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Phytochemistry 63 (2003) 977–983
PHYTOCHEMISTRY
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Neo-clerodane diterpenoids and phenylethanoid glycosides from *Teucrium chamaedrys* L.

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Received 11 December 2002; received in revised form 18 March 2003

Abstract

A *neo*-clerodane type diterpenoid, 12(*S*)-15,16-epoxy-19-hydroxy-*neo*-cleroda-13(16),14-dien-18,6α:20,12-diolide, and two phenylethanoid glycosides, teucrioside-3‴-*O*-methylether and teucrioside-3‴,4‴-*O*-dimethylether were isolated from the aerial parts of *Teucrium chamaedrys*. Their structures were identified on the basis of extensive NMR spectra, LC-ESIMS analysis, and molecular modeling studies.

Keywords: *Teucrium chamaedrys*; Lamiaceae; *neo*-Clerodane; Phenylethanoid; Glycoside; Teucrioside

1. Introduction

Teucrium L. genus, a member of the Lamiaceae, is found throughout the world but mainly abounding in the northern temperate and subtropical regions of the Eastern Hemisphere. Representatives of the genus *Teucrium* have been used for more than 2000 years as medicinal herbs. The ethnopharmacological claims for *Teucrium* species include the use of decoctions or infusions as stimulants, tonics, diaphoretics, diuretics, and treatments of stomach pain, asthma, amenorrhea, leucorrhoea, chronic bronchitis and gout in traditional medicine (Grieve, 1996; Ulubelen et al., 2000). *Teucrium chamaedrys* (Germander), which is one of the most common and highly investigated species in the genus, is marketed for use in weight control, but there have been several reports from European countries, especially from the Mediterranean basin, of hepatotoxicity (Larrey et al., 1992; Dao et al., 1993). As a consequence, preparations containing Germander have been prohibited in France, and the toxicity studies showed that furano *neo*-clerodane diterpenes are responsible for hepatotoxicity (Kouzi et al., 1994). Our continuing interest in the genus *Teucrium* led to the isolation of 13 compounds, including two new phenylethanoid glycosides and a new *neo*-clerodane diterpenoid from *T. chamaedrys*.

2. Results and discussion

Compound **1** (Fig. 1) had the molecular formula $C_{20}H_{24}O_6$ as determined by LC-ESIMS (*m*/*z* 360.9 $[M+H]^+$, 392.9 $[M+Na]^+$, 743.7 $[2M+Na]^+$), and ^{13}C-NMR measurements. Of the nine degrees of unsaturation indicated by the molecular formula of **1**, four were present as multiple bonds (δ 109.3 *d*, 126.7 *s*, 141.7 *d*, 145.7 *d*, 179.5 *s* and 182.8 *s*) indicating the pentacyclic nature of the molecule. Inspection of the ^{1}H NMR spectrum of **1** showed signals for a β-substituted furan ring (two α-furan protons at *δ* 7.56 *s* and 7.61 *s*, and one β-proton at 6.49 *s*), and a secondary methyl group (*δ* 1.03 *d*, *J* = 6.4 Hz). Besides the signals of the furan ring, the remaining downfield signals were due to four protons of three oxygen-bearing carbon atoms (*δ* 3.53, *dd*, *J* = 2.9, 10.5 Hz; *δ* 4.28, 4.66, both *d*, AB system, J_{AB} = 10.9 Hz; *δ* 5.49, *t*, *J* = 8.7 Hz). Taking into account the results of our comprehensive 1D and 2D NMR spectroscopic studies and previously reported spectro-

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doi:10.1016/S0031-9422(03)00378-9

scopic data of related metabolites isolated from the genus *Teucrium* (Popa and Reinbol'd, 1973; Piozzi et al., 1987; Calis et al., 1996; Bedir et al., 1999; Savona et al., 1982), it was evident that **1** possesses a *neo*-clerodane type nucleus.

From the DQF-COSY and HMQC experiments, it was possible to establish 3 sequences excluding the signals ascribable to the furan ring: –CH–CH_2–CH_2–CH_2–CH–, –CH(O)–CH_2–CH–CH_3–, and –CH_2–CH(O)–, corresponding to the C-10→C-4, C-6→C-17, and C-11→C-12 moieties, respectively.

In order to link these three fragments, with the furan and the lactone rings, a ^{1}H–^{13}C-long range correlation spectroscopy (HMBC) experiment was performed. This not only connected the fragments but also facilitated the location of the C-18/C-6 γ-lactone ring (Fig. 2). The relative stereochemistry of the chiral centers in **1** was assigned by a combination of 2D-NOESY data, analysis of coupling constants, as well as molecular modeling studies (Fig. 3). The absolute configuration of C-12 was determined as *S* based on a distinctive dipolar coupling from H-12 to H_2-1 in the NOESY spectrum (Fig. 3). The absence of an nOe interaction between H-12 and H_3-17 further supported the *S* configuration of C-12 (Bruno et al., 1995). Consequently, the structure of compound **1** was established as 12(*S*)-15,16-epoxy-19-hydroxy-*neo*-cleroda-13(16),14-dien-18,6α:20,12-diolide.

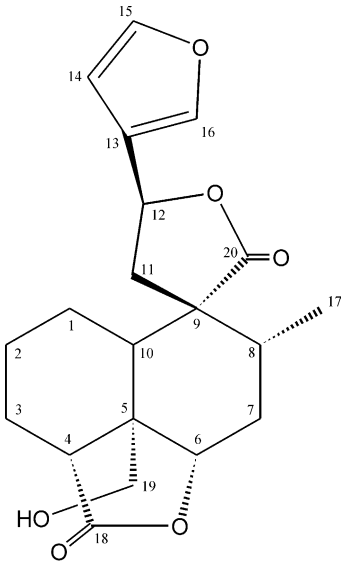


Fig. 1. Structure of compound **1**.

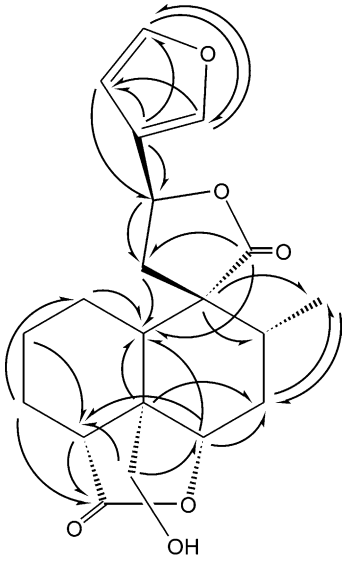


Fig. 2. Selected HMBC of compound **1**.

Compound **2** was obtained as an amorphous, yellowish powder. Its UV spectrum (MeOH) showed λ_{max} at 332, 265.5, and 220 nm indicating its polyphenolic nature. In the IR spectrum (KBr) absorption bands typical for hydroxyls (3400 cm^{-1}), α,β-unsaturated ester (1700 cm^{-1}), olefinic double bond (1630 cm^{-1}) and aromatic rings (1610, 1520 cm^{-1}) were observed.

The positive ion ESI-MS of **2** exhibited a pseudomolecular ion $[M+Na]^+$ at *m/z* 779.6, suggesting the molecular formula $C_{34}H_{44}O_{19}$ which was confirmed by the observation of one methyl, four methylene, 22 methine, and seven quaternary carbon resonances in its ^{13}C NMR spectrum (Table 3). Taking into account the results of our comprehensive ^{1}H- and ^{13}C-NMR studies and previous knowledge derived from metabolites isolated from the genus *Teucrium* (Gross et al., 1988; Bedir and Calis, 1997), the main features of a phenylethanoid glycoside were evident: characteristic signals arising from (*E*)-caffeic acid and 3,4-dihydroxyphenylethyl moieties [protons

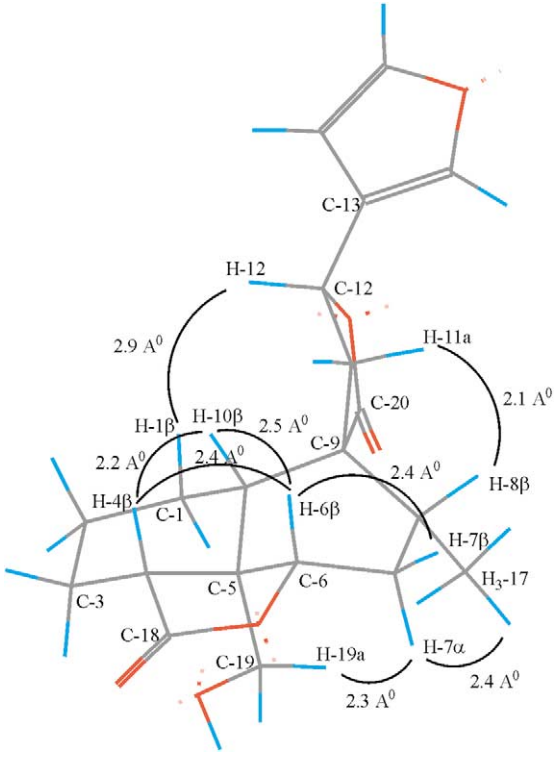


Fig. 3. Interatomic distances and selected NOESY correlations of **1**.

Table 1
^{1}H and ^{13}C NMR spectroscopic data of **1** (in CD_3OD, ^{1}H: 500 MHz, ^{13}C:125 MHz)

H/C	δ_C (ppm)	δ_H (ppm)	*J* (Hz)
1	23.5 *t*	1.49 *m*, 2.16 *m*	
2	23.1 *t*	1.58 *m*, 1.83 *m*	
3	26.4 *t*	1.58 *m*, 2.05 *m*	
4	48.4 *d*	2.39[a]	
5	48.4 *s*		
6	78.0 *d*	3.53 *dd*	2.9, 10.5
7	36.7 *t*	1.69 *dt*, 2.12 *m*,	4.0, 4.5
8	39.1 *d*	1.77 *m*	
9	52.6 *s*		
10	47.7 *d*	1.89 *dd*	8.6, 12.5
11	42.6 *t*	2.38[a], 2.56 *dd*	8.2, 14.2
12	73.7 *d*	5.49 *t*	8.7
13	126.7 *s*		
14	109.3 *d*	6.49 *s*	
15	145.7 *d*	7.56 *s*	
16	141.7 *d*	7.61 *s*	
17	17.3 *q*	1.03 *d*	6.4
18	182.8 *s*		
19	69.1 *t*	4.28 *d*, 4.66 *d*	10.9
20	179.5 *s*		

Assignments confirmed by COSY, HMQC and HMBC experiments.
[a] Signal pattern was unclear due to overlapping.

of aromatic rings (2×ABX systems), two *trans*-olefinic protons (AB system, $J=15.9$ Hz), β-methylene at δ 2.80 (2H, t, $J=7.0$ Hz), and two none-equivalent protons at δ 4.05 and 3.73 (each 1H, *m*) of the α-methylene hydrogen atoms]. Additionally, three anomeric proton resonances appeared at δ 5.40 (*d*, $J=1.8$ Hz, H-1″), 4.88 (d, $J=3.7$ Hz, H-1‴), and 4.37 (*d*, $J=7.9$ Hz, H-1′). The ^{13}C NMR spectrum of **2** supported the triglycosidic structure revealing the anomeric carbon resonances at δ 101.9, 104.3 and 104.2. Complete assignment of each sugar proton system was achieved by DQF-COSY, while the carbons were assigned from HMQC and HMBC spectra. The evaluation of the abovementioned spectra permitted identification of the sugar moieties as α-rhamnopyranosyl, α-lyxopyranosyl, and β-glucopyranosyl (Agrawal et al., 1985; Gross et al., 1988). The caffeoyl unit was positioned at C-4′ of the glucosyl unit on the basis of the strong deshielding of the H-4′ signal of glucose (δ 4.92, *t*, $J=9.2$ Hz). Furthermore, the C-3′ (δ 80.5) resonance of glucose moiety was shifted downfield ca. 4 ppm due to glycosylation, suggesting the attachment of one of the sugar units at this position. Prominent HMBC cross-peaks from H-3′ (δ 3.83) of glucose to the anomeric carbon atom of rhamnose (δ 102.1), from H-2″ (δ 3.95) of rhamnose to the anomeric carbon of lyxose (δ 104.3), from H-4′ (δ 4.92) of glucose to the carbonyl carbon atom (δ 168.2) of the acyl moiety as well as the HMBC correlation observed between H-1′ (δ 4.37) of glucose and the C-α (δ 72.3) carbon atom of the phenethyl alcohol unit permitted the determination of all interfragmental connectivities.

Based upon the above data, the structure of **2** was identified as 2-(3,4-dihydroxyphenethyl)-*O*-α-L-lyxopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-4-*O*-*trans*-caffeoyl-β-D-glucopyranoside, known as teucrioside which was earlier reported from *Teucrium chamaedrys* (Gross et al., 1988; Bedir and Calis, 1997). The comparison of ^{1}H and ^{13}C NMR spectra of **2** with those of an authentic sample of teucrioside as well confirmed its identity (Fig. 4).

The ^{1}H and ^{13}C NMR data of **3** were similar to those of **2**. Compound **3** contains an additional *O*-methyl resonance as indicated from the ^{1}H- and ^{13}C NMR data (δ_H 3.90 *s*; δ_C 56.5). The LC-ESIMS of **3** exhibited a pseudomolecular ion peak at *m*/*z* 771.1 $[M+H]^+$ ($C_{35}H_{46}O_{20}$), which was 14 mass units higher than that of teucrioside, confirming the presence of a methoxyl group. The signals arising from the sugar moieties and phenethyloxy parts of **3** and **2** were superimposible. The major differences were observed for the carbon resonances of the acyl moiety. The assignment of ^{1}H and ^{13}C signals of the phenylpropanoid part of **3**, was facilitated by DQF-COSY and HMQC spectra. The location of the *O*-methyl group was ascertained by an HMBC experiment which showed a long-range correlation between the methoxyl protons (δ 3.90) and C-3‴′ (δ 149.4), suggestive of ferulic acid unit as acyl moiety, as in martynoside (Sasaki et al., 1978). Based on these observations, the structure of **3** was elucidated as teucrioside-3‴′-*O*-methylether {2-(3,4-dihydroxyphenethyl)-*O*-α-L-lyxopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-4-*O*-*trans*-feruloyl-β-D-glucopyranoside}.

The NMR spectral data of **4** also revealed the features of a phenylethanoid glycoside. The ^{1}H and ^{13}C NMR spectra of **4** were almost similar to those of **3**, especially for the phenethyloxy part and sugar moieties, indicating the presence of the same glycosylation pattern. The most significant difference was the presence of an additional *O*-methyl resonance (δ_H 3.81 and 3.89; δ_C 56.7 and 56.8). These were also supported by the positive ion ESI-MS showing a molecular ion peak at *m*/*z* 785.2 $[M+H]^+$. The molecular mass difference of 14 unit verified the presence of an extra methoxyl group. The methoxyl groups of the acyl moiety were assigned to C-3‴′ and C-4‴′ on the basis of the upfield shifts of C-2‴′ and C-5‴′ as compared to **2** (C-2‴′: δ 111.9 for **4**, 115.3 for **2**; C-5‴′: δ 113.0 for **4**, 116.6 for **2**, *ca.* 3 ppm), and the observation of cross peaks in the HMBC spectrum from C-3‴′ and C-4‴′ (δ 149.3 and 150.6, respectively) to methoxyl protons at δ 3.81 and 3.89, respectively (Fig. 5). Moreover, nOe's observed between H-2‴′/H-5‴′ (δ 7.19, 6.84, respectively) and methoxyl protons at δ 3.89 and 3.81, in the 2D-NOESY spectrum, were also supported the location of methoxyl groups at C-3‴′ and C-4‴′. Based on these results, the structure of **4** was established as 2-(3,4-dihydroxyphenethyl)-*O*-α-L-lyxopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-4-*O*-3,4-dimethoxy-*trans*-cinnamoyl-β-D-glucopyranoside.

Teucrin A (Popa and Reinbol'd, 1973), dihydroteugin (Savona et al., 1982), isoteuflidin (Rodriguez et al., 1984), teuflin (Savona et al., 1979), teucvidin (Uchida et al., 1975), teucrin H1 (Gacs-Baitz et al., 1978), 6β-hydroxyteuscordin (Papanov and Malakov, 1982) teucrin G (Reinbol'd and Popa, 1974), and verbascoside (Andary et al., 1982) were also isolated from the aerial parts of *T. chamaedrys*. They were identified on the basis of their LC-ESIMS, and NMR (^{1}H and ^{13}C) data, in comparison with literature values.

3. Experimental

3.1. General

The 1D- and 2D-NMR spectra were obtained on a Bruker Avance DRX 500 FT spectrometer operating at 500 and 125 MHz, respectively. The chemical shift values are reported as parts per million (ppm) units relative to tetramethylsilane (TMS), and the coupling constants are in Hz (in parentheses). For ^{13}C NMR spectra, multiplicities were determined by a distortionless enhancement by polarization transfer (DEPT) experiment. LCMS data were obtained using a Finnigan AQA ThermoQuest instrument in the ESI mode. TLC was done on precoated silica gel 250F plates (Merck); developing system, *n*-hexane:EtOAc:MeOH mixtures (10:10:0.5, 10:10:1 and 10:10:2) for diterpenes, $CHCl_3$–MeOH–H_2O mixtures (80:20:2, 70:30:3 and 61:32:7) for phenylpropanoid glycosides; visualization, vanilin-H_2SO_4 (1%). CC was on silica gel (40 μm, J.T. Baker), reversed phase (RP) (40 μm, PolarPlus, J.T. Baker). The 3 D structure of **1** was built using CS Chem 3D Pro Software (Cambridge Soft.). HRESIMS data were obtained using a Bruker BioApex FT-MS instrument in the ESI(+) mode.

3.2. Plant material

Teucrium chamaedrys (Germander) was purchased from American Mercantile Corporation (Lot No. 7809).

Table 2
^{1}H NMR spectroscopic data of **2–4** (δ_H: 500 MHz, CD_3OD)

H	**2** δ_H (ppm), *J* (Hz)	**3** δ_H (ppm), *J* (Hz)	**4** δ_H (ppm), *J* (Hz)
Phenethyl alcohol			
2	6.70 *d* (1.8)	6.73 *brs*	6.75 *brs*
5	6.67 *d* (8.0)	6.71 *brd* (8.0)	6.82 *brd* (8.0)
6	6.56 *dd* (8.0, 1.8)	6.59 *d* (8.0)	6.69 *d* (8.0)
α	4.05 *m*, 3.73 *m*	4.06 *m*, 3.72 *m*	4.06 *m*, 3.72 *m*
β	2.80 *t* (7.0)	2.81 *t* (7.0)	2.83 *t* (7.0)
Glu			
1′	4.37 *d* (7.9)	4.40 *d* (8.0)	4.38 *d* (8.0)
2′	3.42 *dd* (7.9, 9.2)	3.42 *t* (8.5)	3.42 *t* (8.5)
3′	3.78 *t* (9.2)	3.82 *t* (9.0)	3.82 *t* (9.0)
4′	4.92 *t* (9.2)	4.95 *t* (9.0)	4.93 *t* (9.0)
5′	3.53 *m*	3.55 *m*	3.56 *m*
6′	3.63 *dd* (11.5, 2.5), 3.52[a]	3.66[a], 3.53[a]	3.65[a], 3.50[a]
Rha at $C_{glu\text{-}3}$			
1″	5.40 *d* (1.8)	5.43 *brs*	5.33 *brs*
2″	3.95 *dd* (1.8, 3.1)	3.98 *brs*	3.98 *brs*
3″	3.67 *dd* (3.1, 9.7)	3.70[a]	3.69[a]
4″	3.28 *dd* (9.7, 9.8)	3.31 *t* (9.5)	3.31 *t* (9.5)
5″	3.53[a]	3.55[a]	3.56[a]
6″	1.07 *d* (6.1)	1.10 *d* (6.0)	1.10 *d* (6.0)
Lyx at $C_{rha\text{-}2}$			
1‴	4.88 *d* (3.7)	4.91 *d* (3.5)	4.90 *d* (3.5)
2‴	3.85 *dd* (3.7, 3.6)	3.89[a]	3.90[a]
3‴	3.71 *dd* (3.6, 8.0)	3.72[a]	3.75[a]
4‴	3.74[a]	3.78[a]	3.76[a]
5‴	3.68[a], 3.56[a]	3.73[a], 3.58[a]	3.71[a], 3.56[a]
Acyl moiety			
2⁗	7.05 *d* (2.0)	7.20 *brs*	7.19 *brs*
5⁗	6.79 *d* (8.2)	6.84 *d* (8.0)	6.84 *d* (8.0)
6⁗	6.97 *dd* (8.2, 2.0)	7.10 *brd* (8.0)	6.91 *brd* (8.0)
α′	6.29 *d* (15.9)	6.30 *d* (16.0)	6.39 *d* (16.0)
β′	7.59 *d* (15.9)	7.68 *d* (16.0)	7.67 *d* (16.0)
$OC\underline{H}_3$		3.90 *s*	3.89 *s*, 3.81 *s*

Assignments confirmed by COSY, HMQC and HMBC experiments.
[a] Signal pattern was unclear due to overlapping.

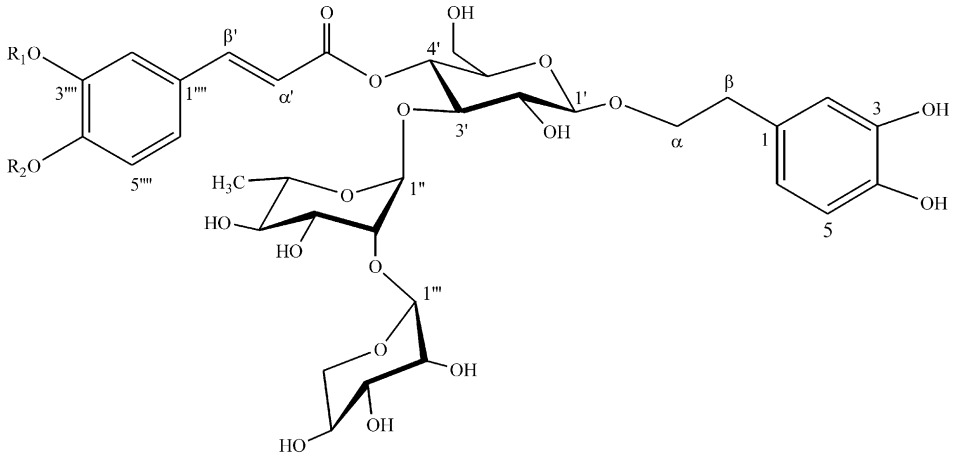
This sample was obtained from Europe and a voucher specimen is deposited at American Mercantile Corporation.

3.3. Extraction and isolation

The powdered herb (2.8 kg) was extracted with acetone (2×8 l, 8 h) and then filtered. The filtrates were combined and evaporated to dryness in vacuo to yield 247.7 g crude extract (yield 8.85%). An aliquot of the acetone extract (204.7 g) was fractionated on flash silica gel (40 μm, 1.4 kg) using a VLC system (Vacuum Liquid Chromatography), eluted with *n*-hexane (4 l), diethylether (6 l), EtOAc (5 l), CH_2Cl_2 (6 l), 50% CH_2Cl_2–acetone (28 l), acetone (6 l), and MeOH (16 l), to yield eleven fractions [Fr-A (28.7 g), Fr-B (20.9 g), Fr-C (7.1 g), Fr-D (6.0 g), Fr-E/H (21.0 g), Fr-I (9.8 g), Fr-J/K (95.0 g)]. Fr-C (7.1 g) was applied to a RP (C-18) column by VLC, employing a H_2O–MeOH (90%→50%) gradient, to yield five further fractions (Fr-C1 to Fr-C5). Fr-C2 (2.8 g) was subjected to flash silica gel chromatography (40 μm, 350 g) with $CHCl_3$–MeOH mixtures (99%→90%) to afford 18 fractions (Fr-C2-1 to Fr-C2-18). Fr-C2-2 (405.8 mg) was applied to a silica column (40 μm, 120 g), eluted with *n*-hexane–EtOAc (1:1) to give 18 fractions (Fr-C2-2a to Fr-C2-2r). Fr-C2-2h (56.5 mg) was purified by Horizon Biotage Inc, HPFC system (High Performance Flash Chromatography) using *n*-hexane–EtOAc mixtures (80:20 to 50:50) on a 12 mm silica cartridge (flow rate: 5 ml/min) to afford TC-5 (teuflin:10.6 mg). Fr-C2-2n comprised TC-6 (teucvidin: 20 mg). Fr-C2-7 (173.5 mg) was loaded onto a RP column (C-18; 30 g, VLC), using MeOH–H_2O (30%→100%), yielding TC-7 (Fr-C2-7c, teucrin H1:11.9 mg) and 10 fractions (Fr-C2-7a to Fr-C2-7h). Fr-C2-7h (33.5 mg) was purified by Horizon Biotage Inc., HPFC System (12 mm silica cartridge, flow rate: 5 ml/min) to give TC-8 (6β-hydroxyteuscordin: 6.6 mg). Fr-C2-8 (160.0 mg) was subjected to flash silica (85 g) CC using *n*-hexane–EtOAc–MeOH (10:10:0.5) afford TC-9 (teucrin G: 14.4 mg, Fr-C 2-8g), and 11 fractions (Fr-C2-8a to Fr-C2-8i). Addition of MeOH to Fr-D (6.0 g) gave a precipitate. The precipitate was filtered off, and washed with MeOH to give a white powder TC-1 (teucrin A: 1.07 g). The mother liquor was dried (4.5 g) and subjected to flash silica CC using $CHCl_3$–MeOH mixtures (100% $CHCl_3$ to 100% MeOH) gradient wise, to yield 31 fractions (Fr-D1 to Fr-D31). Fr-D17 (383.6 mg) was subjected to flash silica CC (100 g), eluted with *n*-hexane–EtOAc–MeOH (10:10:2) to give 10 fractions (Fr-D17a to Fr-D17j). Fr-D17d was pure TC-3 (23.4 mg). Fr-D17g (40 mg) was purified by Horizon Biotage Inc., HPFC System using 12 mm silica cartridge and *n*-hexane–EtOAc (80:20 to 50:50 + 5-drops of MeOH) as an eluent with a flow rate of 5 ml/ min, to afford TC-4 (isoteuflidin: 10.4 mg). Fr-D20 (301 mg) was subjected to Sephadex LH-20 CC using 100% MeOH as the eluent to give 8 fractions (Fr-D20a to Fr-D20i). Fr-D20f to Fr-D20h were combined according to their TLC profiles (198.4 mg), and purified by Horizon Biotage Inc., HPFC System [12 mm silica cartridge, *n*-hexane–EtOAc (80:20 to 50:50 + 5-drops of MeOH), flow rate 5 ml/min] to give TC-2 (dihydroteugin:17.6 mg). Fr-I was partitioned between *n*-butanol and water and the *n*-butanol phase, which was rich in phenylpropanoids, was evaporated to dryness (9.4 g). Reversed phase (C-18) chromatography of 4.2 g of *n*-butanol extract using MeOH–H_2O mixtures (100%H_2O→90%MeOH) gradient, yielded forty two fractions (Fr-I1 to Fr-I42) Re-chromatography of Frs.I19 (1.59 g) by flash silica column using mixtures of $CHCl_3$ and MeOH (95:5 and 90:10), and

Table 3
^{13}C NMR spectroscopic data of **2–4** (δ_C: 125 MHz; CD_3OD)

C	**2** δ_C (ppm)	**3** δ_C (ppm)	**4** δ_C (ppm)
Phenethyl alcohol			
1	131.5 *s*	131.6 *s*	132.9 *s*
2	116.3 *d*	117.2 *d*	117.1 *d*
3	146.2 *s*	146.1 *s*	147.4 *s*
4	144.7 *s*	144.7 *s*	147.1 *s*
5	117.2 *d*	116.4 *d*	116.5 *d*
6	121.3 *d*	121.3 *d*	121.2 *d*
α	72.3 *t*	72.3 *t*	72.3 *t*
β	36.6 *t*	36.6 *t*	36.7 *t*
Glu			
1′	104.3 *d*	104.2 *d*	104.2 *d*
2′	76.2 *d*	76.1 *d*	76.3 *d*
3′	82.0 *d*	81.9 *d*	82.0 *d*
4″	70.6 *d*	70.6 *d*	70.7 *d*
5′	76.1 *d*	76.0 *d*	76.1 *d*
6′	62.4 *d*	62.3 *d*	62.6 *d*
Rha at C_{glu-3}			
1″	101.9 *d*	101.8 *d*	101.9 *d*
2″	80.5 *d*	80.4 *d*	80.5 *d*
3″	72.0 *d*	72.0 *d*	72.1 *d*
4″	74.1 *d*	74.1 *d*	74.3 *d*
5″	70.5 *d*	70.4 *d*	70.6 *d*
6″	18.6 *q*	18.5 *q*	18.9 *q*
Lyx at C_{rha-2}			
1‴	104.3 *d*	104.2 *d*	104.3 *d*
2‴	71.5 *d*	71.4 *d*	71.6 *d*
3‴	72.6 *d*	72.6 *d*	72.8 *d*
4‴	69.0 *d*	68.9 *d*	69.1 *d*
5‴	65.0 *t*	64.9 *t*	65.1 *t*
Acyl moiety			
1⁗	127.7 *s*	127.7 *s*	127.6 *s*
2⁗	115.3 *d*	111.9 *d*	111.9 *d*
3⁗	146.9 *s*	149.4 *s*	149.3 *s*
4⁗	149.8 *s*	150.8 *s*	150.6 *s*
5⁗	116.6 *d*	116.6 *d*	113.0 *d*
6⁗	123.3 *d*	124.4 *d*	124.4 *d*
α′	114.7 *d*	115.1 *d*	115.1 *d*
β′	148.1 *d*	147.9 *d*	147.8 *d*
C=O	168.3 *s*	168.3 *s*	168.1 *s*
OCH_3		56.5 *q*	56.8 *q*, 56.7 *q*



Compounds	R_1	R_2
2 (teucrioside)	H	H
3	Me	H
4	Me	Me

Fig. 4. Structures of phenylethanoid glycosides (**2**–**4**).

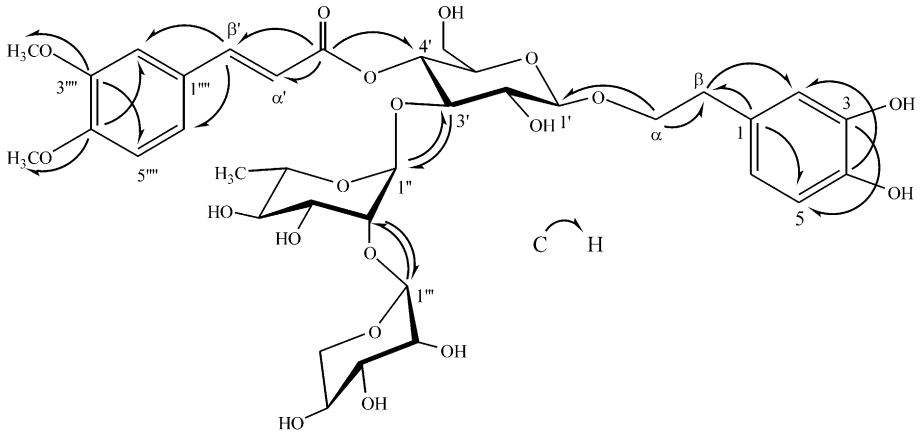


Fig. 5. Selected HMBC of compound **4**.

$CHCl_3$–MeOH–H_20 (80:20:1, 80:20:2, 70:30:3 and 65:35:5), yielded 23 fractions (Fr-I19a to Fr-I19v). Fr-I19r gave compound **2** (teucrioside, 666.0 mg). Fr-I20 (199.5 mg) was applied to a Sephadex LH-20 column, employing 100% MeOH, to give 15 fractions (Fr-I20a-Fr-I20l). Fr-I20j (85.6 mg) comprised verbascoside. Fr-I29 (267.0 mg) was subjected to polyamide gel (28.0 g) column using mixtures of H_2O–MeOH (100%→20%), and MeOH, to afford 29 fractions (Fr-I29a-Fr-I29g). Fr-I29c (33.3 mg) gave compound **3**. Fr-I27 (230.0 mg) was chromatographed on a polyamide gel (28 g) column, eluted with H_2O–MeOH (100%→50%) to give 16 fractions (Fr-I27a-Fr-I-27p), one of which was compound **4** (Fr-I27j, 29 mg).

3.4. Compound *1*

12(*S*)-15,16-Epoxy-19-hydroxy-*neo*-cleroda-13(16),14-dien-18,6α:20,12-diolide. White powder; $[\alpha]_D^{25} + 26.0^\circ$ (*c* 0.001, MeOH); ^{1}H- and ^{13}C-NMR: see Table 1. LCE-SIMS: at *m*/*z* 360.9 $[M+H]^+$, 392.9 $[M+Na]^+$, 743.7 $[2M+Na]^+$. HRESIMS: *m*/*z* 361.1649 $[M+H]^+$ requires 361.1651).

3.5. Teucrioside (*2*)

2-(3,4-Dihydroxyphenethyl)-*O*-α-L-lyxopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-4-*O*-*trans*-caffeoyl-β-D-glucopyranoside. White powder; UV λ_{max} (MeOH)

220, 265, and 332 nm; IR (KBr) ν_{max} 3400, 2924, 1700, 1635, 1610, 1520 cm^{-1}; ^1H- and ^{13}C-NMR: see Table 2 and 3; LCESIMS: at m/z 779.6 $[M+Na]^+$, 757.2 $[M+H]^+$. HRESIMS: m/z 779.2377 $[M+Na]^+$ (requires 779.2375).

3.6. Teucrioside-3''''-O-methylether (*3*)

2-(3,4-dihydroxyphenethyl)-*O*-α-L-lyxopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-4-*O*-*trans*-feruloyl-β-D-glucopyranoside. $\alpha]_D^{25}$ −87.5° (*c* 0.0008, MeOH); IR (KBr) ν_{max} 3373, 2924, 1689, 1629, 1596 and 1516, 1271, 1160, 1122, 1040 cm^{-1}; ^1H and ^{13}C NMR: see Tables 2 and 3; LCESIMS: at m/z 771.1 $[M+H]^+$. HRESIMS: m/z 793.2532 [M + Na] (requires 793.2531).

3.6.1. Teucrioside-3'''',4''''-O-dimethylether (*4*)

2-(3,4-dihydroxyphenethyl)-*O*-α-L-lyxopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-4-*O*-3,4-dimethoxy-*trans*-cinnamoyl-β-D-glucopyranoside. White powder; $\alpha]_D^{25}$ −60.0° (*c* 0.0007, MeOH); IR (KBr) ν_{max} 3400, 1699, 1629, 1595, 1514, 1270, 1155, 1120, 1040 cm^{-1}; ^1H- and ^{13}C-NMR: see Tables 2 and 3; LCESIMS: at m/z 785.2 $[M+H]^+$. HRESIMS: m/z 807.2683 [M + Na] (requires 807.2688).

Acknowledgements

This work was supported in part by the United States Department of Agriculture, ARS Specific Cooperative Research Agreement No. 58-6408-2-0009, and funded by Food and Drug Administration, Grant No: FD-U-002071-01 "Botanical Dietary Supplements: Science-Base for Authentication".

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