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Neo-clerodane diterpenes from *Teucrium fruticans*

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Dedicated to Professor Dr. F. Piozzi on the occasion of his 75th birthday

Abstract

In addition to three out of the four previously reported *neo*-clerodanes already found in *Teucrium fruticans* (fruticolone, isofruticolone and 8β-hydroxyfruticolone), and 6-acetylteucjaponin B (isolated from *T. scordium* and *T. grisebachii*), four new *neo*-clerodanes, namely 7β-hydroxyfruticolone, 11-hydroxyfruticolone, deacetylfruticolone and 6-acetyl-10-hydroxyteucjaponin B were also isolated. The structures were unambiguously elucidated based on extensive NMR spectral studies (one- and two-dimensional experiments). Semi-prep-HPLC proved to be a convenient purification procedure, occasionally being followed by TLC. The new compounds were assayed against *Spodoptera littoralis* and two of them were shown to have potent antifeedant activity.

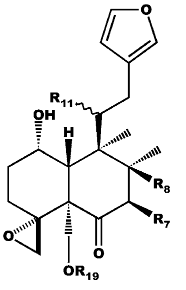
Keywords: *Teucrium fruticans*; Labiatae; Structure elucidation; NMR; *Neo*-clerodane diterpenes; Antifeedant activity

1. Introduction

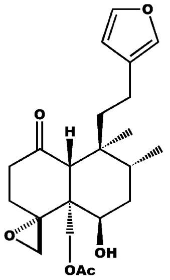
The genus *Teucrium* (family Labiatae) is one of the richest sources of clerodane diterpenes, and the new natural products are conveniently reviewed periodically (Piozzi et al., 1998 and references therein). Most of the reported structures display a furan ring system in the side-chain, with an oxygen-bearing C-12. The isolates from *T. fruticans* (Savona et al., 1978a,b) fruticolone **1**, isofruticolone **2** and 8β-hydroxyfruticolone **3** are exceptional since they don't bear substituents at C-11 and C-12. Only a few other exceptions amongst more than 200 compounds are known (Piozzi et al., 1987, 1998; Piozzi, 1994).

Since *T. fruticans* is a widely distributed species and readily available, it was chosen to evaluate the reversed phase chromatographic behavior of furo-clerodanes. A methylene choride extract of the aerial parts of this plant revealed a complex diterpene fraction on RP-HPLC analysis. The isolation, structural elucidation and antifeedant activity of previously unreported *neo*-clerodane diterpenes, with different substitution on the decalin portion and in the side-chain, is reported. Semi-prep-HPLC proved to be a convenient purification procedure, occasionally being followed by TLC.

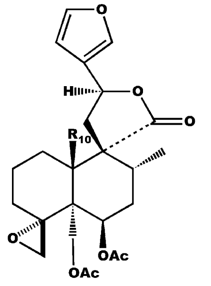
In addition to fruticolone, isofruticolone, and 8β-hydroxyfruticolone (Savona et al., 1978a,b), 6-acetylteucjaponin B **7** previously isolated from *Teucrium scordium* and *T. grisebachii* (Jakupovic et al., 1985;



I



Isofruticolone **2**



II

I		R7	R8	R11	R19
Fruticolone	**1**	H	H	H	Ac
8ß-Hydroxyfruticolone	**3**	H	OH	H	Ac
7ß-Hydroxyfruticolone	**4**	OH	H	H	Ac
11-Hydroxyfruticolone	**5**	H	H	OH	Ac
Deacetylfruticolone	**6**	H	H	H	H

II		R10
6-Acetyl-teucjaponin B	**7**	H
6-Acetyl-10-hydroxyteucjaponin B	**8**	OH

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Tonn et al., 1990) was also present and isolated from *T. fruticans*. The structural elucidation of new *neo*-clerodanes isolated in the present work, 7β-hydroxyfruticolone **4**, 11-hydroxyfruticolone **5**, deacetylfruticolone **6** and 6-acetyl-10-hydroxyteucjaponin B **8** was based on extensive spectral studies (one and two-dimensional NMR experiments). The antifeedant activity against *Spodoptera littoralis* was determined for all isolated compounds.

2. Results and discussion

2.1. Structural elucidation of isolated compounds

Compounds **1**, **2** and **3** showed spectroscopic data identical to those previously reported (Savona et al., 1978a,b). More detailed data for fruticolone **1** with some corrected assignments are included in the tables as a convenient model compound.

Compound **4** was isolated as a white solid, and on the basis of NMR analysis assigned the structure of 7β-hydroxyfruticolone. Several of the described signals of fruticolone **1** (Savona et al., 1978a), were observed in the ^{1}H NMR spectrum of **4** with only minor shift changes [δ 1.14 (3H, *d, J*=6.6 Hz, H-17), 1.42 (3H, *s*, H-20), 2.09 (3H, *s*, OAc), 2.33 and 3.60 (1H each, *d, J*=5.3 Hz and *dd*, *J*=2.2 and 5.3 Hz, respectively, H-18), 4.47 (1H, *m,* H-1), 4.87 and 5.56 (1H each, *d*, *J*=12.7 Hz, H-19), 6.25 (1H, *m*, H-14), 7.21 (1H, *m*, H-16), 7.37 (1H, apparent *t* (=*dd*), *J*=1.7 Hz, H-15)] (Table 1). The ^{13}C NMR and DEPT spectra confirmed the presence of a furan ring [δ 124.2, C-13; 110.7, C-14; 143.1, C-15 and 138.5, C-16], a carbonyl group (δ 207.8, C-6), an acetate (δ 170.8, **CO**Me; 20.9, CO**Me**), two methyl signals (δ 11.0, C-17; 20.6, C-20) and two oxygen-substituted methylene signals for the oxirane ring and C-19 (δ 49.3, C-18; 64.3, C-19) (Table 2).

The presence of two additional proton absorptions in the δ 3–5 region (oxygen substituted carbons) and the disappearance of the one-proton apparent triplet and doublet of doublets attached to C-7 (δ 2.79 and 2.14, respectively, in fruticolone) were the more significant features in the ^{1}H NMR spectrum, pointing out the possible oxidation of that position. The lower field signal appeared as a doublet of doublets at δ 4.33 (*J*=4.2 and 12.2 Hz), and the large coupling constant matched one of the values derived from the splitting pattern for H-8 (doublet of quadruplets signal) and established a *trans*-diaxial relationship between H-7 and H-8. Mutual coupling with the second signal (a doublet at δ 3.63, *J*=4.2 Hz) suggested by the common *J* value, was confirmed by a cross peak in the ^{1}H NMR 2D COSY spectrum, and supported the presence of a hydroxyl group at C-7 with β configuration (equatorial). As expected, no cross peak was observed in the HSQC spectrum for a carbon resonance and the proton signal at δ 3.63, but in the HMBC spectrum cross peaks were observed for C-6, C-7 and C-8.

Again, the features of a structural relationship with fruticolone **1** were present in compound **5**. Its IR spectrum showed the presence of hydroxyl groups (3479 cm^{-1}) and a carbonyl group (1714 cm^{-1}). The ^{1}H NMR spectrum showed furan ring signals [δ 6.34 (1H, *m*, H-14), 7.37 (1H, *m*, H-16), 7.45 (1H, apparent *t* (=*dd*), *J*=1.5 Hz, H-15)], two methyl groups [δ 0.96 (3H, *d, J*=6,6 Hz, H-17), 1.43 (3H, *s*, H-20)], one acetate [δ 2.09 (3H, *s*)], an oxirane ring [δ 2.37 and 3.05 (1H each, *d, J*=5.0 Hz, *dd, J*=2.0 and 5.0 Hz, respectively, H-18)] and an AB system [δ 5.11 and 5.20 (1H each, *d, J*=12.0 Hz, H-19)] (Table 1). The ^{13}C NMR spectrum and DEPT experiments confirmed the presence of a furan ring [δ 121.3, C-13; 110.9, C-14; 143.9, C-15 and 140.4, C-16] as well as a carbonyl group (δ 207.1, C-6), an acetate (δ 170.9, **CO**Me; 21.1, CO**Me**), two methyl signals (δ 16.5, C-17; 17.2, C-20) and the pair of CH_2–O signals (δ 49.3, C-18; 64.5, C-19) (Table 2).

Once more, an additional absorption in the oxygen-substituted carbon region in proton and carbon spectra appeared. The splitting pattern and chemical shift in the ^{1}H NMR [δ 3.88, *dd*] confirmed the presence of a hydroxyl substituent, previously suggested by MS [a protonated molecular ion at *m*/*z* 407 was consistent with a molecular formula of $C_{22}H_{30}O_7$]. The HSQC spectra showed that no hydrogen in the decalin moiety (fruticolone as reference) had been replaced, and the ^{1}H NMR 2D COSY spectrum showed only two correlations for the δ 3.88 signal [δ 2.49 and 2.75]. Both were assigned from HSQC and confirmed by HMBC spectra as protons attached to C-12 which points out the structure of **5** to be 11-hydroxyfruticolone.

Compound **6** was isolated as a colourless oil. Its IR spectrum showed the presence of hydroxyl groups (3433 cm^{-1}) and a carbonyl group (1707 cm^{-1}). The molecular ion at *m*/*z* 348 in the MS spectrum along with the ^{13}C NMR data (Table 2) were in good agreement with the molecular formula $C_{20}H_{28}O_5$ (calculated mass 348.19). The ^{1}H NMR spectrum of **6** was closely similar to that of the co-occurring diterpene fruticolone, except for the lack of the signals for an acetyl moiety and these results suggested that **6** might be the 19-deacetylderivative of **1**. The substitution of an acetoxy group by a hydroxyl group causes the expected upfield shift of the C-19 methylene protons (see Table 1). The structure of **6** as deacetylfruticolone was further supported by correlations found in the ^{1}H NMR 2D COSY, HSQC and HMBC spectra.

In the spectra of compound **7** the more significant features were the missing methyl group singlet (C-20) and the appearance of a triplet at δ 5.38, consistent with a γ-lactone ring which connects C-12 to C-20. Analysis of our spectral data (Table 1) and comparison with those published for montanin C (Fayos et al., 1984), as well as its C-12 epimeric compound, 6-acetylteucjaponin

Table 1
^{1}H NMR spectral data of compounds **1** and **4**–**8** (in $CDCl_3$, at 499.80 MHz)[a]

H	**1**	**4**	**5**	**6**	H	**7**	**8**
1	4.48 *m* ($w_{1/2}=7.5$)	4.47 *m* ($w_{1/2}=7.5$)	4.86 *m* ($w_{1/2}=8.8$)	4.42 *m* ($w_{1/2}=7.5$)	1a	[c]	1.88[d]
2α	1.88 *ddt*[b] (2.7, 4.6, 13.7)	1.89	1.78 *ddt*[b] (3.5, 4.8, 13.9)	1.78	1b	[c]	2.19[d]
2β	1.72 *t*[b]*dd* (3.0, 4.6, 13.7)	1.70 *t*[b]*dd* (2.6, 4.6, 13.9)	1.97	1.88	2a	[c]	1.85[d]
3α	2.68 *t*[b]*dd* (2.2, 4.4, 13.6)	*2.73* *t*[b]*dd* (2.2, 4.1, 13.6)	2.60 *t*[b]*dd* (2.2, 4.3, 13.9)	2.58 *t*[b]*dd* (2.2, 4.6, 13.0)	2b	[c]	2.00[d]
3β	0.99 *ddd* (3.1, 4.6, 13.9)	1.02 *ddd* (3.2, 4.4, 13.9)	*1.01* *ddd* (3.0, 5.0, 13.2)	1.10 *ddd* (2.2, 4.1, 13.6)	3a	[c]	1.11[d]
7α	2.14 *dd* (3.6, 14.1)	4.33 *dd* (4.15, 12.2)	2.48 *m*[d]	2.24 *dd* (3.9, 14.4)	3b	[c]	2.32[d]
7β	2.79 *t*[b] (13.9)	–[e]	2.60 *m*[d]	2.67 *t*[b] (13.6)	6	4.82 *dd* (7.0, 14.0)	5.33 *dd* (5.1, 10.9)
8	1.98 *dqd* (3.3, 6.6, 13.9)	1.68 *dq* (12.2, 6.6)	2.20 *m*	2.10 *m*	7α	[c]	2.31 *ddd* (12.2,13.2,13.2)
10	1.90 *br s*	1.90 *br s*	2.34 *br s*	1.89 *br s*	7β	[c]	1.60 *ddd* (4.6, 4.5, 13.6)
11a	1.75 *ddd* (4.6, 12.7, 14.9)	1.76 *ddd* (4.4,12.4, 15.1)	3.88 *dd* (1.5, 11.0)	1.74	8	[c]	2.17[d]
11b	1.92	1.88	–[e]	1.92	10	[c]	–[e]
12a	2.10 *m*[d]	2.09 *ddd* (4.1, 12.4, 12,7)	2.49 *dd* (11.1, 13.9)	2.15 *m*[d]	11a	2.39 *d* (8.5; H_2)	2.17 *dd* (9.5 14.8)
12b	2.32 *m*[d]	2.29 *ddd* (4.3, 12.4, 12,9)	2.75 *dd* (1.5, 13.7)	2.37 *ddd* (4.8, 14.1, 14.4)	11b		3.00 *dd* (8.5, 14.9)
14	6.26 *m*	6.25 *m*	6.34 *m*	6.26 *m*	12	5.38 *t* (8.5)	5.38 *t* (9.0)
15	7.38 *t*[b] (1.7)	7.37 *t*[b] (1.7)	7.45 *t*[b] (1.5)	7.38 *t*[b] (1.7)	14	6.39 *m*	6.40 *m*
16	7.23 *m*	7.21 *m*	7.37 *m*	7.23 *m*	15	7.46 *m*	7.47 *m*
17	0.94 *d* (6.6)	1.14 *d* (6.6)	0.96 *d* (6.6)	0.94 *d* (6.6)	16	7.46 *m*	7.45 *m*
18a	2.32 *d* (5.6)	2.33 *d* (5.3)	2.37 *d* (5.0)	2.47 *d* (5.1)	17	1.01 *d* (6.6)	1.01 *d* (6.5)
18b	3.50 *dd* (2.4, 5.6)	3.60 *dd* (2.2, 5.3)	3.05 *dd* (2.0, 5.0)	3.60 *dd* (2.0, 4.8)	18	2.27 *d* (3.5)	2.44 *d* (4.4)
19a	4.99 *d* (12.7)	4.87 *d* (12.7)	5.11 *d* (12.0)	4.21 *d* (12.2)	18	3.01 *dd* (2.2, 3.5)	3.18 *dd* (2.2, 4.1)
19b	5.44 *d* (12.7)	5.56 *d* (12.7)	5.20 *d* (12.0)	4.76 *d* (12.4)	19	4.51 *d* (13.0)	4.58 *d* (12.9)
20	1.36 *s*	1.42 *s*	1.43 *s*	1.31 *s*	19	5.27 *d* (13.0)	5.21 *d* (12.9)
19-Ac	2.07 *s*	2.09 *s*	2.09 *s*		6-Ac	1.99 *s*	1.99 *s*
OH		3.63 *d* (4.15)			19-Ac	2.10 *s*	2.10 *s*

[a] δ in ppm; *multiplicity*; (J or $w_{1/2}$) in Hz.
[b] Apparent multiplicity: *t*[b] = *dd*.
[c] Undetermined.
[d] Overlapped signal.
[e] No protons.

Table 2
^{13}C NMR data for compounds **1** and **4**–**8** (in $CDCl_3$, at 75.43 MHz)

C	1	4	5	6	C	7*	8
1	66.8	66.9	67.4	66.4	1	22.4	29.8
2	34.8a	34.8	33.9	34.2	2	24.4	19.5
3	28.5b	28.4	28.2	28.3	3	31.7	31.0
4	61.6	61.7	61.5	62.2	4	64.3	62.6
5	55.1	54.4	54.8	57.0	5	45.0	49.3
6	206.9	207.8	207.1	208.5	6	71.2	69.4
7	55.0	73.8	45.8	45.1	7	32.2	32.5
8	38.4	46.7	36.1	38.1	8	37.6	33.6
9	39.9	40.2	43.2	39.8	9	50.5	57.1
10	52.7	52.8	50.1	51.8	10	52.4	80.2
11	38.6c	38.9	78.1	38.8	11	42.6	38.5
12	18.1	18.0	26.2	18.2	12	71.5	71.9
13	124.4	124.2	121.3	124.5	13	124.8	125.1
14	110.7	110.7	110.9	110.7	14	107.7	108.0
15	143.0d	143.1	143.9	138.5	15	143.8	144.2
16	138.5e	138.5	140.4	143.0	16	139.3	139.6
17	15.4	11.0	16.5	15.5	17	16.1	16.5
18	49.3	49.3	49.3	51.1	18	47.8	50.4
19	64.2	64.3	64.5	64.45	19	61.2	63.2
20	19.2	20.6	17.2	19.1	20	175.8	174.6
19-1	171.1	170.8	170.9		6-1	169.6	170.1
19-2	21.0	20.9	21.1		6-2	20.7	21.2
					19-1	170.0	170.3
					19-2	20.7	21.3

a,b,c,d,e Assignments which are corrected with respect to the previous report [Savona et al., 1978; (a) 38.5, (b) 34.7, (c) 28.6, (d) 138.5, (e) 143.0. (b) and (c) assignments may be interchanged]. *Data reported in the bibliography (Tonn et al., 1990).

B (Tonn et al., 1990), led to the conclusion that compound **7** had the same structure and relative stereochemistry at the chiral centre C-12 of the aforementioned 6-acetylteucjaponin B, being therefore, a (12*S*)-*neo*-clerodan-20,12-olide. This compound was first prepared synthetically (Martínez-Ripoll et al., 1981) and has been reported as natural product from *T. scordium* (Jakupovic et al., 1985) and *T. grisebachii* (Tonn et al., 1990).

The ^1H NMR spectrum of compound **8** showed a structural relationship with 6-acetylteucjaponin B **7** [furan and oxiran rings, CH_2O (C-19) AB system, two acetate groups (δ 2.10 and 1.99; 3H each, s), a γ-lactone ring (triplet, H-12, overlapping a H-6 doublet of doublets]. On the other hand, the presence of a hydroxyl group placed at C-10 was envisaged from the lack of any signal for H-10 in the ^1H NMR spectrum (Table 1), and a quaternary C-O absorption (*δ* 80.2) in the ^{13}C NMR spectrum (Table 2). Downfield shifts for H-6 (*δ* 5.33) and H-11 signals (*δ* 2.17 and 3.00) must be explained by a strong anisotropic effect of the hydroxyl group. In the proposed structure for **8**, a β configuration for the hydroxyl group must be considered from the paramagnetically shifted β carbons with respect to compound **7:** C-1 ($\Delta\delta + 7.4$ ppm), C-5 ($\Delta\delta + 4.3$ ppm) and C-9 ($\Delta\delta + 6.6$ ppm), and the observed differences in the chemical shift of γ-carbons (compound **7** as reference): C-8 ($\Delta\delta - 4.0$ ppm), C-11 ($\Delta\delta - 4.0$ ppm), C-2 ($\Delta\delta - 4.9$ ppm) in ^{13}C NMR spectrum (Table 2) (Whitesell and Minton, 1987). The deshielded resonance of one of the C-11 methylene protons [*δ* 3.00 ppm, H-11], confirms the β configuration assigned for the tertiary hydroxyl group at C-10. Other C-10 hydroxylated *neo*-clerodanes bearing a γ-lactone moiety at C-12/C-20 have been isolated from *T. pestalozzae* (De la Torre et al., 1990), *T. sandrasicum* (Topcu et al., 1996; De la Torre et al., 1997), and *T. oliverianum* (Bruno et al., 1991).

Table 3
Insect antifeedant activity against fifth instar larvae of *Spodoptera littoralis* in dual-choice feeding assays

Compd	Dose (μg/cm^2)	N	FR_{50}* (mean ± SEM)
1	10	5	0.69 ± 0.11
2	10	8	0.78 ± 0.11
3	10	5	0.57 ± 0.07
4	10	6	0.57 ± 0.04
5	10	6	0.45 ± 0.07
6	10	5	1.03 ± 0.07
7	10	5	0.07 ± 0.02
	1	5	0.34 ± 0.06
8	10	5	0.08 ± 0.01
	1	5	0.16 ± 0.02

*Feeding ratio when the consumed area of control disc (CCD) is 50%, [FR = CTD (consumed area of treated disc)/CCD]; SEM: standard error of the mean.

2.2. Antifeedant bioassay

The results of the antifeedant bioassay against *S. littoralis* are reported in Table 3. In this bioassay fruticolone **1** showed low antifeedant activity as well as isofruticolone **2**, which was previously reported as one of the most potent of the *Teucrium fruticans* derived *neo*-clerodanes (Bruno et al., 1999). The hydroxy-fruticolone derivatives **3**, **4** and **5** and the deacetylderivative **6** showed only low antifeedant activity. It is known that both rings in the decalin moiety may be substituted with additional hydroxyl or ester groups, but these do not seem to be essential for antifeedant activity, since the non-substituted analogues often show comparable activities (Klein Gebbink et al., 2002). Surprisingly, the highest antifeedant activity was displayed by the new family of diterpene compounds found in the plant, 6-acetylteucjaponin B, previously described to show potent antifeedant activity against *Tenebrio molitor* larvae (Sosa et al., 1994), and its C-10 hydroxy derivative, showing a moderate activity even at lower dosage.

3. Experimental

3.1. General experimental procedures

^1H NMR (500 MHz), ^{13}C NMR (75 MHz), DEPT, H,H-COSY, H,C-HSQC and HMBC spectra were recorded on Varian (Zug, Switzerland) Inova 500/Unity 300 spectrometers under standard conditions and pulse sequences. Chemical shifts are given in ppm with respect to residual $CHCl_3$ or $CDCl_3$ signals (δ 7.27 and 77.00, respectively). EIMS in positive ion mode were obtained with an HPLC apparatus coupled to an MS detector (HP 1100 instrument from Agilent), using $MeCN–H_2O$ (50:50) at 1 ml/min as mobile phase. Compounds were analyzed by flow injection analysis (FIA). The IR spectra were recorded on a BOMEM MB-120 apparatus as films over KBr. Optical rotations were determined using a Perkin Elmer polarimeter model 341 (on the sodium D line). Silica gel 60 F_{254} plates (Merck) were used for TLC with CH_2Cl_2: TBME (*tert*-butylmethyl ether) 4:1 as mobile phase. Analytical HPLC was performed using a Lichrospher® 100 RP-18, 5 μm column; mobile phase gradient system 1 [H_2O–MeOH (50:50, 0–5 min; 50:50–40:60, 5–10 min; 40:60, 10–15 min; 40:60–50:50, 15–20 min), 1 ml/min, 25 °C]. Semi-preparative HPLC was performed using a Pharmacia Biotech, frac-100 semi-automatic collector (fractions collected every minute); Kromasil 100 C18, 5 μm, 25×1 cm column; mobile phase gradient system 2 [H_2O–MeOH (50:50–30:70, 0–20 min; 30:70–20:80, 20–25 min; 20:80, 25–40 min; 20:80–50:50, 40:45 min), 2 ml/min, 25 °C]. All HLPC work was performed using an Alliance 2695 apparatus with a UV diode-array detector 996 (both from Waters). In order to protect the integrity of the columns, a coupled C-18 guard column was used. All solvents used for extraction and chromatographic procedures were HPLC grade and were purchased from Merck (Darmstadt, Germany). Water used in HPLC mobile phase mixtures was Milli Q (Millipore).

3.2. Plant material

Teucrium fruticans aerial parts (leaves and stems) were collected from IIQAB-CID front yard in March 2003 and dried at 60 °C in an oven with air circulation. A voucher specimen has been deposited in the herbarium of the Institut Botànic de Barcelona (number BC 831501).

3.3. Extraction and isolation of *neo*-clerodane diterpenes

Finely grounded plant material (50 g) was extracted with CH_2Cl_2 (1 l) at room temperature for 1 week, in the dark. After solvent evaporation, the residue (4.9 g) was digested/centrifuged sequentially in 50 ml aqueous MeOH 30%, 60% and 90%. Filtration/elution (intermediate washing step with equal volume of the corresponding solvent mixture) on a 10 g RP C-18 column afforded six fractions. TLC proved the major presence of the diterpene compounds in the fractions eluted and filtered with MeOH 60% and 90%, respectively. The enriched clerodane fraction (65.9 mg) was separated by RP-HPLC (gradient system 2), affording eight fractions (A-H). The ^1H NMR spectrum of the less polar fraction H, suggested the presence of a mixture of fruticolone **1** and isofruticolone **2** later resolved by preparative TLC (21.3 and 2.6 mg, respectively). 11-Hydroxyfruticolone **5** (1.4 mg), 8β-hydroxyfruticolone **3** (19.1 mg) and 6-acetyl-10-hydroxyteucjaponin B **8** (13.4 mg) were obtained as pure compounds in fractions B, C and D, respectively. Fractions E and F were reprocessed by semi-preparative HPLC to afford 6-acetylteucjaponin B **7** (0.7 mg), 6-acetyl-10-hydroxyteucjaponin B **8** (1.2 mg), and 7β-hydroxyfruticolone **4** (1.5 mg). Fraction G was purified by preparative TLC and in addition to isofruticolone **2** and 6-acetyl-10-hydroxyfruticolone **8** in trace amounts, afforded deacetylfruticolone **6** (3.0 mg). Once purified and identified, the retention time of each isolated compound was established (gradient system 1), and its presence in the fresh plant checked, treating 50 g of material by the aforementioned extraction procedure. RP-HPLC conditions as described above (gradient system 1).

3.3.1. 7β-Hydroxyfruticolone (**4**)

White amorphous solid; $[\alpha]_D^{25} + 22.0°$ ($CHCl_3$; *c*. 0.11); IR ν_{max} cm^{-1}: 3471, 2929, 1725, 1453, 1375, 1250, 875; ^1H NMR data: Table 1; ^{13}C NMR data: Table 2; EIMS *m*/*z*: 407 $[M+1]^+$, 329, 312, 311, 299, 283, 281.

3.3.2. 11-Hydroxyfruticolone (**5**)

White amorphous solid; $[\alpha]_D^{25} + 45.0°$ ($CHCl_3$; *c*. 0.12); IR ν_{max} cm^{-1}: 3479, 2936, 1714, 1455, 1374, 1251, 1029, 755; ^1H NMR data: Table 1; ^{13}C NMR data: Table 2; EIMS *m*/*z*: 407 $[M+1]^+$, 389, 329, 311, 299, 293, 283, 281, 265, 216.

3.3.3. Deacetylfruticolone (**6**)

Colourless oil; $[\alpha]_D^{25} + 5.4°$ ($CHCl_3$; *c*. 0.22); IR ν_{max} cm^{-1}: 3443, 2928, 1707, 1645, 1455, 1380, 11247, 1159, 1023, 870, 779, 595; ^1H NMR data: Table 1; ^{13}C NMR data: Table 2; EIMS *m*/*z*: 348 $[M]^+$, 331, 301, 267, 251, 107.

3.3.4. 6-Acetyl-10-hydroxyteucjaponin B (**8**)

White amorphous solid; $[\alpha]_D^{25}$ $+35.2°$ ($CHCl_3$; *c*. 0.13); IR ν_{max} cm^{-1}: 3469, 2941, 1731, 1475, 1371, 1248, 1027, 755; ^1H NMR data: Table 1; ^{13}C NMR data: Table 2; EIMS *m*/*z*: 463 $[M+1]^+$, 407, 403, 385, 343, 325, 311, 307, 297, 281, 279.

3.4. Antifeedant activity bioassay

A binary choice feeding bioassay using leaf disks of lettuce, *Lactuca sativa,* with an area of 1 cm^2 was used to evaluate the activity of the compounds against fifth instar larvae of *Spodoptera littoralis* (Bellés et al., 1985). Compounds to be tested were uniformly distributed on the upper surface of the disk by application of 10 μl acetone solutions (treated disks: TD). Control disks (CD) were analogously treated with acetone. In each replicate, four treated and four control disks were alternatively placed in a covered polyethylene Petri dish (8.5 cm diameter) in the presence of five larvae. Experiments were performed under the same temperature and humidity conditions of the laboratory culture, but in constant darkness.

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References

Bellés, X., Camps, F., Coll, J., Piulachs, M.D., 1985. Insect antifeedant activity of clerodane diterpenoids against larvae of *Spodoptera littoralis* (BOISD.) (Lepidoptera). J. Chem. Ecol. 11, 1439–1445.

Bruno, M., Omar, A.A., Perales, A., Piozzi, F., Rodríguez, B., Savona, G., De la Torre, M.C., 1991. *Neo*-clerodane diterpenoids from *Teucrium oliverianum*. Phytochemistry 30, 275–282.

Bruno, M., Piozzi, F., Rossellia, S., Simmonds, M.S.J., 1999. Antifeedant activity of *neo*-clerodane diterpenoids from *Teucrium fruticans* and derivatives of fruticolone. Phytochemistry 52, 1055–1058.

De la Torre, M.C., Rodríguez, B., Bruno, M., Fazio, C., Husnu, K., Baser, C., Duman, H., 1997. *Neo*-clerodane diterpenoids from *Teucrium sandrasicum*. Phytochemistry 45, 1653–1662.

De la Torre, M.C., Rodríguez, B., Bruno, M., Savona, G., Piozzi, F., Perales, A., Torres, M.R., Servettaz, O., 1990. Two C-10 oxygenated *neo*-clerodane diterpenes from *Teucrium pestalozzae*. Phytochemistry 29, 2229–2233.

Fayos, J., Pascual, C., Perales, A., Piozzi, F., Rico, M., Rodríguez, B., Savona, G., 1984. Correct structures of montanin C, teupolin I and 12-*epi*-teucvin, three (12R)-neoclerodan-20,12-olides isolates from *Teucrium* species. J. Org. Chem. 49, 1789–1793.

Jakupovic, J., Baruah, R.N., Bohlmann, F., Quack, W., 1985. New clerodane derivatives from *Teucrium scordium*. Planta Med. 51, 341–342.

Klein Gebbinck, E.A., Jansen, B.J.M., de Groot, A., 2002. Insect antifeedant activity of clerodane diterpenes and related model compounds. Phytochemistry 61, 737–770.

Martínez-Ripoll, F.J., Rodríguez, B., García Alvarez, M.C., Savona, G., Piozzi, F., Paternostro, M.P., Hanson, J.R., 1981. The absolute stereochemistry of some clerodane diterpenoids from *Teucrium* species. J. Chem. Soc. Perkin Trans. I, 1186–1190.

Piozzi, F., 1994. Further researches on the furoclerodanes from *Teucrium* species. Heterocycles 37, 603–626.

Piozzi, F., Bruno, M., Rosselli, S., 1998. Further furoclerodanes from *Teucrium* genus. Heterocycles 48, 2185–2203.

Piozzi, F., Rodríguez, B., Savona, G., 1987. Advances in the chemistry of the furanoditerpenoids from *Teucrium* species. Heterocycles 25, 807–841.

Savona, G., Passannanti, S., Paternostro, M.P., Piozzi, F., 1978a. Two new diterpenoids from *Teucrium fruticans*. J. Chem. Soc. Perkin Trans. I, 356–359.

Savona, G., Paternostro, M.P., Piozzi, F., Hanson, J.R., Siverns, M., 1978b. 8β-Hydroxyfruticolone, a diterpenoid from *Teucrium fruticans*. Phytochemistry 17, 320–322.

Sosa, M.E., Tonn, C.E., Giordano, O.S., 1994. Insect antifeedant activity of clerodane diterpenoids. J. Nat. Prod. 57, 1262–1265.

Tonn, C.E., Caltabiano, J.A., Gianello, J.C., Giordano, O.S., 1990. Two *neo*-clerodane diterpenoids from *Teucrium grisebachii*. Rev. Latinoamer. Quim 21, 52–54.

Topcu, G., Eris, C., Che, C.T., Ulubelen, A., 1996. C-10 Oxygenated *neo*-clerodane diterpenes from *Teucrium sandrasicum*. Phytochemistry 42, 775–778.

Whitesell, J.K., Minton, M.A., 1987. Stereochemical Analysis of Alicyclic Compounds by C-13 NMR Spectroscopy. Chapman and Hall Ltd, New York.