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C-10 OXYGENATED NEO-CLERODANE DITERPENES FROM *TEUCRIUM SANDRASICUM*

G. TOPCU,*† C. ERIŞ,† C-T. CHE‡ and A. ULUBELEN‡§

†TUBITAK, Marmara Research Center, Research Institute for Basic Sciences, P.O. Box 21, 41470, Gebze, Turkey; ‡Department of Chemistry, The Hong Kong University of Science & Technology, Clear Water Bay, Kowloon, Hong Kong; §Faculty of Pharmacy, University of Istanbul, 34452 Istanbul, Turkey

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Abstract—From the aerial parts of *Teucrium sandrasicum*, three new C-10 oxygenated neo-clerodane diterpenes were isolated. The structures of the compounds were established by spectral data including spin decoupling, ^{1}H–^{1}H COSY, HETCOR, COLOC and NOESY experiments.

INTRODUCTION

There are 27 *Teucrium* species in Turkey, eight of which are endemic. They are used in folk medicine as a treatment for diabetes [1]. As a part of our studies on the terpenoids of Turkish *Teucrium* species [2–3], we have now investigated the aerial parts of *T. sandrasicum* O. Schwarz, a species endemic to Turkey. The acetone extract of the plant yielded three new C-10 oxygenated neo-clerodane derivatives, sandrasin A (**1**), 6-deacetylsandrasin A (**2**) and sandrasin B (**3**) whose structures were established on the basis of spectroscopic evidence including spin decoupling, COSY, HETCOR, COLOC and NOESY experiments.

RESULTS AND DISCUSSION

The HREIMS of sandrasin A (**1**) gave a molecular formula $C_{24}H_{30}O_{10}$ (*m/z* 478.1832, calcd. 478.1838). The UV spectrum showed a maximum at 208 nm. The IR spectrum was consistent with the presence of hydroxyl(s) (3390 cm^{-1}), acetyl group(s) (1720, 1710, 1245 cm^{-1}), a γ-lactone ring (1760 cm^{-1}) and a β-substituted furan ring (3150, 3120, 1508 and 880 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of sandrasin A (**1**) (Table 1) showed signals attributed to a tertiary methyl group (δ_H 1.20, 3H, *s*; δ_C 26.0), a β-substituted furan ring with two α-protons [δ_H 7.47, *d*, J = 1.2 Hz; δ_C 139.6 (C-16), δ_H 7.41, *t*, J = 1.2 Hz; 144.2 (C-15)] and a β-proton [δ_H 6.33, *d*, J = 1.2 Hz; δ_C 108.0 (C-14)] in addition to a quaternary C singlet [δ_C 124.9 (C-13)], 4α,18-spiro-oxirane ring protons [δ_H 3.02, *d*, J = 3.5 Hz and 2.40, *br d*, J = 3.5 Hz (overlapped with the signal of H-11); δ_C 51.6 (C-18), 62.9 (C-4)] and a 20,12-lactone group [δ_H 5.38 *br t*, J = 9.0 Hz, H-12β; δ_C 72.2 (C-12), 174.6 (C-20)] similar to those of other neo-clerodane diterpenoids isolated from some *Teucrium* species [4–8]. The signals at δ_H 5.42 (*dd*, J = 12 and 5 Hz, H-6β) and δ_C 67.3, and at δ_H (5.20, *br d*, J = 13 Hz, H-19a) and 4.40 (*br d*, J = 13 Hz, H-19b), and δ_C 63.6 indicated that two acetyl groups were located at C-6 and C-19, the acetyl signals being at δ_H 1.92 (3H, *s*) and 2.03 (3H, *s*) and δ_C 21.20 for two acetyl methyls and at 170.6 for two carbonyl groups. The couplings of H-6 with the protons of C-7 [δ 2.75 (1H, *t*, J = 12.5 Hz, H-7α) and 1.72 (1H, *dd*, J = 12.5 and 5 Hz, H-7β)] indicated the α position of the acetyl group at C-6 which was confirmed by spin decoupling and COSY experiments. The relationships between H-12 and the protons of C-11 [δ 2.52 (1H, *dd*, J = 9, 15 Hz, H-11β) and 3.12 (1H, *dd*, J = 9, 15 Hz, H-11α)] and between H-19a and H-19b, and H-18a and 18b, and also the sequence H-14–H-16 were also revealed by COSY experiment. From its ^{13}C NMR spectrum, there were two tertiary hydroxyl groups in the molecule, one at δ 75.8 corresponding to C-8, the other at δ 81.9 which was placed at C-10, owing to paramagnetic shifts on C-1 ($\Delta\delta$ + 7.9), C-5 ($\Delta\delta$ + 3.8) and C-9 ($\Delta\delta$ + 5.2) with respect to those of closely related neoclerodane derivatives either lacking or with an α-hydroxyl group at C-10 [9–11]. The β-configuration assignment of this hydroxy group was supported by its deshielding effect on one of the C-11 methylene protons [H-11α (*pro*-S), δ 3.12] which is close to the 10β-hydroxyl group. The relative configurations at C-6, C-10 and C-12 were deduced from the NOESY results, thus two hydroxyl groups at δ 4.90 (C-10 OH) and δ

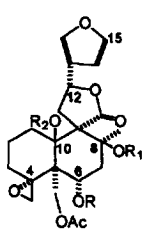
*Author to whom correspondence should be addressed.

Table 1. ^1H and ^{13}C NMR data of compounds **1**–**3** (in $CDCl_3$)

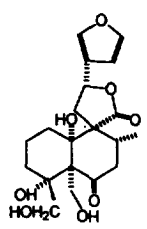
	1		**2**		**3**	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C*
1	1.18, 2.20	31.8 *t*	1.18, 2.16	30.6 *t*	#†	25.4 *t*
2	1.80, 2.05	18.8 *t*	1.78, 2.00	18.9 *t*	#†	14.9 *t*
3	1.75, 1.90	29.4 *t*	1.75, 1.85	29.3 *t*	#†	21.0 *t*
4	–	62.9 *s*	–	63.9	–	74.8 *s*
5	–	49.8 *s*	–	51.6 *s*	–	51.9 *s*
6	5.42	67.3 *d*	4.36	64.9 *d*	–	197.0 *s*
7	1.72, 2.75	38.2 *t*	186, 2.52	39.3	2.28, 3.55	37.7 *t*
8	–	75.8 *s*	–	76.0 *s*	1.58	31.2 *d*
9	–	59.3 *s*	–	59.6 *s*	–	53.8 *s*
10	–	81.9 *s*	–	81.5 *s*	–	84.0 *s*
11	2,52, 3.12	34.8 *t*	2.52, 3.16	34.9 *t*	1.85, 2.78	33.0 *t*
12	5.38	72.2 *d*	5.39	72.1 *d*	5.41	71.2 *d*
13	–	124.9 *s*	–	125.0 *s*	–	119.2 *s*
14	6.33	108.0 *d*	6.39	108.0 *d*	6.40	107.3 *d*
15	7.41	144.2 *d*	7.42	144.1 *d*	7.39	143.2 *d*
16	7.47	139.6 *d*	7.49	139.6 *d*	7.47	139.4 *d*
17	1.20	26.0 *q*	1.25	26.1 *q*	1.00	15.3 *q*
18	2.40, 3.02	51.6 *t*	2.62, 3.32	51.6 *t*	3.70, 3.86	69.8 *t*
19	4.40, 5.20	63.6 *t*	4.54, 5.08	63.8 *t*	3.78, 3.98	59.2 *t*
20	–	174.6 *s*	–	174.8 *s*	–	174.2 *s*
C=O |	–	170.6 *s*	–	170.8 *s*	–	–
CH_3	1.92	21.2 *q*	2.03	21.1 *q*	–	–
C=O |	–	170.6 *s*	–	–	–	–
CH_3	2.03	21.2 *q*	–	–	–	–

*In $CDCl_3$ + acetone-d_6.
†Overlapped signals.

3.44 (C-8 OH) showing NOEs with H-6β, indicated their β orientations, H-6β also showed a NOE with H-18a (δ 3.02). The *R* configuration of H-12 was deduced by the observation of a NOE between Me-17 and H-12; this stereochemistry has been studied extensively by other investigators [12, 13]. The β orientation of H-12 was also supported by the NOE between H-11β (δ 2.52) and H-12. The C-10 oxygenated neoclerodanes are rare, they were first isolated from a Turkish *Teucrium* species by Spanish scientists [11, 14]. The ^{13}C NMR (APT) spectrum of **1** indicated the presence of three methyl quartets as two signals, seven methylene triplets, five methine doublets and nine quaternary carbons (as eight signals) for 24 carbon atoms. The protons and carbons were unambiguously assigned by HETCOR (Table 1) and COLOC experiments. The locations of the C-8 and C-10 hydroxyl groups followed from the COLOC experiment which showed correlations between H-19 (δ 5.20) and C-10, and between H-1 (δ 1.18) and C-9 as well as C-8. Because the hydroxyl groups were tertiary, acetylation under normal conditions was not possible, but when carried out for 48 hr with heating, a product with four acetyl groups was obtained (**1a**). The acetyl signals were at δ 1.98 (3H, *s*) and 2.09 (9H, *s*). The remaining ^1H NMR signals of **1a** resonated with more or less the same frequencies as those of **1** (see Experimental).



1 R=Ac, R_1=R_2=H
1a R=R_1=R_2=Ac
2 R=R_1=R_2=H



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The second new compound (**2**) had a molecular formula $C_{22}H_{28}O_9$ as deduced from its high-resolution its EI-MS (*m/z* 436.1728, calcd. 436.1733). The signals in the ^1H and ^{13}C NMR spectra of compound **2** were similar to those of compound **1** with only one of the acetyl signals missing (Table 1). All the NMR signals were similar in both compounds **1** and **2**; however, due to the lack of an acetyl group at C-6, H-6β was shifted upfield to δ_H 4.36, *dd*, J = 3.5 and 12 Hz and δ_C 64.9 (C-6) in **2**. The relationships between H-6β and the C-7 protons were deduced from spin decoupling and COSY experiments, which revealed all of the ^1H–^1H correlations in compound **2**. Acetylation of **2** yielded compound **1**, whose spectral data were the same as those of authentic **1**, while acetylation of **2** for 48 hr by heating

yielded **1a** which had the same spectral data as those of compound **1a** prepared from **1**, thus indicating the relationship between these two compounds. The ^{13}C NMR (APT and DEPT) showed the presence of two methyl quartets, seven methylene triplets, five methine doublets and eight carbon singlets for 22 carbon atoms. Unambiguous assignment of the protons and carbons was possible by HETCOR (Table 1) and COLOC experiments. The NOESY results showed the same stereochemistry for **2** as in compound **1**. All the spectral evidence showed that the acetyl group at C-6 is missing in compound **2**, therefore it was named 6-deacetylsandrasin A.

The third new compound, sandrasin B (**3**), had a molecular formula $C_{20}H_{26}O_8$ (*m/z* 394.1622, calcd. 394.1627). The spectral data of sandrasin B (**3**) had similarities and differences compared with data from compounds **1** and **2**. The spiro-oxirane ring at C-4 was opened up to form a β-CH_2OH and an α-OH groups, as indicated by the NOESY experimental data. A NOE was observed between the C-19 protons and the C-4 α-OH group. This was supported by the reports [15] that 4-α,18-epoxy-6α-hydroxy- or 6-oxo-neo-clerodanes appear to be biosynthetically transformed into 4α-hydroxy derivatives. No acetyl group was present in compound **3**, while furan ring signals were observed at similar frequencies [δ_H 7.47, *d*, $J = 1.2$ Hz; δ_C 139.4 (C-16), δ_H 7.39, *d*, $J = 1.2$ Hz; δ_C 143.2 (C-15), δ_H 6.40, *d*, $J = 1.2$ Hz; δ_C 107.3 (C-14)] together with a quaternary C singlet at δ 119.2 (C-13). The C-19 methylene group was observed at δ_H 3.78 (*d*, $J = 12.5$, H-19a) and 3.98 (*d*, $J = 12.5$ Hz, H-19b) and δ_C 59.2 (C-19) indicating a hydroxymethylene instead of an acetoxymethylene group. The C-18 methylene signals were at δ_H 3.70, (*d*, $J = 11$ Hz, H-18a), 3.86 (*d*, $J = 11$ Hz, H-18b) and δ_C 69.8 (C-18) and C-4 at δ 74.8. The C-8 methyl group was observed as a doublet at δ 1.00 (3H, *d*, $J = 7$ Hz), indicating the lack of the tertiary hydroxyl group at C-8, and H-8 was at δ 1.58 as a multiplet while the C-7 protons were at δ 2.28 (1H, *dd*, $J = 2.5$ and 13 Hz, H-7β) and 3.55 (1H, *t*, $J = 13$ Hz, H-7α). The chemical shift of H-12 was similar to those of compounds **1** and **2** [δ_H 5.41, *t*, $J = 8.5$ Hz; δ_C 71.2 (C-12)] and C-11 signals were at δ_H 2.78 (*dd*, $J = 9$ and 15 Hz, H-11α) and 1.85 (*dd*, $J =$ 8.5 and 15 Hz, H-11β), and δ_C 33.0 (C-11). The chemical shift of the C-11 protons indicated an α-hydroxyl group instead of a β-hydroxyl at C-10, which was confirmed by NOESY correlations; thus, NOE interaction was observed between the C-10 α-OH and C-19 protons and also between the 4α-OH and H-19. The NOE correlation between Me-17 and H-12 indicated the *R* configuration for the latter. No secondary hydroxyl signal was observed in the molecule from its ^1H and ^{13}C NMR spectra and the presence of a carbonyl group at δ 197.0 indicated that an oxo group replaced the hydroxyl group at C-6, which was supported by biogenetic considerations and also by the chemical shift of C-8 at δ 3.12 [16]. If the oxo group was at C-7, the latter signal (δ 31.2) would be shifted downfield to *ca* 40–50 ppm [17–19]. The spectral data indicated the given structure for sandrasin B (**3**).

EXPERIMENTAL

General. ^1H and ^{13}C NMR: Bruker 200 MHz for COLOC and NOESY Joel JNM Ex-400; HREI-MS: VG ZabSpec.

Plant material. The aerial parts of *T. sandrasicum* were collected at an altitude of 1150 m from Muğla (Köyceğiz, Sandras mountain), south-western Turkey, in June 1993. The plant was identified by Dr Kerim Alpinar (Istanbul), a voucher specimen (ISTE 65135) is deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul.

Extraction and isolation of the compounds **1–3**. The dried and powdered aerial parts of the plant (2.2 kg) were extracted with distilled Me_2CO at room temp., filtered and evapd to dryness *in vacuo*. The crude residue (134 g) was fractionated on a silica gel column (5 × 70 cm) eluting with petrol, followed by a gradient of $CHCl_3$ up to 100%, followed by EtOAc and EtOH both to 100%. The $CHCl_3$–EtOAc (4:1) frs gave 22 mg (**1**), 28 mg (**2**) and 15 mg (**3**).

Forced acetylation of compounds **1** *and* **2**. In order to acetylate the tertiary hydroxyl groups, compounds **1** and **2** (10 mg each) were separately dissolved in 1 ml pyridine then 1 ml Ac_2O was added and the soln heated on a water bath for 48 hr at 80°.

Sandrasin A (**1**). $[\alpha]_D$ +7° (*c* 0.5, $CHCl_3$). UV λ_{max}^{MeOH} (log ε) nm: 208 (3.6), 235 (sh); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3390, 3150, 3120, 2980, 2840,1760, 1720, 1710, 1508, 1480, 1360, 1270, 1245, 1200, 1090, 1030, 950, 880; ^1H and ^{13}C NMR: Table 1; HREI-MS *m/z* (rel. int.): 478.1832 $[M]^+$ (12), 461 $[M - H_2O + 1]^+$ (23), 419 $[M - OAc]^+$ (25), 358 $[M - 2 \times HOAc]^+$ (80), 340 $[358\text{-}H_2O]^+$ (57), 254 (50), 213 (55), 203 (70), 177 (100), 108 (85), 91 (93), 79 (85).

Acetyl derivative of **1** (**1a**). IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3050, 2940, 1760, 1740, 1445, 1360, 1240, 1080, 950, 875; ^1H NMR: δ 5.40 (1H, *dd*, $J = 3.5$, 12 Hz, H-6β), 5.37 (1H, *t*, $J = 8.5$ Hz, H-12), 2.40 (1H, *d*, $J = 3.5$ Hz, H-18a), 3.08 (1H, *d*, $J = 3.5$ Hz, H-18b), 4.4 (1H, *d*, $J = 13.5$ Hz, H-19a), 5.26 (1H, *d*, $J = 13.5$ Hz, H-19b), 6.33 (1H, *d*, $J = 1.2$ Hz, H-14), 7.40 (1H, *d*, $J =$ 1.2 Hz, H-15), 7.43 (1H, *d*, $J = 1.2$ Hz, H-16), 1.98 (3H, *s*, OAc), 2.09 (9H, *s*, 3 × OAc), 1.20 (3H, *s*, Me-17).

6-*Deacetylsandrasin A* (**2**). $[\alpha]_D$ +19.4° (*c* 0.3, $CHCl_3$). UV λ_{max}^{MeOH} (log ε) nm: 208 (3.6), 235 (sh); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3430, 3150, 3090, 2960, 2880, 1760, 1730, 1620, 1510, 1480, 1370, 1260, 1240, 1180, 1040, 880, 780; ^1H and ^{13}C NMR: Table 1; HREI-MS *m/z* (rel. int.): 436.1728 $[M]^+$ (2), 418 $[M - H_2O]^+$ (8), 376 $[M - HOAc]^+$ (6), 358 $[M - HOAc - H_2O]^+$ (28), 345 $[M - CH_2OAc - H_2O]^+$ (40), 192 (60), 176 (50), 94 (100), 81 (68).

Acetyl derivative of **2** (**1**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3390, 3150, 2980, 1760, 1720, 1510, 1480, 1360, 1270, 1245,

1200, 1090, 1030, 950, 875; ^{1}H NMR ($CDCl_3$) δ: 5.43 (1H, *dd*, $J = 3.5$, 12 Hz, H-6β), 5.39 (1H, *t*, $J =$ 8.5 Hz, H-12), 2.40 (1H, *d*, $J = 3.5$ Hz, H-18a), 3.04 (1H, *d*, $J = 3.5$ Hz, H-18b), 4.40 (1H, *d*, $J = 13.5$ Hz, H-19a), 5.22 (1H, *d*, $J = 13.5$ Hz, H-19b), 6.36 (1H, *d*, $J = 1.2$ Hz, H-14), 7.41 (1H, *d*, $J = 1.2$ H-15), 7.48 (1H, *d*, $J = 1.2$ Hz, H-16), 1.93 (3H, *s*, OAc), 2.03 (3H, *s*, OAc), 1.22 (3H, *s*, Me-17).

Tetraacetyl derivative of **2** (**1a**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3050, 1760, 1740, 1445, 1360, 1240, 1080, 950, 875; ^{1}H NMR ($CDCl_3$) δ: 5.40 (1H, *dd*, $J = 3.5$, 12 Hz, H-6β), 5.37 (1H, *t*, $J = 8.5$ Hz, H-12), 2.40 (1H, *d*, $J = 3.5$ Hz, H-18a), 3.08 (1H, *d*, $J = 3.5$ Hz, H-18b), 4.40 (1H, *d*, $J = 13.5$ Hz, H-19a), 5.26 (1H, *d*, $J =$ 13.5 Hz, H-19b), 6.35 (1H, *d*, $J = 1.2$ Hz, H-14), 7.39 (1H, *d*, $J = 1.2$ Hz, H-15), 7.43 (1H, *d*, $J = 1.2$ Hz, H-16), 1.98 (3H, *s*, OAc), 2.07 (9H, *s*, 3 × OAc), 1.20 (3H, *s*, Me-17).

Sandrasin B (**3**). $[\alpha]_D$ −12.5° ($c = 0.1$, $CHCl_3$), UV λ_{max}^{MeOH} (log ε) nm: 208 (3.5). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 2900, 2890, 1760, 1500, 1450, 1340, 1180, 1020, 880, 800, 740; ^{1}H and ^{13}C NMR: Table 1; HREI-MS *m/z* (rel. int.): 394.1622 $[M]^+$ (100), 341 (10), 311 (18), 283 (22), 255 (34), 231 (23), 203 (34), 161 (57), 109 (53), 81 (86), 60 (53).

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