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NEO-CLERODANE DITERPENOIDS FROM THREE SPECIES OF *TEUCRIUM*

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Key Word Index—*Teucrium rivas-martinezii*, *T. divaricatum* subsp. *divaricatum*, *T. asiaticum*; Labiatae; diterpenoids; neo-clerodane derivatives; teucrasiatin.

Abstract—Three species of the genus *Teucrium* have been investigated. Two of them (*T. rivas-martinezii* and *T. divaricatum* subsp. *divaricatum*) contain known neo-clerodane diterpenoids, and the other one (*T. asiaticum*) contains a new neo-clerodane derivative, teucrasiatin, together with another previously known diterpene. The structure of teucrasiatin [(12*S*, 20*S*)-19-acetoxy-4α,18;15,16-diepoxy-6-oxo-*neo*-cleroda-13(16),14-diene-20,12-hemiacetal] was established by chemical and spectroscopic means. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

A large number of neo-clerodane diterpenoids have been isolated during the last few years from plants belonging to the genus *Teucrium* [1–4]. Interest in these compounds has been stimulated by their biological activity as antifeedants against some economically important lepidopterous pests [1, 5–10], as well as by their hepatotoxicity [11]. In continuation of our search for new insect antifeedants from natural sources [4, 6–8, 10, 12, 13], we report here on the identification of several neo-clerodanes from other *Teucrium* species and the structure elucidation of a new diterpenoid, teucrasiatin (**1**), found in the acetone extract of *T. asiaticum*.

RESULTS AND DISCUSSION

Teucrium rivas-martinezii, a species endemic in South-east Spain [14], yielded the already known neo-clerodane diterpenoids 19-acetylgnaphalin (**3**) [15] and 19-acetylteulepicin [16], and *T. divaricatum* subsp. *divaricatum* gave teucrin A [17], all of them previously isolated from other *Teucrium* species [1–3].

From the acetone extract of the aerial parts of *T. asiaticum* (an endemic species of the Balearic Islands), we have isolated two neo-clerodane derivatives, one of which, teucrasiatin (**1**), is a new compound. The other one was auropolin, a diterpenoid isolated for the first time from *T. polium* subsp. *aureum* [18] and also found, together with teuflin, in *T. asiaticum* [19].

Teucrasiatin (**1**) has the molecular formula $C_{22}H_{28}O_7$. Its ^1H and ^{13}C NMR spectra (Tables 1 and 2, respectively) showed characteristic signals for a β-substituted furan, a 4α,18-oxirane, a 19-acetoxyl group, a C-17 secondary methyl group, a C-6 ketone and a spiro-20,12-hemiacetal function involving the C-9, C-11, C-12 and C-20 carbons, identical with those found in several neo-clerodanes isolated from *Teucrium* species [12, 13, 15–20].

Treatment of teucrasiatin (**1**) with acetic anhydride–pyridine gave the monoacetyl derivative **2** ($C_{24}H_{30}O_8$), the IR spectrum of which was devoid of hydroxyl absorptions and whose ^1H NMR spectrum (Table 1) showed a paramagnetic shift of the H-20 signal ($\Delta\delta$ +0.76) with respect to **1**. Oxidation of **1** with chromium trioxide-pyridine yielded 19-acetylgnaphalin, the structure of which (**3**), including its neo-clerodane absolute configuration [21], is well known [15, 22, 23]. These transformations established that teucrasiatin possessed the structure and absolute stereochemistry depicted in **1**, except for the configuration at its C-20 asymmetric centre.

The 20*S* stereochemistry of teucrasiatin (**1**) was established by NOE experiments. Irradiation at δ 1.02 (Me-17 protons) caused positive NOE enhancements, among others, in the signals of the H-20 (3%), H_A-11 (2%), H-14 (2%) and H-16 (1%) protons. Moreover, irradiation at δ 5.55 (H-20) produced a NOE enhancement (5%) in the signal of the Me-17 protons. These results established that Me-17, the furan protons, H_A-11 and the C-20 hemiacetalic protons are on the same side of the plane defined by the 20,12-hemiacetal ring [24, 25]. This conclusion was also supported by comparing the chemical shifts of the C-8 and C-10 carbons

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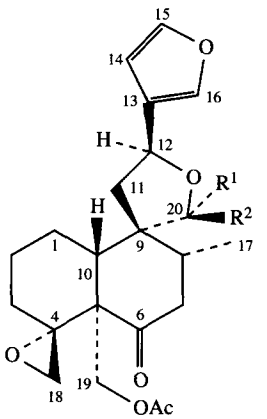

	R^1	R^2
1	OH	H
2	OAc	H
3	O	
4	H	OAc

of 20-*O*-acetylteucrasiatin (**2**) with those of gnaphalidin (**4**) [15, 22]. Thus the observed differences [Table 2: $\Delta\delta = \delta(\mathbf{2}) - \delta(\mathbf{4}) = +1.9$ ppm (C-8) and -1.3 ppm (C-10)] were similar to those found ($\Delta\delta$ +1.7 and -3.3 ppm for C-8 and C-10, respectively) between teulanigin and 20-epi-teulanigin, two 20-*O*-acetyl-20,12-hemiacetal-neo-clerodanes epimers at C-20 [26].

Finally, it is of interest to note that the C-20 configuration of gnaphalidin (**4**) was not initially established [15] and shortly afterwards this structural feature was defined as (20*S*)-20-*O*-acetyl-20,12-hemiacetal without sound arguments [22, 23]. Now, taking into account the structures of teucrasiatin (**1**) and its (20*R*)-20-*O*-acetyl derivative* (**2**), it is evident that the configuration at C-20 of gnaphalidin (**4**) depicted in previous communications [22, 23] was correct, because **2** and **4** are epimers at C-20.

EXPERIMENTAL

Mps: uncorr. Collection of the plant materials: *T. rivas-martinezii*, June 1995, at Minateda, near Hellín, Albacete, Spain; *T. divaricatum* subsp. *divaricatum*, July 1991, at Mitiline, Greece; *T. asiaticum*, June 1989, between Sóller and Pollensa, Majorca, Spain. Voucher specimens were deposited in the Herbarium of the Royal Botanic Garden, Madrid (*T. rivas-martinezii*), and the 'Dipartimento di Biologia' of the University of Milan, Italy (*T. divaricatum* subsp. *divaricatum* and *T. asiaticum*).

Extraction and isolation of the diterpenoids. Dried and finely powdered *T. rivas-martinezii* Alcaraz, Garre, Martínez-Parras et Peinado [14] aerial parts (320 g) were extracted with Me_2CO (2 l × 3) at room temp. for 1 week. The extract (15.2 g) was subjected to CC (silica gel, Merck No. 7734, deactivated with 10% H_2O, w/v, 400 g) eluting with petrol and petrol–EtOAc mixts. The frs eluted with EtOAc–petrol (1:1) yielded 19-acetylgnaphalin (**3**, 206 mg) [15] and elution with EtOAc–petrol (2:1) gave 19-acetylteulepicin (250 mg) [16].

Dried aerial parts of *T. divaricatum* Sieber subsp. *divaricatum* Celak (410 g) were extracted as above. The extract (22 g) was chromatographed (CC) as above giving teucrin A (25 mg, eluted with EtOAc) [17] as the sole detectable diterpene constituent.

Dried and powdered *T. asiaticum* L. (syn. *T. lancifolium* Boiss.) aerial parts (194 g) were extracted as above yielding an extract (20 g), which was subjected to CC as above giving teucrasiatin (**1**, 250 mg, eluted with EtOAc–petrol 1:1) and auropolin (2 g, eluted with EtOAc-petrol 2:1) [18].

The previously known diterpenoids, 19-acetylgnaphalin, 19-acetylteulepicin, teucrin A and auropolin, were identified by their physical (mp, $[\alpha]_D$) and spectroscopic (^{1}H NMR, MS) data and by comparison (mmp, TLC) with authentic samples.

Teucrasiatin (**1**). Mp 230–232° (EtOAc–*n*-hexane); $[\alpha]_D^{18}$ +6.6° ($CHCl_3$; *c* 0.334). IR ν_{max}^{KBr} cm^{-1}: 3350 (hydroxyl), 3140, 3130, 3120, 1500, 875 (furan), 3040 (oxirane), 1735, 1250 (OAc), 1715 (ketone), 2950, 2880, 1470, 1380, 1220, 1160, 1025, 980, 810; ^{1}H NMR: Table 1; ^{13}C NMR: Table 2; EI-MS (70 eV, direct inlet) *m/z* (rel. int.): 404 $[M]^+$ (0.3), 386 $[M - H_2O]^+$ (0.7), 344 $[M - HOAc]^+$ (0.6), 331 $[M - CH_2OCOMe]^+$ (10), 313 (20), 285 (15), 219 (18), 205 (12), 202 (12), 201 (13), 191 (20), 175 (22), 163 (47), 162 (30), 161 (29), 159 (32), 147 (43), 145 (44), 134 (75), 119 (25), 105 (40), 95 (81), 93 (100), 91 (48), 81 (86), 77 (73), 69 (89), 55 (79), 43 (70). (Found: C, 65.41; H, 6.86. $C_{22}H_{28}O_7$ requires: C, 65.33; H, 6.98%.)

20-O-*Acetylteucrasiatin* (**2**) *from teucrasiatin* (**1**). Treatment of **1** (26 mg) with Ac_2O–pyridine (1:1, 5 ml) for 48 hr at room temp. yielded **2** (21 mg, after crystallization from EtOAc–*n*-hexane): mp 196–198°; $[\alpha]_D^{18}$ +47.9° ($CHCl_3$; *c* 0.401). IR ν_{max}^{KBr} cm^{-1}: 3150, 3125, 1595, 1500, 875 (furan), 3050 (oxirane), 1750, 1735, 1240 (OAc), 1715 (ketone), 2990, 1480, 1455, 1370, 1155, 1095, 1020, 980, 830, 755; ^{1}H NMR: Table 1; ^{13}C NMR: Table 2; EI-MS (70 eV, direct inlet) *m/z* (rel. int.): $[M]^+$ absent, 386 $[M - HOAc]^+$ (2), 343 (13), 326 $[M - 2HOAc]^+$ (4), 313 (19), 297 (18), 255 (18), 216 (42), 205 (23), 189 (32), 145 (37), 121 (33), 105 (44), 95 (88), 94 (70), 91 (73), 81 (95), 79 (62), 77 (51), 69 (57), 55 (65), 43 (100). (Found: C, 64.81; H, 6.69. $C_{24}H_{30}O_8$ requires: C, 64.56; H, 6.77%.)

*In accordance with the convention of Canh, Ingold and Prelog, a 20*R* (or 20*S*) carbon atom of a neo-clerodane-20,12-hemiacetal must be defined as 20*S* (or 20*R*) when the hemiacetalic hydroxyl group is acetylated, although the C-20 absolute configuration is the same in both cases.

Table 1. ^{1}H NMR spectral data of compounds **1** and **2***

H	1†	2‡	*J* (Hz)	1†	2‡
1α	2.45 *qd*	2.25§	1α, 1β	14.5	§
1β	§	2.18§	1α, 2α	4.9	§
2α	§	2.00§	1α, 2β	14.5	§
2β	1.40 *m*	1.44 *m*	1α, 10β	14.5	§
3α	~2.10§	2.00§	1β, 2β	~14	§
3β	1.19 *dt*	1.26 *dt*	2α, 3β	4.9	4.2
7α	2.69 *t*	2.57 *dd*	2β, 3α	4.9	§
7β	2.24 *dd*	2.38 *dd*	2β, 3β	4.9	4.2
8β	1.82 *ddq*	1.89 *ddq*	3α, 3β	13.4	13.6
10β	~2.10§	2.18§	3α, 18B	2.0	1.7
11A‖	1.89 *dd*	1.96 *dd*	7α, 7β	14.3	15.2
11B¶	2.30 *dd*	2.44 *dd*	7α, 8β	14.3	13.5
12	5.23 *dd*	5.12 *dd*	7β, 8β	3.4	3.8
14	6.40 *dd*	6.38 *dd*	8β, 17	6.8	6.9
15	7.42 *t*	7.39 *t*	11A, 11B	13.1	13.2
16	7.40 *m*	7.41 *m*	11A, 12	10.2	9.9
Me-17	1.02 *d*	1.08 *d*	11B, 12	7.1	7.3
18A**	2.33 *d*	2.39 *d*	14, 15	1.8	1.8
18B††	3.28 *dd*	3.09 *dd*	14, 16	0.9	0.8
19A	4.72 *d*	4.16 *d*	15, 16	1.8	1.8
19B	4.88 *d*	4.76 *d*	18A, 18B	5.4	5.2
20	5.55 *d*‡‡	6.34 *s*	19A, 19B	12.5	11.6
OAc	2.05 *s*	2.18 *s*	20, 20 (OH)	2.4§§	—
	—	2.02 *s*			
20 (OH)	2.92 *d*§§				

*In $CDCl_3$. Chemical shifts are reported with respect to residual $CHCl_3$ (δ 7.25). Spectral parameters were obtained by first order approximation. All these assignments were in agreement with HMQC spectra.

†At 300 MHz.

‡At 500 MHz.

§Overlapped signal.

‖H_A-11 is the pro-*R* hydrogen and it was distinguished by NOE experiments.

¶Pro-*S* hydrogen.

***Exo* hydrogen with respect to ring B.

††*Endo* hydrogen with respect to ring B.

‡‡Collapsed into a singlet after addition of D_2O.

§§Disappeared after addition of D_2O.

Table 2. ^{13}C NMR spectral data of compounds **1**, **2** and **4**

C	1*	2*	4†	C	1*	2*	4†
1	23.7 *t*	23.9 *t*	23.3 *t*	13	124.9 *s*	124.4 *s*	125.7 *s*
2	24.9 *t*	24.9 *t*	24.9 *t*	14	108.7 *d*	108.6 *d*	108.4 *d*
3	32.9 *t*	32.6 *t*	33.0 *t*	15	143.5 *d*	143.5 *d*	143.4 *d*
4	60.9 *s*	60.7 *s*	61.1 *s*	16	139.4 *d*	139.4 *d*	139.4 *d*
5	54.8 *s*	54.2 *s*	54.3 *s*	17	17.5 *q*	17.4 *q*	18.0 *q*
6	206.9 *s*	205.8 *s*	205.6 *s*	18	49.9 *t*	50.6 *t*	49.2 *t*
7	46.2 *t*	46.5 *t*	45.0 *t*‡	19	64.5 *t*	63.6 *t*	61.8 *t*
8	43.6 *d*	43.2 *d*	41.7 *d*	20	99.5 *d*	98.3 *d*	97.8 *d*
9	53.5 *s*	53.0 *s*	51.6 *s*	OAc	171.2 *s*	170.6 *s*	170.4 *s*
10	53.8 *d*	52.6 *d*	55.1 *d*		20.9 *q*	169.6 *s*	169.3 *s*
11	44.4 *t*	44.5 *t*	45.7 *t*‡		—	21.6 *q*	21.3 *q*
12	70.8 *d*	72.4 *d*	71.1 *d*		—	20.8 *q*	20.7 *q*

*At 125.7 MHz in $CDCl_3$. Chemical shifts are reported with respect to the solvent (δ_{CDCl_3} 77.0). Multiplicities were determined from DEPT and HMQC spectra.

†Taken from ref. [22] (25.2 MHz, $CDCl_3$).

‡These assignments may be reversed.

19-*Acetylgnaphalin* (**3**) *from teucrasiatin* (**1**). CrO_3–pyridine oxidation of **1** (15 mg) in the usual manner gave **3** [11 mg, mp 226–228° (EtOAc–*n*-hexane); $[\alpha]_D^{18}$ +81.4° ($CHCl_3$; *c* 0.217)] identical in all respects (^1H NMR, MS, TLC, mmp) with 19-acetylgnaphalin [lit. [15]: mp 227–229° (EtOAc–*n*-hexane); $[\alpha]_D^{22}$ +82° ($CHCl_3$; *c* 0.315)].

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