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Teucrol, a decarboxyrosmarinic acid and its 4′-*O*-triglycoside, teucroside from *Teucrium pilosum*

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Abstract

Teucrol, a decarboxy rosmarinic acid and its triglycoside, teucroside, 9′-decarboxyrosmarinic acid-4′-*O*-α-rhamnosyl-(1‴→6‴)-*O*-β-galactosyl-(1‴→4″)-*O*-α-rhamnoside, are two new natural phenolics which have been isolated and identified from the aqueous alcohol extract of the whole plant of *Teucrium pilosum* (Decne) Asch. & Schweinf. (Lamiaceae). Structures were determined by conventional methods of analysis and confirmed by MS and NMR spectral analysis, including 2D-homonuclear COSY experiment.

Keywords: *Teucrium pilosum*; Lamiaceae; Teucrol; 9′-decarboxyrosmarinic acid; Teucroside; 9′-decarboxyrosmarinic acid 4′-*O*-α-rhamnosyl (1‴→6‴)-*O*-β-galactosyl-(1‴→4″)-*O*-α-rhamnoside; NMR

1. Introduction

Plants of the family Lamiaceae are known for their high contents of flavonoids, essential oils and esters of caffeic acids (Banthorpe et al., 1989; Nawwar et al., 1989; Bisset, 1994; El-Ansari et al., 1995). The family is represented in Egypt by 15 genera, including 42 species, among which the genus *Teucrium* is of restricted occurrence (Täckholm, 1974) and only four of its species are growing wild in the western mediterranean coastal region and Sinai proper. In Egypt, infusion of the tender parts of these plants are used for treating dyspeptic complaints and biliary upsets (Boulos, 1983). As a part of our investigation of the constitutive phenolics of Lamiaceae we here describe the isolation and structure elucidation of the new phenolic ester, 9′-decarboxyrosmarinic acid, 3′,4′-dihydroxy-β-phenylethyl caffeate, or teucrol (**1**) and its triglycoside, 9′-decarboxyrosmarinic acid 4′-*O*-α-rhamnosyl-(1⁗→6‴)-*O*-β-galactosyl-(1‴→4″)-*O*-α-rhamnoside, teucroside, (**2**) from the aqueous ethanolic whole plant extract of *T. pilosum*.

The new compound (**1**) is of special interest as it represents the first reported occurrence of a caffeate ester of 3,4-dihydroxy-β-phenyl- ethanol. The related 2-(3,4-dihydroxyphenyl)ethenyl caffeate was reported to occur in callus cultures of several Lamiaceae species (Banthorpe et al., 1989). Its triglycoside (**2**) also, is interesting as it contains the new trioside, α-*O*-rhamnopyranosyl-(1→6)-β-*O*-galactopyranosyl (1→4)-α-*O*-rhamnopyranose. However, different glycosides of 3,4-dihydroxy-β-phenylethanol acylated with caffeic acid at their sugar moieties (e.g. orobanchin), have been previously reported to occur in *Syringa vulgaris* and *Echinacea angustifolia* (Stoll et al., 1950; Harborne, 1966; Birkofer et al., 1968).

2. Results and discussion

Ground dry shrublets of *T. pilosum* were exhaustively extracted with ethanol:H_2O (3:1). Compounds (**1 & 2**) were isolated and purified by applying a combination of polyamide column chromatography followed by Sephadex LH-20 columns.

Compound (**1**), isolated as an off-white amorphous powder was found to exhibit chromatographic properties [fluorescent blue spot on paper chromatograms (PC) under UV light, changing to bright canary yellow colour on fuming with ammonia, high R_f-values in organic solvents and medium R_f-values in aqueous solvents], result of $FeCl_3$ colour reaction (intense green) and UV spectral maxima in MeOH ($250_{shoulder}$, 290, 332) typical

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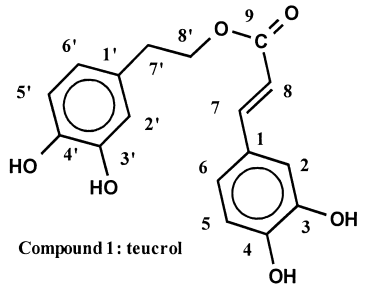
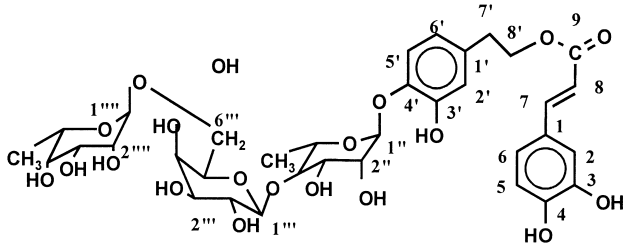


Compound 1: teucrol



Compound 2: teucroside

for caffeate esters (Harborne, 1973). On normal acid hydrolysis of (**1**), (2N aqueous HCl, 3 h, 100°C), caffeic acid [comparative paper chromatograms (CoPC), UV spectra, ^{1}H and ^{13} C NMR] and 3,4-dihydroxy-β-phenylethanol (chromatographic properties, UV spectra, EI-MS and ^{1}H NMR) were produced as the only phenolic products. Besides that, (**1**) exhibited a $[M-H]^-$ at m/z 315, in negative FAB-MS, corresponding to M_r of 316. These results suggested the compound to be a caffeate ester of 3,4-dihydroxy-β-phenylethanol, most probably the 9′-decarboxyrosmarinic acid. To find out how both the caffeic acid and the dihydroxy-β-phenylethanol are incorporated in the molecule of (**1**), NMR spectral measurements (DMSO-d_6, room temperature) were then undertaken. Resonances in the ^{1}H NMR spectrum were assigned on the basis of the mode of splitting and chemical shift values. The assignments were then confirmed by ^{1}H-^{1}H COSY experiments. In the recorded 1D-spectrum, the recognition of two distinct sp^3 methylenic proton triplets of coupling constant, $J=7$ Hz, at δ ppm 2.68 and 3.88, each integrated to two protons, attributable to the α- and β- methylene groups in the proposed dihydroxy-β-phenylethyl moiety of (**1**), together with the recognition, in the aromatic region, of the proton resonances pattern of 1,3,4-trisubstituted benzene, expected from the phenyl ring of the phenylethyl moiety [δ ppm 6.62 (*dd*, $J=8$ Hz, $J=2$ Hz, H-6′); 6.72 (*d*, $J=2$ Hz, H-2′); 6.8 (*d*, $J=8$ Hz, H-5′)], confirmed not only the identity of this moiety, but also the esterification of its alcoholic group, which resulted in the downfield shift of the resonance of the geminal methylenic protons ($\Delta\delta=0.36$ ppm), in comparison with the corresponding resonance (at δ ppm = 3.52) in the spectrum of free 3,4-dihydroxy-β-phenylethanol (see experimental). The spectrum also, showed the characteristic pattern of a caffeate proton resonances [δ ppm: 6.23 (*d*, $J=16$ Hz, H-α), 6.86 (*d*, $J=8$ Hz, H-5), 6.96 (*dd*, $J=8$ Hz, 2 Hz, H-6), 7.o7 (*d*, J = 2 Hz, H-2), 7.62 (*d*, $J=16$ Hz, H-β)], thus supporting the proposed structure of compound (**1**) to be 3′,4′-dihydroxy-β-phenylethyl caffeate, or decarboxyrosmarinic acid.

The ^{13}C NMR data of (**1**) are in accordance with the proposed structure, whereby the α- and β- sp^3 methylenic carbons 7′, 8′ of the hydroxylated β-phenylethyl moiety were recognized from the resonances at δ ppm 38.7 and 66.0, respectively. The latter, when compared with the corresponding resonance in the spectrum of free 3,4-dihydroxy-β-phenylethanol was found to be shifted downfield ($\Delta\delta=2.3$ ppm), thus confirming esterification of the connected alcoholic group. The present ester, in the molecule of (**1**), was further confirmed by the recognizable upfield position of the caffeate carbonyl carbon at δ 165.8 ppm, in comparison with the chemical shift (168.2 ppm) of the same carbon in the spectrum of the free acid. Assignments of the remaining resonances, in the spectrum of (**1**) were made straightforward after direct comparison with the ^{13}C NMR data of appropriate model compounds, e.g. rosmarinic acid (Kelley and Carmach, 1976) and were found to agree well with the proposed structure of (**1**), thus finally confirming its identity to be 9′-decarboxyrosmarinic acid, 3,4-dihydroxy-β-phenylethyl caffeate, or teucrol, a new caffeate ester which has not been reported previously to occur in nature.

The second new phenolic (**2**), separated as a faint brown amorphous powder, has exhibited chromatographic properties (light blue fluorescence on PC under UV light which turning yellow with ammonia vapour, medium R_f -values in organic solvents and high R_f -values in aqueous solvents), $FeCl_3$ reaction (intense green) and UV maxima in MeOH ($250_{shoulder}$, 287, 228), which suggested its close relation to compound (**1**), and that it is, most probably, a salt or a glycosylated derivative of (**1**).

On negative FAB-MS, compound (**2**) exhibited a molecular ion peak at $[M-H]^-$: 769, corresponding to a molecular weight of 770 amu. On normal acid hydrolysis, (**2**) yielded caffeic acid and 3,4-dihydroxy-β-phenylethanol (CoPC, UV, ^{1}H and ^{13}C NMR spectral data) as the only phenolic components together with rhamnose and galactose (CoPC). However, (**2**) resisted the effect of the enzyme β-galactosidase in acetate buffer of pH = 5.1 at 37°C for 48 h. On mild acid hydrolysis (0.1 N aqueous HCl, 30 min, 100°C), (**2**) yielded, among other phenolics, decarboxy rosmarinic acid (isolated from an ethyl acetate extract of the aqueous hydrolysate by preparative PC and identifed by CoPC, UV and FAB-M spectral analysis). The data, given above led to the conclusion that compound (**2**) is a dirhamnosyl-monogalactosyl

decarboxy rosmarinic acid. However, the attachment sites were still unkown at this stage. This view was then supported through NMR spectral analysis which determined the sites of attachment of the sugar moieties to the phenolic moiety in the molecule of (**2**).

The recorded ^{1}H NMR spectrum (DMSO-d_6, room temp.) revealed two distinct patterns of proton resonances, the first typical for decarboxy rosmarinic acid and was found to contain well-separated signals of this moiety, except those of the 8′-methylenic protons which were determined by ^{1}H-^{1}H COSY experiment to be located at δ ppm 3.85, but overlapped with other sugar proton signals. The second pattern is characteristic for sugar protons and was found to contain two distinct α-$^1C_4$-rhamnose anomeric proton resonances at δ ppm 5.12 and 4.50, each as a doublet of $J=2$ Hz, together with one β-$^4C_1$-galactose anomeric proton resonance at δ ppm 4.82 (*d*, H = 8.5 Hz), and two rhamnose methyl proton doublets ($J=6$ Hz) at δ ppm 0.95 and 1.04 as well (Nawwar et al.). Other sugar proton resonances appeared in this pattern as a broad intense multiplet at δ ppm 3.30–3.85. The relatively upfield position of the anomeric protons of the galactose and one of the rhamnose moieties would suggest that they are connected, in the molecule of (**2**), through sugar alcoholic groups and not through phenolic hydroxyls. Connection through the latter would cause these two resonances to be shifted relatively downfield as it is the case with the second anomeric rhamnose proton located at δ ppm 5.12. Similar chemical shifts are known in association with flavonoid glycosides (Harborne, 1994). These data together with the results of enzymic hydrolysis would suggest that the three sugar moieties are arranged in the form of a rhamnosylgalactosyl–rhamnoside chain which is linked to the phenolic moiety of (**2**) through a phenolic hydroxyl group, to result in a decarboxy rosmarinic acid rhamnosylgalactosylrhamnoside structure. Ambiguity about the sites of attachment was then unraveled through ^{13}C NMR analysis.

Structural elucidation of (**2**) was finally achieved by ^{13}C NMR spectroscopy, including off-resonance and ^{1}H-^{13}C COSY, which allowed the full assignments of all carbon resonances. The received ^{13}C NMR data unambiguouly identified (**2**) as a decarboxyrosmarinic acid derivative bearing a 4′-*O*-substituent. This followed, immediately from the direct comparison with the ^{13}C NMR spectrum of the aglycone (**1**). In the aromatic region, both spectra have exhibited close similarity. However a distinction can be made, because of the upfield shift ($\Delta\delta = 1.7$ ppm) of the C-4′ carbon resonance of the 3′,4′-dihydroxy-β-phenylethyl moiety of (**2**) and the accompanying downfield shifts of the resonances of its *ortho* carbons, C-3′ and C-5′ ($\Delta\delta = 1.0$ & 1.1 ppm, respectively) and of the resonance of the *para*- carbon, C-6′ ($\Delta\delta = 1.7$ ppm) as well. Similar shifts are well-known from the ^{13}C NMR data of phenolic glycosides (Nawwar et al., 1982). The number and characteristic shifts of the ^{13}C sugar signals indicated the presence of two rhamnose and one galactose, all existing in the pyranose form. The presence of two rhamnose moieties was concluded from two sp^3 methyl resonances at δ ppm 18.0 and 19.4. The positions of these signals indicated that the former belongs to the primary rhamnose moiety, being directly attached to the 4′-phenolic hydroxyl of the aglycone, because attachment to sugar hydroxyls would shift the methyl rhamnose signals downfield to ca 21 (Markham et al., 1978), as in the case of the latter methyl signal, at δ ppm 19.4. The α-configuration of the rhamnose moieties followed from their C-1 chemical shift values of their resonances at δ ppm 101.4 and 100.6, while the β-configuration of the galactose moiety was concluded from the 102.5 ppm chemical shift position of its anomeric carbon. That the sugar core in compound (**2**) contains a robinobiosyl, [rhamnosyl-(1→6)-galactosyl] moiety was concluded from the existence, in the recorded ^{13}C NMR spectrum, of the pattern of carbon resonances, which characterises this moiety, together with six additional sugar carbon resonances, attributable to the primary rhamnoside unit of that sugar core. The measured chemical shifts indicated that this unit is substituted, by the robinobiosyl moiety at its carbon number four, whose resonance was shifted, therefore downfield to δ ppm 79.0 [in comparison with the chemical shift of the corresponding resonance in the spectrum of free rhamnose (Breitmaier and Voelter, 1978)]. An accompanying γ-upfield shift caused by the galactosyl C-1, in the robinobiosyl moiety was also detected for the resonances of C-3 and C-5 (δ ppm 70.50 and 70.56) of the primary rhamnoside moiety, thus confirming the structure of the sugar core in (**2**), to be α-*O*-rhamnopyranosyl-(1‴→6‴)-β-*O*-galactopyranosyl-(1‴→4″)-α-*O*-rhamnopyranoside, which represents a new natural trioside. Off-resonance and ^{1}H-^{13}C COSY experiments finally confirmed this identity. Consequently, compound (**2**) is identified to be 3′,4′-dihydroxy-β-phenylethyl caffeate-4′-α-*O*-rhamnopyranosyl-(1⁗→6‴)-β-*O*-galactopyranosyl-(1‴→4″)-α-*O*-rhamnopyranoside, teucroside, a new phenolic trioside which has not been reported before to occur in nature.

3. Experimental

NMR:Jeol EX-270 spectrometer, 270 MHz (^{1}H NMR) and 67.5 MHz (^{13}C NMR), respectively. ^{1}H resonances were measured relative to TMS and ^{13}C NMR resonances to DMSO-d_6 and converted to TMS scale by adding 39.5. Typical conditions: spectral width = 5000 Hz for ^{1}H and 20000 Hz for ^{13}C, 32 K data points and a flip angle of 45°C. FAB-MS: Finnigan MM-7070 instrument (VG Analytical). PC (descending): Whatman no. 1 paper, using solvent systems: (1)

H_2O; (2) 15% HOAc; (3) BAW (*n*-BuOH-HOAc-H_2O, 4:1:5, upper layer); (4) C_6H_6-*n*-BuOH-H_2O-pyridine (1:5:3:3, upper layer). Solvents 3 and 4 were used for sugar analysis.

3.1. Plant material

Fresh shrublets of *Teucrium pilosum* Decne, were collected from Wadi El-Raha, South Sinai, Egypt, during June 1998 and authenticated by Dr. I. El-Garf, Department of Botany, Faculty of Sciences, Cairo University. A voucher specimen is deposited at the herbarium of the NRC, Cairo.

3.2. Isolation and identification

The ground meal (2.5 kg) of the dried shrublets was extracted by being refluxed with EtOH:H_2O (3:1) over a boiling water bath (three extractions, each for 8 h, with 4 l). The concd. extract was applied to a polyamide 6S (Riedel-de-Haën, Seelze Hannover, Germany) column (1500 cm long, 5 cm internal diameter) and eluted with H_2O followed by H_2O–EtOH mixtures of decreasing polarities to yield, among nine desorbed fractions, a phenolic one, eluted by H_2O–EtOH mixture (80:20) and appearing on the column as a bright blue band under UV light. Repeated column fractionation over Sephadex LH-20, using *n*-BuOH saturated with water for elution afforded individual pure samples of **1**, (122 mg) and **2** (101 mg).

3.3. 3,4-Dihydroxy-β-phenylethyl caffeate, 9′-decarboxy-rosmarinic acid, teucrol, (*1*)

R_f- values:0.46 (H_2O), 0.54 (HOAc), 0.84 (BAW). UV λ_{max} (nm) in MeOH: $250_{shoulder}$, 290, 332. M_r 316,-ve FAB-MS $[M-H]^-$: 315. Normal acid hydrolysis of **1** gave caffeic acid and 3,4- dihydroxy-β-phenyl ethanol. Caffeic acid: R_f- values: 0.25 (H_2O), 0.56 (HOAc), 0.81 (BAW); UV λ_{max} (nm) in MeOH: 218, 245,298,325; ^1H NMR: δ ppm 6.2 (*d*, *J* = 16 Hz, H-8), 6.76 (*d*, *J* = 7.5 Hz, H-5), 6.88 (*dd*, *J* = 7.5 Hz and 2.5 Hz, H-6), 6.98 (*d*, J = 2.5 Hz, H-2), 7.48 (*d*, *J* = 16 Hz, H-7); ^{13}C NMR: δ ppm: 126.1 (C-1), 115.2 (C-2), 144.9 (C-3), 148.4 (C-4), 116.2 (C-5), 121.5 (C-6), 146.2 (C-7), 115.2 (C-8), 168.0 (C-9). 3,4-dihydroxy-β-phenyl ethanol: R_f- values: 0.64 (H_2O), 70.0 (HOAc), 0.88 (BAW); EI-MS: *m*/*z* (rel. inten.): 155 (9), 153 (7), 136 (100), 109 (32), 81 (31); ^1H NMR: δ ppm 2.58 (t, *J* = 7 Hz, H-7′), 3.52 (t, *J* = 7 Hz, H-8′), 6.60 (dd, *J* = 7.5 Hz and 2.5 Hz, H-6), 6.68 (*d*, *J* = 2.5 Hz, H-2), 6.76 (*d*, *J* = 7.5 Hz, H-5); ^{13}C NMR″ δ ppm: 38.2 (C-7), 63.7 (C-8), 131.3 (C-1), 115.6 (C-2), 143.5 C-3), 145.2 (C-4), 115.8 (C-5), 121.5 (C-6). ^1H NMR of **1**: 3′,4′-dihydroxy-β-phenylethyl moiety: δ ppm 2.68 (t, *J* = 7 Hz, H-7′), 3.88 (t, *J* = 7 Hz, H-8′), 6.62 (*dd*, *J* = 8 Hz, 2 Hz, H-6′), 6.72 (*d*, *J* = 2 Hz, H-2′), 6.8 (*d*, *J* = 8 Hz, H-5′); caffeate moiety: δ ppm 6.23 (*d*, *J* = 116 Hz, H-8), 6.86 (d, J = 8 Hz, H-5), 6.96 (*dd*, J = 8 Hz and 2 Hz, H-6), 70.7 (D, *J* = 2 Hz, H-2), 7.62 (*d*, *J* = 16 Hz, H-7). ^{13}C NMR of (**1**): 3′,4′-dihydroxy-β-phenylethyl moiety: δ ppm 38.7 (C-7′), 66.0 (C-8′), 132.4 (C-1′), 116.0 (C-2′), 143.7 (C-3′), 145.0 (C-4′), 115.4 (C-5′), 121.1 (C-6′); caffeate moiety: δ ppm 146.0 (C-7), 114.2 (C-8), 166.5 (C-9), 125.8 (C-1), 114.0 (C-2), 144.86 (C-3), 149.0 (C-4), 116.5 (C-5), 121.8 (C-6).

3.4. Teucrol-4′-O-α-rhamnopyranosyl-(1‴→6‴)-β-O-galacto-pyranosyl-(1‴→4″)-α-O-rhamnopyranoside, teucroside (*2*)

R_f- values: 0.83 (H_2O), 0.87 (HOAc), 0.54 (BAW). λ_{max} (nm) in MeOH: $250_{shoulder}$, 287, 328. M_r: 770,-ve FAB-MS $[M-H]^-$: 769. Normal acid hydrolysis gave caffeic acid and 3,4-dihydroxy-β-phenylethanol (CoPC) in the ethyl acetate extract of the hydrolysate and galactose and rhamnose (CoPC) in the aqueous hydrolysate left after ethyl acetate extraction. Mild acid hydrolysis : Prep. PC on Whatman paper no. 3MM, using BAW as solvent system, for the ethyl acetate extract of the hydrolysate (40 mg of **2,** 0.1 N HCl, 30 min, at 100°C), and inspection under UV light of the dried developed chromatograms to define the analysed band, folowed by extraction of the bands allowed the isolation of 9-decarboxyrosmarinic acid (identified as given above). Enzymic hydrolysis: 12 mg of **2,** were treated with β-galactosidase (BDH) at pH 5.0 for 48 h at 37°C followed by CoPC of the product in solvent 1, 2 and 3 to prove the recovery of the compound unchanged. 1D-^1H and ^1H-^1H COSY NMR: 9′-decarboxyrosmarinic acid moiety: δ ppm 2.65 (t, *J* = 7 Hz, H-7), 3.85 (hidden by sugar proton resonances), 6.45 (dd, *J* = 8 Hz and 2 Hz, H-6′), 6.62 (*d*, *J* = 8 Hz, H-5′), 6.65 (*d*, *J* = 2 Hz, H-2′), 6.15 (*d*, *J* = 16 Hz, H-α), 6.7 (*d*, *J* = 7.5 Hz, H-5), 6.83 (*dd*, *J* = 7.5 Hz and 2 Hz, H-6), 7.00 (*d*, *J* = 2 Hz, H-2), 7.42 (*d*, *J* = 16 Hz, H-β); teucrioside moiety, α-*O*-rhamnopyranosyl-(1⁗→6‴)-β-*O*-galactopyranosyl-(1‴)-α-rhamnopyranoside:δ ppm 5.12 (*d*, *J* = 2 Hz, α-rhamnoside H-1‴), 4.82 (*d*, *J* = 8.5 Hz, β-galactopyranosyl H-1‴), 4.5 (*d*, *J* = 2 Hz, α-rhamnopyranosyle H-1⁗), 3.15-3.9 (m, 8′ methylenic and sugar protons overlapped with exchangable proton resonances), 0.95 (*d*, *J* = 6 Hz, rhamnose CH_3), 1.04 (d, *J* = 6 Hz, rhamnose CH_3). ^{13}C NMR: 9′-decarboxyrosmarinic acid: δ ppm 38.5 (C-7′), 66.6 (C-8′), 131.7 (C-1′), 116.4 (C-2′), 145.8 (C-3′), 142.7 (C-4′), 115.6 (C-5′), 119.7 (C-6′), 145.9 (C-7), 114.8 (C-8), 165.8 (C-9), 125.6 (C-1), 113.8 (C-2), 145.1 (C-3), 148.7 (C-4), 115.9 (C-5), 121.7 (C-6); Teucrioside moiety: δ ppm 100.6 (C-1⁗), 70.7 (C-2⁗), 70.4 (C-3⁗), 72.0 (C-4⁗), 68.9 (C-5⁗), 19.4 (C-Me), 102.5 (C-1‴), 71.8 (C-2‴), 73.0 (C-3‴), 68.5 (C-4‴), 74.5 (C-5‴), 67.5 (C-6‴), 101.4 (C-1″), 70.6 (C-2″), 70.5 (C-3″), 79.0 (C-4″), 70.6 (C-5″), 18.0 (C-Me).

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