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EUGENYLGLUCOSIDE, A NEW NATURAL PHENYLPROPANOID HETEROSIDE FROM *MELISSA OFFICINALIS*

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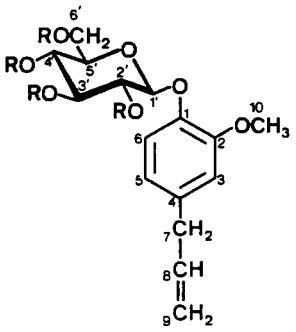
ABSTRACT.—Eugenylglucoside [**1**] has been isolated in pure state from *Melissa officinalis* leaves. The main physicochemical constants of this compound have been established and compared to those of the synthesized substance.

In our continuing investigation of the secondary metabolites of *Melissa officinalis* L. leaves (1,2), our attention was directed toward heterosides of monoterpenes and phenylpropanoids. The presence of iridoid glycosides in the Lamiaceae has been stated previously (3), and their distribution and taxonomic significance have also been reviewed (4). In our research, we were unable to find any iridoid glucosides in *M. officinalis* leaves, but we firmly established the presence of heterosides with volatile aglycones (1). The lack of iridoids in *M. officinalis* leaves strengthens the classification of the Lamiaceae made by Wunderlich (5). The occurrence of various monoterpenic heterosides in plants has also been reviewed (6).

The subject of this paper is the isolation and characterization of pure eugenylglucoside [**1**], previously obtained as a mixture with glucosides of benzyl alcohol, β-phenylethyl alcohol, nerol, geraniol, and geranic acid (7).

Dried leaves of *M. officinalis* were extracted with MeOH and purified by cc on Al_2O_3 (1). The aqueous eluate was cleared of polysaccharides, and the heterosides were fractionated by reversed-phase cc. After acetylation, the heterosides were finally purified by Si gel chromatography. Compound **2** was obtained as colorless plates from EtOH.



1 R=H
2 R=Ac

Synthesis of **2** (for reference purposes) was done by means of a Koenigs and Knorr reaction, with α-acetobromoglucose in an alkaline H_2O/Me_2CO solution of the phenol (8).

Deacetylation of **2** was done with NaOMe (9). Enzymic hydrolysis of **1** with β-glucosidase in Sørensen's pH 5 buffer liberated glucose as the sugar fraction and eugenol as the aglycone, both identified by gc-ms (1).

The ^{1}H nmr of **2** shows a doublet at δ 3.35 (H-7 protons, $J_{7,8} = 6.5$), a singlet at δ 3.80 (methyl H-10), two double doublets at δ 5.08 (H_a-9, *cis* position) and at δ 5.10 (H_b-9, *trans* position) ($J_{9a,8} = 12$, $J_{9b,8} = 16$, $J_{9a,9b} = 2$), a multiplet at δ 5.96 (H-8),

aromatic associated signals at δ 6.70 (m, 2H) and at δ 7.05 (d, 1H), and the expected signals for sugar protons.

Assignment of the signals in the ^{13}C nmr of **1** (see Table 1) was made through comparison of chemical shifts published for aromatic substances (10).

TABLE 1. ^{13}C-nmr Spectral Data[a] of Compound **1**.

Carbon	
C-1	144.89 s
C-2	148.89 s
C-3	112.98 d
C-4	133.45 s
C-5	120.30 d
C-6	115.52 d
C-7	39.02 t
C-8	137.89 d
C-9	115.52 t
C-10	55.64 q
C-1′	100.28 d
C-2′	73.23 d
C-3′	76.97 d[b]
C-4′	69.70 d
C-5′	76.84 d[b]
C-6′	60.67 t

[a]Measured in DMSO-d_6 at 90.53 MHz. The chemical shifts are given in δ-scale relative to TMS. s = singlet; d = doublet; t = triplet; q = quartet.

[b]Assignments may be reversed.

Eugenol is a constituent of several essential oils, but it does not appear to occur in the steam volatile fraction of *M. officinalis* leaves (11,12). The presence of a heteroside of this phenylpropanoid has been recorded in cell cultures of *Ocimum basilicum*, but the genuine compound was neither isolated nor identified (13). In a recent paper, isolation of eugenylglucoside [**1**] from *Citrus* peelings has been reported (14). However, the nmr characteristics published in this earlier work do not compare with our determinations made on both the synthetic and the isolated products. The compound isolated from *Citrus* is said to lower blood pressure (14). Eugenol esters and ethers have been reported to possess a spasmolytic activity (15). Biological tests to study a possible antispasmodic effect of **1** are now underway.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were recorded with a Tottoli apparatus and are uncorrected. Optical rotation was measured at 21° in EtOH with a Polartronic I polarimeter. Uv spectra were recorded on a Lambda 3 uv/vis Spectrophotometer (Perkin-Elmer). ^1H- and ^{13}C-nmr spectra were recorded for compound **2** in $CDCl_3$ on a Bruker WP 200 at 200 MHz and for compound **1** in DMSO-d_6 on a Bruker AM 360 at 90.53 MHz. Chemical shifts are reported in ppm (δ scale) with TMS as internal standard. Preparative scale hplc was made with a Jobin-Yvon Modulprep using reversed phase Lichroprep RP-8 (15–25 μm, Merck). Tlc was performed on Si gel plates (Merck) with the following solvent systems: (a) *n*-PrOH-PhMe-HOAc-H_2O (25:20:10:10) for the heterosides, and (b) CH_2Cl_2-Me_2CO (10:1) for the acetylated derivatives, using anisaldehyde as spray reagent.

PLANT MATERIAL.—*M. officinalis* was obtained from the Botanical Garden in Geneva, Switzerland. A voucher specimen is deposited in our laboratory (A.M.). Leaves were dried at room temperature in a dark place.

ISOLATION OF EUGENYLGLUCOSIDE [**1**].—Powdered, dried leaves (2.5 kg) were extracted with MeOH and purified by chromatography on Al_2O_3 (1). The aqueous eluate was concentrated, and free sugars were eliminated by preparative scale hplc on an RP-8 column with H_2O as eluent. Heterosides were obtained by subsequent MeOH elution of the RP-8 column. The heterosides-containing MeOH fraction was purified by cc on Si gel (70–230 mesh, Merck) with H_2O-saturated EtOAc, containing 1–2% MeOH, as eluent. Fractionation of the purified heterosides was made with a Lobar® RP-8 column (B size, Merck) using an MeOH/H_2O elution gradient; **1** was eluted with 50% MeOH. After acetylation (with Ac_2O in C_5H_5N), **2** was purified on a Lobar® Si gel column (B size, Merck) with PhMe-Me_2CO (25:1) as eluent and finally crystallized in EtOH.

DEACETYLATION (9).—Compound **2** was dissolved in 5 ml 0.5% NaOMe in anhydrous MeOH, and the mixture was kept at room temperature for 20 min. The solution was neutralized with Dowex 50W X4 ion exchange resin (20–50 mesh, in anhydrous MeOH) and chromatographed on Lobar® RP-8 column with 50% aqueous MeOH to give pure **1**.

SYNTHESIS OF **2** (8).—α-Acetobromoglucose (Merck) (4.12 g) was dissolved at room temperature in 16 ml Me_2CO, and added, with stirring, to a solution of 0.82 g eugenol (Roth) in 4 ml Me_2CO and 7 ml 0.725 N KOH. In a few moments, the pH of the resulting solution dropped below 8.0. Then, 7 ml of 0.725 N KOH was added dropwise to the mixture, at a rate sufficient to maintain the pH above 8.0. After the last addition, the reaction mixture was set aside for 2 h as soon as the pH was approximately 7.0. The solution was then extracted with C_6H_6, and the organic layer evaporated. The residue was purified by cc on Lobar® Si gel (Merck) with PhMe-Me_2CO (25:1) and crystallized in EtOH.

ENZYMIC HYDROLYSIS.—Described elsewhere (1).

EUGENYLGLUCOSIDE TETRAACETATE [**2**].—Mp 122–123°; R_f (solvent system b) 0.73; ^{1}H nmr δ 2.03, 2.10 (6H each, s, 2 acetyls each), 3.35 (2H, d, $J_{7,8}=6.5$, H-7), 3.76 (1H, ddd, $J_{4',5'}=10$, $J_{5',6a'}=2.5$, $J_{5',6b'}=5$, H-5′), 3.80 (3H, s, OMe), 4.17 (1H, dd, $J_{6a',6b'}=12$, H_a-6′), 4.29 (1H, dd, H_b-6′), 4.91 (1H, m, $J_{1',2'}\sim 8$, H-1′), 5.08 (1H, dd, $J_{9a,8}=12$, $J_{9a,9b}=2$, H_a-9), 5.10 (1H, dd, $J_{9b,8}=16$, H_b-9), 5.27 (3H, m, H-2′, H-3′, H-4′), 5.96 (1H, ddt, H-8), 6.70, 7.05 (2H, m, and 1H, d, aromatic ring H-3, H-5, H-6).

EUGENYLGLUCOSIDE [**1**].—Mp 130–131°; R_f (solvent a) 0.73; $[\alpha]^{21}D=-45.85°$ ($c=1.03$ in EtOH); uv λ max (MeOH) 276, 225 nm (log ε 3.37, 3.88); ^{13}C nmr see Table 1.

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