d*
8	131 98 *s*
9	133 79 *s*
10	124 26 *s*
11	181.39 *s*
12	155 12 *s*
13	129 16 *s*
14	182 74 *s*
15	25.37 *d*
16	19 63 *q*[c]
17	19 45 *q*[c]
18	21 25 *q*
19	179 73 *s*
20	140.80 *d*[a]

SFORD multiplicities are in parenthesis.

[a,b,c] Values may be interchanged

(**3**) as a yellow crystalline product mp 207–215°, $[\alpha]_D^{20} = +426.47$ (pyridine, *c* 0 034), UV λ_{max}^{MeOH} nm (ε) 200 (8000), 277 (4340), 330 (5060), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3361, 1778, 1656, 1602; ^1H NMR see Table 1, ^{13}C NMR see Table 2; MS *m/z* (rel. int.) 340 $[M]^+$ (100), 312 (12), 284 (8), 269 (14).

Catalytic hydrogenation of **3** Anastomosine (**3**, 70 mg) in EtOAc (10 ml) was hydrogenated using Pd-C (5%, 14 mg) as catalyst for 2 hr The catalyst was removed by filtration and the residue obtained after removal of the solvent, was disolved in Me_2CO and aerated The crystalline product obtained (**4**) showed mp 218–220° from CH_2Cl_2–petrol, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3408, 1768, 1642, ^1H NMR ($CDCl_3$) see Table 1, MS *m/z* (rel int) 344 $[M]^+$ (100), 298 (18), 95 (40), 55 (40), 41 (46)

Reductive methylation of **3** Anastomosine (**3**, 170 mg, 0.494 mmol) was hydrogenated at room temp in EtOAc (10 ml) using Pd-C (5%, 50 mg) as catalyst for 6 hr After the usual work-up the product of the reaction was dissolved in Me_2CO (40 ml) and treated with freshly dist $(MeO)_2SO_2$ (0 2 ml, 1.5 mmol) and K_2CO_3 (2 g) The reaction mixture was stirred for 40 min under reflux and 16 hr at room temp., $(MeO)_2SO_2$ (0 2 ml) and K_2CO_3 (2 g) were added and the reflux continued for 2 hr The reaction mixture was filtered, the solvent removed under red pres. and the residue stirred with H_2O for 3 hr and extracted with EtOAc The organic phase was washed, dried and the solvent removed The crude product obtained was separated by flash chromatography over silica gel The less polar product (**6**, 16 4 mg, 9%) was obtained as a yellow non crystalline product UV λ_{max}^{MeOH} nm (ε) 200 (7200), 265 (3000), 300 (1450), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1722, 1646, 1602, 1118, ^1H NMR ($CDCl_3$) see Table 1, MS *m/z* (rel int) 372 $[M]^+$ (100), 312 (37), 297 (27), 272 (34), 257 (18)

The second product obtained, **7** (22.4 mg, 11 68%), showed IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1764, 1602, 1118, ^1H NMR ($CDCl_3$) see Table 1, MS *m/z* (rel int.) 388 $[M]^+$ (100, 373 (30 4), 95 (17), 91 (16)

The most polar compound **5** was obtained as a yellow non-crystalline product (28 8 mg, 16 28%) UV λ_{max}^{MeOH} nm (ε) 200 (4200), 273 (3200), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1768, 1654, 1602, 1117, ^1H NMR ($CDCl_3$) see Table 1, MS *m/z* (rel int) 358 $[M]^+$ (100), 313 (5), 297 (8), 243 (10), 207 (6), 95 (20), 91 (13), 53 (12).

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