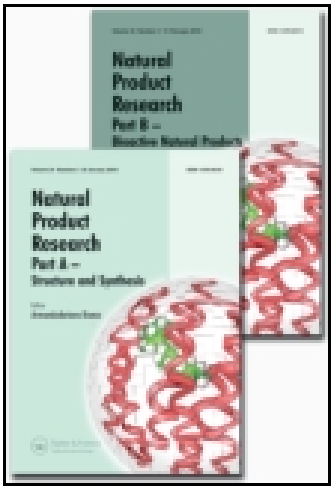

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Benzophenone *O*-glycosides from *Hypericum elegans*

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Elegaphenonoside, a new benzophenone *O*-rhamnoside, together with two known benzophenone *O*-glycosides, namely hypericophenonoside and neoannulatophenonoside, were isolated from the aerial parts of *Hypericum elegans* Stephan ex Willd. The structure of the new compound was established as 3′,5′,6-trihydroxy-4-methoxybenzophenone-2-*O*-α-L-rhamnopyranoside by means of chemical and physical evidence. In addition, the presence of kaempferol, quercetin, isoquercitrin, norathyriol, I-3,II-8-biapigenin, quercitrin, hyperoside and rutin were established in this plant.

Keywords: *Hypericum elegans* Stephan ex Willd; elegaphenonoside; benzophenone *O*-glycosides

1. Introduction

Hypericum elegans Stephan ex Willd is a herbaceous plant growing on the Balkan Peninsula, Mala Asia, Eastern and Middle Europe (Jordanov & Kožucharov, 1970). No detailed phytochemical investigation of *H. elegans* has been undertaken so far. The presence of the naphthodianthrones hypericin and pseudohypericin (Kitanov, 2001), quercitrin (Kitanov & Blinova, 1987), and the xanthone *C*-glucosides mangiferin and isomangiferin (Kitanov & Nedialkov, 1998) in this species were established by TLC screening. This article is a part of an extensive investigation of *H. elegans* and deals with the isolation and structural elucidation of elegaphenonoside, a new benzophenone *O*-rhamnoside.

2. Results and discussion

The plant material was extracted with chloroform and then with methanol. The combined methanolic extract was subjected to a series of chromatographic procedures that led to the isolation of elegaphenonoside (**1**), along with two known benzophenone *O*-glycosides, as well as seven flavonoids and a xanthone.

Elegaphenonoside (**1**) was isolated as optically active white needles with $[\alpha]_D^{25}$ −25.8. Its molecular formula was established as $C_{20}H_{22}O_{10}$ by means of FTMS data (*m*/*z* 423.1287 $[M+H]^+$). The IR spectrum of **1** showed absorption bands of hydroxyls (3356 cm^{-1}),

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chelated carbonyl (1620 cm^{-1}) and aromatic double bonds (1583 cm^{-1} and 1462 cm^{-1}). The bathochromic shift of the band at 336 nm in the UV spectrum with $AlCl_3$ ($\Delta = 36$ nm) revealed the presence of a free hydroxyl group in the *ortho* position to the carbonyl function. Acid hydrolysis of **1** gave 2,3′,5′,6-tetrahydroxy-4-methoxybenzophenone (annulatophenone) and L-rhamnose. The signals in the ^1H and ^{13}C spectra were assigned unambiguously using 2D NMR techniques, i.e. COSY, long-range COSY, NOESY, HETCOR and COLOC. Multiplicities were revealed by DEPT experiments. The ^1H-NMR spectrum of compound **1** showed two doublets at δ_H 6.19 and δ_H 6.38, belonging to the hydrogens of ring A, as well as a triplet and a doublet at δ_H 6.46 and δ_H 6.62, belonging to the hydrogens of ring B. In addition, the signal of the methoxyl group appeared at δ_H 3.81. These showed cross-peaks with the signals at δ_C 96.1 (C-5), 94.6 (C-3), 107.9 (C-4′), 108.2 (C-2′ and C-6′) and 56.0 (MeO at C-4), respectively. Furthermore, the signals of eight quaternary carbons, belonging to the aglycone moiety appeared at δ_C 198.8 (C=O), 165.4 (C-4), 161.1 (C-6), 159.6 (C-3′ and C-5′), 158.6 (C-2) 143.3 (C-1′) and 110.3 (C-1) in the ^{13}C-NMR spectrum of **1**, as well. Both ^1H and ^{13}C signal patterns were similar to that of 2-*O*-glycosides of annulatophenone previously isolated from *H. annulatum* (Momekov et al., 2006; Nedialkov & Kitanov, 2002). The relatively small coupling constant ($J = 1.8$ Hz) of the doublet at δ_H 5.26, attributed to the anomeric proton in the sugar moiety, suggested its α-configuration. The signals of the other sugar protons appeared as multiplets at δ_H 3.40–3.49 (H-2″, H-5″) and 3.28–3.35 (H-4″) as well as a double doublet at δ_H 3.15–3.19 (H-3″) and a doublet at δ_H 1.19 (CH_3-6″). These showed cross-peaks with signals of six sugar carbons at δ_C 100.5 (C-1″), 73.6 (C-4″), 72.0 (C-3″), 71.5 (C-2″), 70.9 (C-5″) and 18.0 (C-6″), respectively. The later carbon pattern is typical for α-L-rhamnopyranosides (Agrawal, 1992). Computer aided molecular modelling of **1** was in good agreement with NOESY experiments (Figure 1), where the distances between correlating protons were 0.23–0.26 nm (2.3–2.6 Å). Thus, compound **1** was identified as 3′,5′,6-trihydroxy-4-methoxybenzophenone-2-*O*-α-L-rhamnopyranoside, named elegaphenonoside, which is a new natural product. In addition, hypericophenonoside, neoannulatophenonoside, kaempferol, quercetin, isoquercitrin, norathyriol, I-3,II-8-biapigenin,

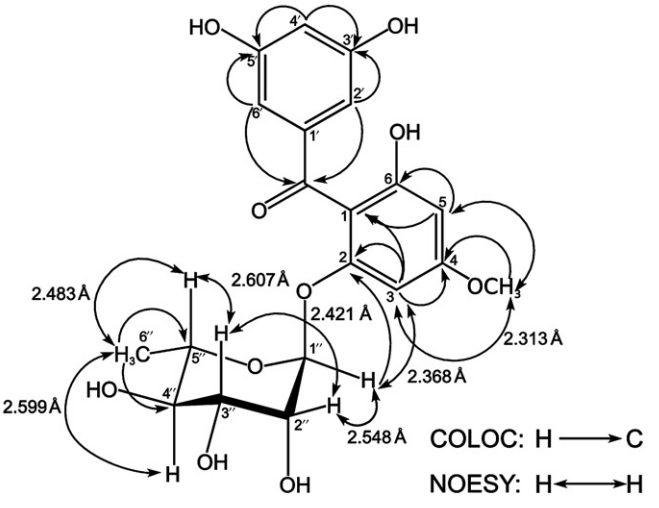

Figure 1. Selected COLOC and NOESY correlations of **1**.

quercitrin, hyperoside and rutin were also isolated and identified by the usual techniques. Benzophenone *O*-glycosides are rarely encountered in nature. Hypericophenonoside and neoannulatophenonoside have been previously isolated only from *H. annulatum* Moris. (Kitanov & Nedialkov, 2001; Momekov et al., 2006). This article reports the occurrence of benzophenone *O*-glycosides in *Hypericum* species for the second time.

3. Experimental

3.1. *General experimental procedures*

Melting points (m.p.) were measured on a Kofler hot-stage microscope and are uncorrected. Optical rotations in MeOH were obtained using a Perkin–Elmer 241 polarimeter. UV spectra were run in MeOH on a Specord UV-VIS instrument; ε values are given in parentheses. IR spectra were recorded with a Shimadzu FTIR-8101 M. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker ARX 300 apparatus at 300 and 75 MHz, respectively, in CD_3OD, using tetramethylsilane (TMS) as internal standard. Electrospray mass spectra (ESIMS) were recorded with a Bruker Esquire-LC ESI-ion trap in positive mode. High-resolution fourier transform mass spectrum (FTMS) was taken on a Bruker FT-ICR with electrospray ionisation in methanol containing formic acid. Column chromatography (CC) was conducted using silica gel 60 (0.063–0.2 mm) and LiChroprep C-18 (0.04–0.063 mm, an overpressure of 0.8–1.0 bar); flash chromatography (an overpressure of 0.8–1.0 bar) and vacuum liquid chromatography were performed with silica gel 60 (0.04–0.063). All adsorbents were purchased from Merck KGaA (Darmstadt, Germany).

3.2. *Collection of biological material*

The aerial parts of *H. elegans* Stephan ex Willd were collected during the flowering season from wild habitat at v. Balgarevo, Kavarna in June 2004. A voucher specimen (No. 153305) was deposited at the Herbarium of the Botany Institute of Sofia (SOM).

3.3. *Extraction and isolation*

The air-dried and powdered aerial parts (606 g) were refluxed with $CHCl_3$ (6 × 1 L) at 70°C for 2 h each time and then extracted with hot MeOH (10 × 1 L) at 70°C. The crude MeOH residue (141.30 g) was subjected to CC on silica gel. A stepwise gradient of CH_2Cl_2 and CH_2Cl_2–MeOH mixtures (100 : 0 → 82 : 18, steps of 1%) were used as mobile phase. A total of 86 fractions (250 mL each) were collected. Based on TLC the identical of these were combined to give a total of 18 pooled fractions (I–XVIII). Fraction XII was subjected to gel filtration on Sephadex LH-20, eluted with MeOH–H_2O mixtures (50 : 50 → 100 : 0, in steps of 10%, 250 mL of each mixture). A total of 50 fractions (30 mL each) were collected. The fractions 28–30 (80% MeOH) were pooled and subjected to CC on LiChroprep C-18 using MeOH–H_2O mixtures (25 : 75 → 40 : 60, in steps of 1%, 250 mL of each mixture) as mobile phase. Gel filtration over Sephadex LH-20 (eluent MeOH) was used as a final purification step, which gave 723.9 mg of compound **1**. Similar chromatographic procedures of fractions XIII–XV gave pure hypericophenonoside and neoannulatophenonoside. In addition, kaempferol, quercetin, isoquercitrin, norathyriol, I-3,II-8-biapigenin, quercitrin, hyperoside and rutin were also isolated by the usual techniques.

3.4. *Acid hydrolysis of compound 1*

A solution of compound **1** (10.0 mg) in 1N HCl (2 mL) was stirred at 100°C under reflux in a reaction flask for 4 h. Reaction mixture was transferred to separating funnel, diluted to 10 mL with water and extracted with water saturated EtOAc (3 × 5 mL). Both layers were separately evaporated to dryness *in vacuo*. The EtOAc residue was subjected to gel filtration on Sephadex LH-20 and eluted with MeOH. The aglycone of **1** was obtained as pale yellow powder and was identified as 2,3′,5′,6-tetrahydroxy-4-methoxybenzophenone on the basis of the melting point, co-TLC with an authentic sample, UV, IR and ESIMS (Kitanov & Nedialkov, 2001). The sugar was identified as L-rhamnose by means of co-TLC with an authentic sample on cellulose (Merck), mobile phase EtOAc-pyridine-H_2O (12 : 5 : 4) and spots were visualised with anisidine phtalate reagent (heating to 110°C for 3–5 min).

3.5. *Molecular modelling*

The optimisation of molecular geometry of **1** was done by *ab initio* calculation mode, using GAMESS-US (Schmidt et al., 1993), Version 24 March 2007 R6, on a 32 bit Linux 2.6 box. The parameters were set to as follows: self-consistent field wavefunction was set to Restricted Hatree Fock (RHF), the initial molecular geometry was given as Gaussian style internals, Gaussian basis was set to Pople's STO-NG minimal basis set, the number of Gaussians was set to 3, C1 was chosen as a symmetry group, all other parameters were set to defaults. The Molden program package Version 4.6 (Schaftenaar & Noordik, 2000) was used for drawing initial geometry coordinates as well as for visualising calculation results and for measuring intra molecular distances.

3.5.1. *Elegaphenonoside (**1**)*

White needles: m.p. 221–223°C; $[\alpha]_D^{25}$ −25.8 ($c = 2.01$, MeOH); IR (nujol) ν_{max}: 3356.0, 2924.4, 2953.4, 2855.0, 1620.4, 1462.2, 1583.7; UV (MeOH) λ_{max}: 300 (4.25); (+$AlCl_3$): 336, 371sh; (+NaOAc): 285, 371sh; ^{1}H-NMR (300 MHz, CD_3OD): 6.62 (2H, d, $J = 2.3$ Hz, H-2′ and H-6′), 6.46 (1H, t, $J = 2.3$ Hz, H-4′), 6.38 (1H, d, $J = 2.2$ Hz, H-3), 6.19 (1H, d, $J = 2.2$ Hz, H-5), 5.26 (1H, d, $J = 1.8$ Hz, H-1″), 3.81 (3H, s, CH_3O-4), 3.40–3.49 (2H, m, H-2″ and H-5″), 3.28–3.35 (1H, m, H-4″), 3.17 (1H, dd, $J = 3.4$, 9.4 Hz, H-3″), 1.20 (3H, d, $J = 6.1$ Hz, C-6); ^{13}C-NMR (75 MHz, CD_3OD): 198.8 (C=O), 165.4 (C-4), 161.1 (C-6), 159.5 (C-3′ and C-5′), 158.5 (C-6), 143.3 (C-1′), 110.3 (C-1), 108.2 (C-2′ and C-6′), 107.9 (C-4′), 100.5 (C-1″), 96.1 (C-5), 94.6 (C-3), 73.6 (C-4″), 72.0 (C-3″), 71.5 (C-2″), 70.9 (C-5″), 56.0 (CH_3O-4), 18.0 (C-6″); ESIMS: m/z 461 $[M + K]^+$, 445 $[M + Na]^+$, 423 $[M + H]^+$, 405 $[M - H_2O + H]^+$, 277 $[M_{agl}]^+$; FTMS: Calculated for $[C_{20}H_{22}O_{10} + H]^+$ 423.1286, found 423.1287; calculated for $[C_{20}H_{22}O_{10} + Na]^+$ 445.1105, found 445.1105; calculated for $[(C_{20}H_{22}O_{10})_2 + Na]^+$ 867.2318, found 867.2317.

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