Neoclerodanes from *Teucrium orientale*

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Two new neoclerodane diterpenoids, 6-deacetyl-teucrolivin A (5) and 8β-hydroxy-teucrolivin B (6), were isolated from the aerial parts of *Teucrium orientale*, along with four already known neoclerodane diterpenoids, teucrolivin A (1), teucrolivin B (2), teucrolivin C (3) and teucrolivin H (4), previously isolated from *Teucrium oliverianum*. Their structures were elucidated on the basis of spectroscopic evidence and chemical transformations. Compounds 1—3 were assayed for antifeedant activity against *Spodoptera littoralis*, *S. frugiperda* and *Heliocoverpa armigera*. Teucrolivin A was the most potent of the three compounds tested.

Key words Lamiaceae; *Teucrium orientale*; structure elucidation; *neo*-clerodane diterpenoid; antifeedant activity

The chemistry of the genus *Teucrium* (Lamiaceae) has been investigated by our group for the last forty years. It contains more than 300 species,[1)] many of them occurring in the Mediterranean region.[2)] To date, the only diterpenoids isolated from the aerial parts of *Teucrium* are those with a neoclerodane skeleton. More than 220 diterpenes have been described, all differing in the functional groups on the neoclerodane or 19-nor-neoclerodane skeleton.[3—6)] These secondary metabolites are of interest because of their ecological role as antifeedant against some species of insects,[7—9)] and potential role in the medicinal properties of the plants.[10—18)]

As part of our investigations of this genus, we report here the results of the phytochemical analysis of *Teucrium orientale* L., a species occurring in the Lebanon–Syria–Israel–Jordan area. The sample of *Teucrium orientale* now investigated comes from Lebanon, where is named "oja'dah sharqiyah". It is botanically similar to *Teucrium oliverianum* (Ging. ex Benth.) R.Br., a species occurring in the area from Jordan to Saudi Arabia from which many compounds were isolated.[19—24)]

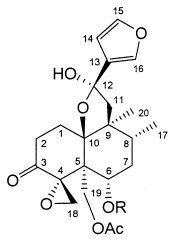
Many species of *Teucrium* are known for their utilization in traditional folk medicine and are claimed to exhibit interesting biological properties such as hypoglycaemic, hypolipidemic, antioxidant, antipyretic, anti-inflammatory, antiulcer, antitumor and antibacterial.[10—18)] Some species are used as a tonic or to treat various ailments like stomach and intestinal problems, colds and for feminine sterility.[25)]

In Lebanon, an infusion of the flowers of *Teucrium orientale* is used in folk medicine as as hypoglycaemic, vermifuge, antipyretic and to treat stomach and intestinal problems.

Column chromatography was used to isolate six neoclerodane diterpenoids: four of them are already known, having been isolated from *Teucrium oliverianum*: teucrolivin A (**1**), teucrolivin B (**2**), teucrolivin C (**3**),[19)] teucrolivin H (**4**).[21)] Two neoclerodanes are new products: they were ascribed the structures of 6-deacetyl-teucrolivin A (**5**) and of 8β-hydroxy-teucrolivin B (**6**), on the basis of spectroscopic evidence and chemical transformations.

Compound **5** ($C_{22}H_{28}O_8$) had an IR spectrum which was consistent with the presence of hydroxyl groups (3490, 3450 cm^{-1}), a furan ring (1505, 874 cm^{-1}), a ketone (1725 cm^{-1}), an oxirane (3040 cm^{-1}) and an acetate (1745, 1229 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of compound **5** (Tables 1, 3) suggested a structure closely related to teucrolivin A (**1**),[19)] the main difference being the upfield shift of the signal of H-6β proton [δ_H 4.09 (1H, dd, J=12.0, 3.8 Hz)] of compound **5** with respect to that of teucrolivin A. Therefore we assigned compound **5** the structure, 6-deacetyl-teucrolivin A. In agreement with the above conclusions, acetic anhydride-pyridine treatment of compound **5** gave teucrolivin A (**1**), allowing us also to assign to compound **5** the absolute configuration of neoclerodane since the absolute configuration of teucrolivin A is well known.[19)]

The ^{1}H- and ^{13}C-NMR spectra (Tables 2, 3) of the second new diterpenoid (**6**) ($C_{24}H_{32}O_{10}$) revealed the presence of a β-substituted furan, an acetoxymethylene group at the C-19 position and a 4α-18 oxirane ring identical with those found in compound **5**. There were also signals for an equatorial



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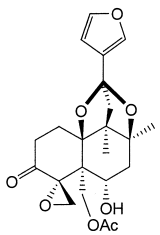
5 R = H



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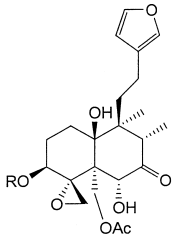

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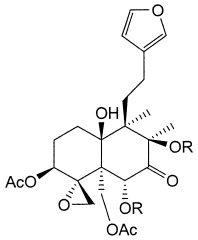

6 R = H
7 R = Ac

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secondary acetoxyl group at the C-3β position [δ_H 5.20 (1H, dd, J=11.0, 5.3 Hz, H-3α)], a C-7 ketone function [δ_C 208.56 (C-7)], a secondary C-6 hydroxyl group [δ_H 5.39 (1H, br s, H-6β)], a tertiary (C-20) methyl group, a tertiary (C-17) methyl group geminated to a hydroxyl group [δ_H 1.34 (3H, s, Me-17; δ_C 17.04 (C-17)], [δ_C 84.33 (C-8)], a C-10 tertiary hydroxyl group [δ_C 82.27 (C-10)] and three hydroxyl groups [δ_H 5.59 (1H, s); 4.75 (1H, s); 3.73 (1H, s)] that disappeared after addition of D_2O. In order to assign the relative configuration to all the stereogenic centers a ROESY[26] (Rotating frame Overhauser effect spectroscopy) experiment was carried out (Table 2). The correlation between H-18a and H-6 confirmed the β-orientation of the latter. Furthermore, the correlation between Me-20 and H-1α showed that they must be close, in a *cis* 1,3-diaxial relationship, and, consquently the hydroxy group at C-10 has to be β-orientated. Finally, the correlation between Me-20 and Me-17 and the absence of correlation between Me-17 and H-6β clearly indicated a β-orientation of the hydroxy group at C-8. These facts allowed us to assign a structure of 8β-hydroxy-teucrolivin B to compound **6**.

Table 1. ^{1}H-NMR Spectral Data of Compound **5** (600 MHz, $CDCl_3$)

H	ppm
1α	2.21 ddd (J=14.0, 12.0, 5.8 Hz)
1β	2.49 ddd (J=14.0, 8.0, 1.7 Hz)
2α	2.64 ddd (J=17.2, 5.8, 1.7 Hz)
2β	2.84 ddd (J=17.2, 12.0, 8.0 Hz)
6β	4.09 dd (J=12.0, 3.8 Hz)
7α	1.25 ddd (J=13.4, 12.0, 11.4 Hz)
7β	1.48 ddd (J=13.4, 3.8, 3.8 Hz)
8β	1.75 ddq (J=11.4, 3.8, 7.0 Hz)
11α	2.33 d (J=13.8 Hz)
11β	2.45 d (J=13.8 Hz)
14	6.38 dd (J=1.5, 1.2 Hz)
15	7.48 br s
16	7.48 br s
Me17	0.80 d (J=7.0 Hz)
18a	3.42 d (J=5.0 Hz)
18b	2.91 d (J=5.0 Hz)
19a	4.85 d (J=12.0 Hz)
19b	4.31 d (J=12.0 Hz)
Me20	1.07 s
OAc	2.00 s

Acetic anhydride-pyridine treatment of **6** at room temperature for 1 d, yielded compound **7** ($C_{28}H_{34}O_{12}$) that showed the presence of four acetoxyl groups [δ_H 2.21 (3H, s), 2.14 (3H, s), 2.10 (3H, s), 2.02 (3H, s)] and of a hydroxyl group [ν_{OH} 3562 cm^{-1}], [δ_H 4.24 (1H, s)] that disappeared after addition of D_2O. The downfield shift of H-6β [δ_H 6.03 (1H, br s)] clearly showed that the α-hydroxyl group at C-6 of compound **6** was acetylated. In order to determinate the position of the fourth acetoxyl group a HMBC (heteronuclear multiple-bond correlation)[27] experiment was carried out (Table 2). The tertiary methyl group at δ_H 1.57 (Me-17) showed correlations with the quaternary carbons at δ_C 92.12 (C-8), δ_C 199.25 (C-7) and δ_C 50.94 (C-9) and on the other hand the tertiary methyl group at δ_H 0.89 (Me-20) correlated with the three quaternary carbons at δ_C 92.12 (C-8), δ_C 50.94 (C-9) and δ_C 82.59 (C-10). Furthermore the hydroxyl group [δ_H 4.24] showed clear correlations with the two quaternary carbons at δ_C 50.94 (C-9) and δ_C 82.59 (C-10), and with the methylenic carbon at δ_C 28.23 (C-1). From all the above data it was evident that compound **7** possessed an acetoxyl group at C-8 and a free hydroxyl group at C-10.

The absolute configuration of 8β-hydroxy-teucrolivin B (**6**) was not ascertained but is believed to belong to the neoclerodane series like teucrolivins A (**1**), B (**2**) and C (**3**), co-occurring in the same species.

The known compounds were identified by direct comparison (TLC, ^{1}H- and ^{13}C-NMR, IR and $[\alpha]_D$) with compounds previously isolated from *Teucium oliverianum* in our laboratory. Copies of the original spectra are obtainable from the corresponding author. ^{13}C-NMR data of teucrolivin H (**4**) are included in Table 3 as they were not previously reported.[21]

A binary choice feeding bioassay using either glass-fibre discs (*Spodoptera littoralis*, *S. frugiperda* and *Heliocoverpa*

Table 2. ^{1}H-NMR Spectral Data of Compounds **6** and **7** (600 MHz, $CDCl_3$)

H (ppm)	**6**	ROESY	**7**	HMBC
1α	2.02 o.s.	H-3α, H-19a, Me-20	o.s.	
3α	5.20 dd (J=11.0, 5.3 Hz)	H-19a, H-1α	5.25 dd (J=10.5, 5.4 Hz)	C-18, C-4, 169.68 (C=O)
6β	5.39 br s	H-18a	6.03 br s	C-5, C-19, 169.33 (C=O), C-7
12a	2.58 m		2.62 m	C-11, C-14, C-13, C-16
12b	2.55 m	Me-20	2.57 m	C-11, C-14, C-13, C-16
14	6.30 br d (J=1.4 Hz)		6.32 br d (J=1.6 Hz)	C-13, C-16, C-15
15	7.37 br d (J=1.4 Hz)		7.41 br d (J=1.6 Hz)	C-14, C-13, C-16
16	7.27 br s		7.29 br s	C-14, C-13, C-15
Me-17	1.34 s	Me-20	1.57 s	C-9, C-8, C-7
18a	3.17 d[a] (J=3.0 Hz)	H-6β	2.91 d (J=3.8 Hz)	C-4
18b	3.10 d[b] (J=3.0 Hz)		2.89 d (J=3.8 Hz)	C-4
19a	4.45 d (J=12.2.0 Hz)	Me-20, H-1α, H-19, H-3α	4.42 d (J=12.2 Hz)	C-5, C-4, C-6, C-10, 170.63 (C=O)
19b	3.97 d (J=12.2 Hz)	Me-20, H-19a	4.29 d (J=12.2 Hz)	C-5, C-4, C-6
Me-20	0.79 s	Me-17, H-1α, H-12, H-19b, H-19a	0.89 s	C-11, C-9, C-10, C-8
8-OAc			2.21 s	167.85 (C=O)
19-OAc	2.11 s		2.14 s	170.63 (C=O)
6-OAc			2.10 s	169.33 (C=O)
3-OAc	2.03 s		2.02 s	169.68 (C=O)
OH[c]	5.59 s		4.24 s	C-1, C-9, C-10
OH[c]	4.75 s			
OH[c]	3.73 s			

a) This is the endo hydrogen with respect to ring B. *b*) This is the exo hydrogen with respect to ring B. *c*) Disappeared after addition of D_2O. o.s. overlapped signal.

armigera) was used to evaluate the activity of the compounds against the final stadium larvae of Lepidoptera.[28] The compounds isolated from *Teucrium orientale* differed in their potency on the feeding behaviour of the larvae.

Table 4 shows that the neo-clerodane teucrolivin A (**1**) was the most potent antifeedant against all three species of Lepidoptera. It was the only compound tested with the epoxy as well as the C-6 and C-19 acetate moieties. The activity declined in teucrolivin B (**2**) and teucrolivin C (**3**), compounds that lack the acetate moiety at C-6. A similar trend in decreasing activity from compound **1** to compound **3** was observed for all three species of insects. These data support earlier suggestions that the presence of the epoxy as well as the acetate contributes to the potency of the compounds. However, we cannot attribute the antifeedant activity of the neo-clerodanes to the presence or absence of this one specific functional group on the molecule,[9] other functional groups are important to the antifeedant and astringent properties of these molecules.

Table 3. ^{13}C-NMR Spectral Data of Compounds **4**, **5**, **6** and **7** (150.9 MHz, $CDCl_3$)

C	**4**	**5**	**6**	**7**
1	29.67 t	28.20 t	27.45 t	28.23 t
2	25.23 t	36.43 t	25.09 t	24.99 t
3	203.74 s	204.26 s	66.30 d	66.41 d
4	64.69 s	64.39 s	61.29 s	62.94 s
5	48.48 s	48.62 s[a)]	55.53 s	55.86 s
6	65.20 d	68.86 d	71.54 d	70.10 d
7	25.76 t	33.62 t	208.56 s	199.25 s
8	86.07 s	33.85 d	84.33 s	92.12 s
9	52.25 s	49.29 s[a)]	49.58 s	50.94 s
10	82.96 s	89.81 s	82.27 s	82.59 s
11	46.21 t	50.58 t	31.31 t	32.38 t
12	103.02 s	100.82 s	22.31 t	22.39 t
13	121.38 s	132.00 s	124.98 s	124.36 s
14	108.65 d	108.14 d	110.74 d	110.56 d
15	143.27 d	143.93 d	142.90 d	143.17 d
16	140.33 d	138.26 d	138.56 d	138.67 d
17	24.89 q	16.04 q	17.04 q	15.55 q
18	52.59 t	52.51 t	46.10 t	45.82 t
19	61.03 t	60.89 t	63.26 t	62.52 t
20	12.86 q	15.28 q	19.00 q	16.36 q
Ac	169.96 s	169.90 s	171.15 s	170.63 s
			169.74 s	169.68 s
				169.33 s
				167.85
	20.58 q	20.48 q	20.85 q	21.45 q
			20.95 q	20.93 q
				20.93 q
				20.69 q

a) These values may be reversed. Multiplicities were established by DEPT pulse sequence.

Experimental

General Experimental Procedures IR spectra were determined with a Perkin Elmer 257 instrument. Optical rotation were recorded on Jasco P-1010 polarimeter. ^{1}H- and ^{13}C-NMR spectra were obtained on Bruker AMX-600 operating at 600.13 and 150.9 MHz for proton and carbon respectively. DEPT experiments were acquired on a Bruker AMX-300 spectrometer. Measurements were made on solution in $CDCl_3$, chemical shifts were referred to TMS set at 0 ppm, and coupling constants are given in Hz. Heteronuclear two-dimensional ^{1}H–^{13}C correlations, one-bond HMQC (heteronuclear multiple quantum correlation) and long-range HMBC (heteronuclear multiple bond correlation), were carried out in the ^{1}H-detected mode with broad-band decoupling in the ^{13}C domain. ROESY experiments were obtained using as spin-lock a continuous low power transmitter pulse and mixing time of 0.2 and 0.4 s, using standard BRUKER pulse sequence. MS were recorded on a Finnigan TSQ70 instrument (70 eV, direct inlet). Elemental analysis was carried out with a Perkin-Elmer 240 apparatus. Flash chromatography was performed by using silica gel (Merck, 0.040—0.063 mesh).

Plant Material Aerial parts of *T. orientale* L. were collected in June 2001, at Chagoura, Cèdres de Bècharrè, Lebanon, at 2000 msl and voucher specimens are deposited in the Herbarium, Botanischer Garten und Botanisches Museum, Freie Universitat, Berlin (leg. and det. N. Arnold *s.n.*, confirm. Th. Raus) and also in the Herbarium, Botanical Garden, Palermo, Italy under the number PAL 2001-1456.

Extraction and Isolation Dried and finely powdered *T. orientale* parts (1153 g) were extracted with Me_2CO (3×5 l) at room temp. for 1 week. The residue (90 g) obtained by removal of the solvent at reduced pressure, was chromatographed on a silica gel (Merck, Art. 7754, deactivated with 15% H_2O, 500 g) column, packed in petroleum ether, using a petroleum ether–EtOAc gradient solvent system (0→80% EtOAc in petroleum ether, total 6 l) followed by EtOAc (1 l) and a mixture of EtOAc and MeOH (9 : 1, 1 l). The fraction eluted with petroleum ether–EtOAc 80% was purified by column chromatography (CH_2Cl_2–MeOH 2%) to afford in order of increasing polarity teucrolivin A (**1**, 50 mg), 6-deacetyl-teucrolivin A (**5**, 7 mg), teucrolivin H (**4**, 20 mg), teucrolivin B (**2**, 360 mg) and 8β-hydroxy-teucrolivin B (**6**, 10 mg). The fraction eluted with petroleum EtOAc was purified by column chromatography (CH_2Cl_2–MeOH 4%) to afford clean teucrolivin C (**3**, 83 mg).

6-Deacetyl-teucrolivin A (**5**): Amorphous solid. $[\alpha]_D^{25}$ +39.60° (c=1.0, $CHCl_3$). IR (film) cm^{-1}: 3490, 3450, 3040, 2950, 2950, 2874, 1745, 1725, 1505, 1460, 1383, 1273, 1229, 1190, 1159, 1136, 1037, 974, 945, 874. ^{1}H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Table 3. EI-MS *m/z*: 420 $[M]^+$ (1), 403 (10), 329 (8), 311 (9), 279 (52), 219 (17), 201 (199), 174 (18), 167 (50), 149 (100). *Anal.* Calcd for $C_{22}H_{28}O_8$: C, 62.84; H, 6.71. Found: C, 62.66; H, 6.61.

Teucrolivin A (**1**) from 6-Deacetyl-teucrolivin A (**5**): Acetic anhydride-pyridine (2 ml, 2 : 1) treatment of **5** (7 mg) at room temperature for 24 h gave after the usual work up 6 mg of teucrolivin A (**1**) identical in all respects (TLC, ^{1}H- and ^{13}C-NMR, IR and $[\alpha]_D$) with the previously described compound.

8β-Hydroxy-teucrolivin B (**6**): Amorphous solid. $[\alpha]_D^{25}$ +7.47° (c=1.52, $CHCl_3$). IR (nujol) cm^{-1}: 3385, 1740, 1720, 1460, 1377, 1248, 1159, 1041, 1024, 947, 893, 874. ^{1}H-NMR spectral data: see Table 2. ^{13}C-NMR spectral data: see Table 3. EI-MS *m/z*: 480 $[M]^+$ (4), 462 (59), 447 (16), 402 (20), 368 (46), 311 (46), 266 (62), 218 (100), 189 (60), 161 (50), 148 (42). *Anal.* Calcd for $C_{24}H_{32}O_{10}$: C, 59.99; H, 6.71. Found: C, 59.88; H, 6.59.

Acetylation of 8β-Hydroxy-teucrolivin B (**6**): Acetic anhydride–pyridine (2 ml, 2 : 1) treatment of **6** (10 mg) at room temperature for 24 h gave after the usual work up 10 mg of the tetraacetyl (**7**) as an amorphous solid; $[\alpha]_D^{25}$ +3.63° (c=1.10, $CHCl_3$). IR (film) cm^{-1}: 3562, 3020, 2950, 2925, 2854,

Table 4. Effect of Neo-clerodanes on the Feeding Behaviour of Final Stadium Lepidopteran Larvae

Neo-clerodane[d)]	Feeding index[a)] mean±S.E.M.[b)] (FI_{50})[c)]		
	1	**2**	**3**
Spodoptera littoralis	54±10.4* (3 ppm)	37±9.5* (650 ppm)	29±7.8 (>1000 ppm)
Spodoptera frugiperda	53±9.2* (nc)	36±11.7[e)] (nc)	18±11.1 (nc)
Helicoverpa armigera	52±11.4* (70 ppm)	25±12.3[e)] (>1000 ppm)	22±8.3 (>1000 ppm)

a) Feeding index=((C−T)/(C+T))%, C=amount of control discs, T=amount of treatment discs eaten after an 18 h bioassay. *b*) Feeding index (mean±S.E.M.) at 100 ppm. *c*) Estimated concentration (ppm) to give feeding index of 50%, nc=not calculated. *d*) Figure 1 contains structures of compounds. *e*) Previously published in ref. 29. *=significant $p<0.05$, Wilcoxon matched pairs test, n=20.

1750, 1738, 1725, 1655, 1502, 1435, 1369 1250, 1235, 1168, 1117, 1085, 1041 975, 930, 897, 874. ^{1}H-NMR spectral data: see Table 2. ^{13}C-NMR spectral data: see Table 3. EI-MS *m/z*: 564 $[M]^+$ (1), 547 (3), 504 (6), 444 (2), 385 (12), 367 (10), 308 (18), 281 (16), 265 (21), 248 (40), 218 (38), 177 (100), 121 (38), 109 (76). *Anal.* Calcd for $C_{28}H_{34}O_{12}$: C, 59.77; H, 6.09. Found: C, 59.68; H, 6.12.

Antifeedant Activity Bioassay Compounds **1**—**3** were tested in a binary glass-fibre choice test[28] for antifeedant activity. The three compounds were all tested at 100 ppm against 18—24 h old final stadium larvae of the three species of Lepidoptera. Lower concentrations were also tested against larvae of *S. littoralis* and *H. armigera*. Regression analysis was used to estimate the FI_{50}. Each concentration was tested against 5—20 larvae per species.

Acknowledgements Work in Palermo was supported by Italian Government project PRIN 2003. Insects were cultured in UK under conditions specified in a Defra license.

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