Pergamon 0031-9422(95)00302-9 Phytochemistry, Vol. 40, No. 6, pp. 1737–1741, 1995
Elsevier Science Ltd
Printed in Great Britain
0031-9422/95 $9.50 + 0.00

NEO-CLERODANE DITERPENOIDS FROM *TEUCRIUM YEMENSE*

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(*Received* 13 *February* 1995)

IN HONOUR OF PROFESSOR ANTONIO G. GONZALEZ

Key Word Index—*Teucrium yemense*; Labiatae; *neo*-clerodane; diterpenoids; flavone; NMR.

Abstract—The aerial parts of *Teucrium yemense* yielded four new *neo*-clerodane diterpenoids, namely, 6β-*O*-acetyl-3β-hydroxyteucroxylepin, teucryemin, 19-*O*-acetylteucryemin and teucryeminone, in addition to the known flavone cirsiliol. The structures of the new compounds were elucidated from their spectral data, by chemical derivatization and by comparison with closely related compounds.

INTRODUCTION

Diterpenoids of the genus *Teucrium* have been the subject of numerous phytochemical investigations, due to their diverse biological activities [1]. *Teucrium yemense* Defl. grows widely in the southern part of Saudi Arabia. Examination of the aerial parts of this plant led to the isolation and characterization of four new *neo*-clerodane diterpenes, which are the subject of this paper.

RESULTS AND DISCUSSION

The EtOAc extract of the aerial parts of *T. yemense* was partitioned between MeCN and *n*-hexane. Chromatography of the MeCN fraction using a combination of column chromatography (silica gel) and centrifugal preparative TLC (Chromatotron®), led to the isolation of four new *neo*-clerodane diterpenes.

Compound **4**, $C_{22}H_{28}O_8$, gave rise to a ^{1}H NMR spectrum (Table 1) that suggested a structure closely related to 3-*O*-desacetylteugracilin A (**7**), a *neo*-clerodane diterpenoid isolated from *T. gracile* [2]. The only difference being that compound **4** has an acetyl group at C-6 instead of C-19. The presence of an acetyl group at C-6 in **4** caused a downfield shift of the respective proton (δ5.92, *d*, J = 2.2 Hz) relative to that of **7** (δ4.16) [2]. The H-19 protons of compound **4** appeared as two double doublets at δ5.07 (J = 12.8, 4.3) and 4.54 (J = 12.8, 5.2 Hz) due to further coupling with the OH group. Acetylation of **4** yielded the acetate **6**, which was identical with the acetylation product of teugracilin A [2], thus confirming the structure and stereochemistry of **4**, which we have named teucryemin.

Compound **5**, $C_{24}H_{30}O_9$, was found to have the same general NMR features as **4**, but its H-19 protons appeared as two down-field doublets, suggesting the presence of an acetate group at C-19 (see Tables 1 and 2). Its structure was firmly established by correlation with **4**. Thus on acetylation it gave the same product, namely, **6**.

Compound **1**, $C_{22}H_{28}O_8$, gave rise to ^{1}H and ^{13}C NMR data that were very close to those of teucroxylepin (**2**), a diterpenoid isolated from *Teucrium oxylepis* subsp. *marianum* [3]. The fact that lactonization occurred between C-19 and C-20 was shown by comparison of the chemical shift values of C-12, C-19 and C-20, at δ63.3, 70.9 and 171.7, respectively, with those of **2**, as well as other similar compounds [4–6].

A significant difference between **1** and **2** was the presence of an equatorially-disposed hydroxyl group at C-3 of **1**, as indicated by the ^{1}H NMR (DMSO-d_6) signal at δ3.92 (*dd*, J = 11.8, 5.2 Hz, H-3α). Also, while C-6 in **2** is hydroxylated, the H-6α ^{1}H NMR signal of **1** at δ4.61 (*br t*, J = 3.6, 3.6 Hz) suggested the presence of an axial acetate group, instead. Comparison of the ^{13}C NMR data of **3**, the diacetyl derivative of **1**, to those of the diacetyl derivative of teucroxylepin [3], added further support to the structure of **1**, since the only observed differences were for the C-1, C-2, C-3, C-4, C-5 and C-18 carbon resonances, due to the hydroxylation of C-3. The structure of compound **1** was further confirmed by correlation with compound **4**. This was accomplished by treating compound **4** with K_2CO_3 [3], followed by acidification then acetylation to give a product indistinguishable from **3**. Therefore, compound **1** is 6β-*O*-acetyl-3β-hydroxyteucroxylepin.

Compound **8**, $C_{24}H_{28}O_9$, showed almost identical ^{1}H and ^{13}C NMR spectra to **9**, the reported acetylation product of teulepicin [2, 6]. While most signals had almost identical chemical shift values, it was observed that the proton chemical shift value of the Me-17 group was at a slightly lower-field position in **8** than in **9**. More remarkably, the carbon chemical shift values of C-8, C-9

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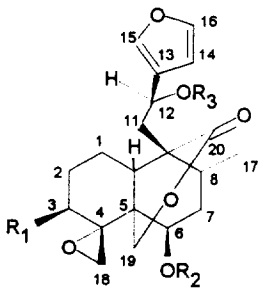
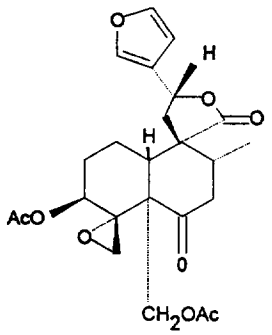


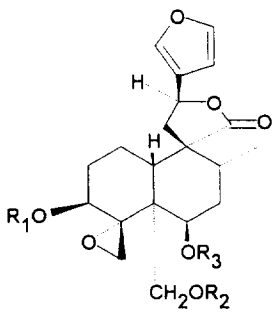
1 R_1=OH, R_2=Ac, R_3=H

2 R_1=R_2=R_3=H

3 R_1=OAc, R_2=R_3=Ac



8

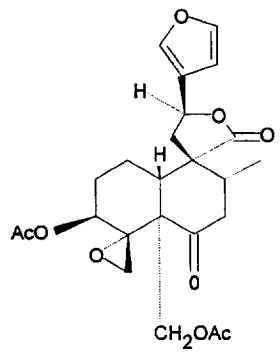


4 R_1=R_2=H, R_3=Ac

5 R_1=H, R_2=R_3=Ac

6 R_1=R_2=R_3=Ac

7 R_1=R_3=H, R_2=Ac



9

and C-13 were at lower field values in **8** than **9** [2], while that of C-10 was more deshielded in **9** than **8**. Furthermore, Compound **8** was found to have different physical data from those of **9**: $[\alpha]_D$ = + 9.6° ($CHCl_3$, *c* 0.224) and mp 136–138° (EtOAc-*n*-hexane), $[\alpha]_D^{18}$ = + 36.6° ($CHCl_3$, *c* 0.172) and mp 189–190° (EtOAc–*n*-hexane), for **8** and **9** [6], respectively.

The above differences in the chemical shift values between **8** and **9** suggested that the two compounds were epimeric at C-12, as previously shown in related compounds [7–9]. This would require compound **8** to have a 12*R* configuration. This was substantiated by studying the NOESY spectra, which clearly indicated that H-12 and H-17 were on the same side of the molecule [5, 7, 8]. Also, irradiation of the Me-17 protons of **8** produced about a 5% enhancement of the intensity of the H-12 proton [7, 8]. As a control, similar NOE studies were performed on compound **4**, but no enhancement of the H-12 signal was observed, instead there was enhancement of the H-14 furanic proton at δ6.64, as previously reported [5, 7] for other 12*S* diterpenes. Compound **8** has been named teucryeminone.

The presence of diterpenoids with different stereochemistry at C-12 in the same plant is not uncommon, as it has been previously reported in other species of the genus *Teucrium*, namely, *T. montanum* [10] and *T. pyrenaicum* [9].

EXPERIMENTAL

Mps: uncorr.; IR: KBr and thin film; ^1H and ^{13}C NMR: 300 and 75 MHz, respectively, in $CDCl_3$ (unless otherwise stated), TMS as int. standard; standard Varian software was used for 2D NMR COSY, HETCOR and NOESY (using 500 MHz), which aided structural assignments; EIMS: 70 eV; $[\alpha]_D$: at ambient temp., centrifugal preparative TLC (CPTLC): Chromatotron® instrument, Harrison Research Inc., model 7924, silica gel P_{254} plate (4 mm, flow rate 4 ml min^{-1}); TLC: silica gel 60 F_{254}, $CHCl_3$–MeCN

Table 1. ^{1}H NMR spectral data (300 MHz, $CDCl_3$, unless otherwise stated) and coupling constants (in Hz) for diterpenoids **1**, **3–6** and **8**

H	1	3	4*	5	6	8
3α	4.03†	5.19 *dd*	4.88 *dd*	4.35 *dd*	5.28 *dd*	5.44‡
6α	4.61 *br t*	4.57 *t*	5.92 *d*	5.12 *br d*	5.07 *br s*	—
7α	1.53 *td*	1.53 *td*	1.87 *m*	1.80 *m*	1.65 *m*	3.33 *t*
7β	1.93 *dt*	1.97 *dt*	2.44 *m*	2.18 *dt*	2.28 *m*	2.14 *m*
8β	2.21 *m*	2.24 *m*	1.97 *m*	1.84 *m*	1.87 *m*	2.15 *m*
10β	2.95 *dd*	2.60 *m*	2.60 *m*	2.27 *dd*	2.36 *dd*	2.02 *m*
11A	2.20 *dd*	2.55 *dd*	2.52 *dd*	2.42 *dd*	2.47 *dd*	2.35 *dd*
11B	2.42 *dd*	2.61 *dd*	2.59 *dd*	2.51 *dd*	2.50 *dd*	2.56 *dd*
12	4.86 *br d*	6.07 *dd*	5.60 *t*	5.39 *t*	5.38 *t*	5.40 *t*
14	6.44 *m*	6.41 *dd*	6.62 *m*	6.38 *m*	6.38 *dd*	6.36 *dd*
15	7.41 *m*	7.38 *dd*	7.69 *dd*	7.43 *t*	7.43 *t*	7.45 *m*
16	7.44 *m*	7.44 *m*	7.84 *m*	7.45 *m*	7.45 *m*	7.46 *m*
17Me	0.83 *d*	0.84 *d*	0.99 *d*	0.98 *d*	0.97 *d*	1.18 *d*
18A	2.49 *d*¶	2.49 *d*¶	3.19 *d***	2.84 *d*¶	2.70 *d*¶	2.60 *d*¶
18B	3.23 *d***	3.10 *d***	3.35 *d*¶	2.88 *d***	2.88 *d*[b]	3.25 *d***
19A	4.00 *d*	4.03 *d*	4.54 *dd*	4.86 *d*	4.74 *d*	4.81 *d*
19B	4.64 *d*	4.69 *d*	5.07 *dd*	4.98 *d*	4.80 *d*	5.50 *d*
OAc	2.13 *s*	2.12 *s*	2.10 *s*	2.07 *s*	2.08 *s*	2.05 *s*
	—	2.06 *s*	—	2.09 *s*	2.06 *s*	1.98 *s*
	—	2.02 *s*	—	—	1.99 *s*	—
OH	—	—	6.77 *br d*	—	—	—
	—	—	5.76 *t*	—	—	—
J (Hz)						
3α, 2α	§	4.5	5.1	3.5	4.5	§
3α, 2β	§	11.2	9.8	11.7	10.7	§
6α, 7α	3.6	2.5	2.2	2.1	—	—
6α, 7β	3.6	2.5	§	§	§	§
7α, 8β	§	12.2	§	§	§	14.0
7β, 8β	4.0	4.3	§	§	§	3.6
8β, 17	6.8	6.8	6.7	6.7	6.6	6.5
10β, 1α	12.8	§	§	13.2	13.1	§
10β, 1β	4.3	§	§	4.5	3.7	§
11A, 11B	16.1	16.5	14.2	14.2	14.4	14.0
11A, 12	9.3	10.0	8.6	8.6	8.7	8.7
11B, 12	3.1	3.0	8.7	8.6	8.5	8.7
14, 15	§	1.9	1.6	1.7	1.7	1.8
14, 16	§	0.8	0.7	0.8	0.8	1.0
15, 16	§	1.8	1.7	1.7	1.8	1.9
18A, 18B	4.0	4.1	6.0	5.2	5.2	5.9
19A, 19B	13.9	14.0	12.7	13.2	13.0	13.4
19A, OH	—	—	4.3	—	—	—
19B, OH	—	—	5.5	—	—	

*Spectrum run in pyridine-d_5.

†Signal resonated as double doublet (δ3.92, J = 5.5, 11.8 Hz) in DMSO-d_6, other signals are comparable to those in $CDCl_3$ spectrum.

‡Signal resonated as double doublet (δ5.63, J = 5.3, 12.0 Hz) in C_6D_6, other signals are comparable to those in $CDCl_3$ spectrum.

§Overlapped signals (J = unresolved).

¶*Exo* hydrogen with respect to ring [2].

**_Endo_ hydrogen with respect to ring [2].

(6:4) as solvent system, with visualization using *p*-anisaldehyde/H_2SO_4 as spray reagent.

Plant material. The aerial parts of *Teucrium yemense* Defl. [11] were collected in Abha, Saudi Arabia, in August 1993. A voucher specimen was deposited in the herbarium of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Isolation of the diterpenoids. The dried ground aerial parts (650 g) were percolated with EtOAc (10 l) at room temp. The EtOAc extract (28.5 g) was partitioned between MeCN and *n*-hexane. The MeCN fraction (10 g) was chromatographed on a silica gel column (type 60, 190 g) and eluted with *n*-hexane–EtOAc (1:9) yielding three frs, namely fr. A (0.83 g), fr. B (1.39 g) and fr.

Table 2. ^{13}C NMR data (75 MHz, $CDCl_3$, unless otherwise stated) for diterpenoids **1**, **3**–**6** and **8***

C	1	3	4†	5	6	8
1	23.3 *t*	22.5 *t*	22.5 *t*	22.1 *t*	21.5 *t*	21.1 *t*
2	30.9 *t*	29.1 *t*	31.7 *t*	31.9 *t*	30.9 *t*	30.6 *t*
3	67.4 *d*	68.2 *d*	66.5 *d*	66.0 *d*	68.6 *d*	66.2 *d*
4	62.2 *s*	59.4 *s*	66.2 *s*	63.9 *s*	62.0 *s*	62.0 *s*
5	40.4 *s*	40.6 *s*	46.9 *s*	54.3 *s*	45.2 *s*	53.6 *s*
6	70.3 *d*	69.9 *d*	70.6 *d*	69.6 *d*	69.9 *d*	205.5 *s*
7	32.7 *t*	32.3 *t*	33.8 *t*	30.6 *t*	29.5 *t*	43.6 *t*‡
8	30.5 *d*	30.3 *d*	33.3 *d*	33.1 *d*	33.0 *d*	43.3 *d*
9	49.4 *s*	49.0 *d*	52.2 *d*	51.7 *d*	51.5 *d*	51.9 *d*
10	38.6 *d*	37.5 *d*	47.5 *d*	47.1 *d*	46.0 *d*	52.6 *d*
11	36.3 *t*	32.9 *t*	44.8 *t*	45.1 *t*	44.7 *t*	43.5 *t*‡
12	63.3 *d*	64.3 *d*	72.1 *d*	71.8 *d*	71.7 *d*	71.8 *d*
13	130.1 *s*	125.5 *s*	126.3 *s*	125.0 *s*	125.0 *s*	124.9 *s*
14	108.3 *d*	108.6 *d*	108.9 *d*	107.9 *d*	107.9 *d*	107.8 *d*
15	143.8 *d*	143.6 *d*	144.8 *d*	144.2 *d*	144.2 *d*	144.5 *d*
16	138.5 *d*	139.8 *d*	140.6 *d*	139.0 *d*	139.5 *d*	139.2 *d*
17	16.1 *q*	15.9 *q*	16.5 *q*	16.0 *q*	16.1 *q*	17.4 *q*
18	48.4 *t*	48.8 *t*	45.4 *t*	45.0 *t*	46.3 *t*	42.4 *t*
19	70.9 *t*	70.6 *t*	61.7 *t*	62.2 *t*	62.8 *t*	62.8 *t*
20	171.7 *s*	170.7 *s*	177.6 *s*	176.7 *s*	176.4 *s*	175.8 *s*
OAc	169.8 *s*	169.7 *s*	169.7 *s*	170.8 *s*	170.7 *s*	171.0 *s*
	—	169.5 *s*	—	169.0 *s*	169.2 *s*	169.4 *s*
	—	169.5 *s*	—	—	168.8 *s*	—
	21.5 *q*	21.3 *q*	21.5 *q*	21.6 *q*	21.4 *q*	20.8 *q*
	—	21.1 *q*	—	21.1 *q*	20.9 *q*	20.7 *q*
	—	20.9 *q*	—	—	20.9 *q*	—

*Multiplicities of the carbon signals of all compounds were determined by APT and DEPT experiment.

†Spectrum run in pyridine-d_5.

‡Interchangeable signals.

C (0.98 g). Fr. A, on concentration, gave 105 mg of a yellow solid, mp 295–296°, (lit. mp 279°C [12]), which was identified as cirsiliol by direct comparison with an authentic sample. Fr. B (1 g) was subjected to CPTLC (4 mm silica gel plate) using toluene–EtOAc–HOAc (65 : 35 : 1) as eluent to afford teucryeminone (**8**) (75 mg, R_f 0.56), followed by fr. B-1 (270 mg), containing a mixture of **1** and **5**. This mixture was separated by reversed-phase CC using MPLC over RP C-18 (25–40 μm, flow rate 5 ml min^{-1}) using MeOH–H_2O (1 : 1) as the solvent system to give 6β-*O*-acetyl-3β-hydroxyteucroxylepin (**1**) (150 mg, R_f 0.27), followed by 19-*O*-acetylteucryemin (**5**) (45 mg, R_f 0.34). Fr. C, on the other hand, afforded teucryemin (**4**) by crystallization from Me_2CO (260 mg, R_f 0.13).

6β-O-Acetyl-3β-hydroxyteucroxylepin (**1**). Pale yellow gum; $[\alpha]_D$ −67.3° ($CHCl_3$, *c* 0.224). IR ν_{max}, film, cm^{-1}: 3450 (br OH), 1720 (δ-lactone); ^1H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): 420 $[M]^+$ (6) and 360 $[M - HOAc]^+$ (12).

Teucryemin (**4**). Needles from Me_2CO, mp 199–200°; $[\alpha]_D$ − 23° (MeOH, *c* 0.106). IR ν_{max}^{KBr}, cm^{-1}: 3460 br (OH), 1760 (δ-lactone); ^1H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): $[M]^+$ absent, 402 $[M–H_2O]^+$ (0.4), 372 $[M–CH_2O–H_2O]^+$ (2.5) and 342 $[M–HOAc–H_2O]^+$ (2).

19-O-*Acetylteucryemin* (**5**). Needles from $CHCl_3$–MeOH, mp 178–180°; $[\alpha]_D$ + 5.5° (MeOH, *c* 0.108). IR ν_{max}^{KBr} cm^{-1}: 3450 br (OH), 1735 (δ-lactone), 1715 (OAc), 1235; ^1H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): $[M]^+$ absent, 389 $[M–CH_2OAc]^+$ (0.5) and 342 $[M–2 \times HOAc]^+$ (2).

Teucryeminone (**8**). Needles from *n*-hexane–EtOAc, mp 136–138°; $[\alpha]_D$ + 9.6° ($CHCl_3$, *c*. 0.224). IR ν_{max}^{KBr} cm^{-1}: 1735 (δ-lactone), 1715 (OAc), 1235; ^1H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): 460 $[M]^+$ (3).

Acetylaton of compounds **1**, **4** and **5**. Compounds **1**, **4** and **5** (30 mg each) were treated separately with 5 ml pyridine and 2.5 ml Ac_2O at room temp. for 12 hr. Regular work-up of the reaction mixture of **1** yielded compound **3** as plates (25 mg), while compounds **4** and **5** afforded the same compound **6** as needles (27 mg each).

3β-Acetoxy-6β, 12S-O-diacetylteucroxylepin (**3**). Plates from $CHCl_3$–MeOH, mp 202–203°; $[\alpha]_D$ − 33° ($CHCl_3$, *c* 0.21); IR ν_{max}^{KBr} cm^{-1}: 1735 (δ-lactone), 1720 (OAc), 1230;

^{1}H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): $[M]^+$ absent, 462 $[M-C_2H_2O]^+$ (0.7).

3β,19-O-Diacetylteucryemin (**6**). Needles from $CHCl_3$–MeOH, mp 119–121°; $[\alpha]_D$ − 9° ($CHCl_3$, *c* 0.33) lit. [2] mp for an amorphous solid 85–95°, $[\alpha]_D$ − 9.9° ($CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1750 (δ-lactone), 1720 (OAc), 1230; ^{1}H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): $[M]^+$ absent, 342 $[M - 2 \times HOAc-C_2H_2O]^+$ (0.1).

Acknowledgements—The authors thank Dr C. D. Hufford, department of Pharmacognosy, University of Mississipi, U.S.A. for measurement of the 300 MHz 1D and 2DNMR spectra; Dr K. Zaw, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois, U.S.A. for the 500 MHz NOESY spectra; Dr A. Nahrstedt, Institute für Pharm. Biologie und Phytochemie Munster, Germany for EIMS spectra and Dr S. Abedin for identification of the plant material.

REFERENCES

1. Harborne, J. B. and Baxter, H. (1993) *Phytochemical Dictionary, A Handbook of Bioactive Compounds from Plants*, p. 645. Taylor & Francis, London.
2. Bruno, M., Dominguez, G., Lourenco, A., Piozzi, F., Rodríguez, B., Savona, G., de la Torre, M. C. and Arnold, N. A. (1991) *Phytochemistry* **30**, 3693.
3. Cuadrado, M. J. S., de la Torre, M. C., Rodriguez, B., Bruno, M., Piozzi, F. and Savona, G. (1991) *Phytochemistry* **30**, 4079.
4. Lourenco, A., de la Torre, M. C., Rodriguez, B., Bruno, M., Piozzi, F. and Savona, G. (1991) *Phytochemistry* **30**, 613.
5. de la Torre, M. C., Pascual, C., Rodriguez, B., Bruno, M., Piozzi, F., Savona, G. and Perales, A. (1986) *Phytochemistry* **25**, 1397.
6. Savona, G., Piozzi, F., Servettaz, O., Rodriguez, B., Hueso-Rodriguez, J. A. and de la Torre, M. C. (1986) *Phytochemistry* **25**, 2569.
7. Pascual, C., Fernandez, P., Garcia-Alvarez, M. C., Marco, J. L., Fernandez-Gadea, F., de la Torre, M. C., Hueso-Rodriguez, J. A., Rodriguez, B., Bruno, M., Paternostro, M., Piozzi, F. and Savona, G. (1986) *Phytochemistry* **25**, 715.
8. Fayos, J., Fernandez-Gadea, F., Pascual, C., Perales, A., Piozzi, F., Rico, M., Rodriguez, B. and Savona, G. (1984) *J. Org. Chem.* **49**, 1789.
9. Garcia-Alvarez, M. C., Marco, J. L., Rodriguez, B., Savona, G. and Piozzi, F. (1982) *Phytochemistry* **21**, 2559.
10. Malakov, P. Y., Papanov, G. Y. and Mollov, N. M. (1979) *Z. Naturforsch.* **34B**, 1570.
11. Collenette, S. (1985) *An Illustrated Guide to the Flowers of Saudi Arabia*, p. 283. Scorpion Publisher Ltd, London.
12. Iinuma, M., Matsuura, S. and Kusuda, K. (1980) *Chem. Pharm. Bull.* **28**, 708.