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OMBUIN 3-SULPHATE FROM *FLAVERIA CHLORAEFOLIA*

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Abstract—One novel flavonoid sulphate ester and two known non-sulphated flavonoids were isolated from the leaves of *Flaveria chloraefolia*. The novel sulphated conjugate is 7,4′-dimethylquercetin (ombuin) 3-sulphate, and the known compounds are 6-methoxyquercetin and its 3-glucoside. The structure of ombuin 3-sulphate was established by UV spectroscopy, acid hydrolysis followed by EIMS of the aglycone, as well as enzymatic synthesis.

INTRODUCTION

The genus *Flaveria* is known to be a rich source of sulphated flavonols [1–9]. In fact, *F. bidentis* accumulates a number of flavonoid mono- to tetrasulphate esters, among which are isorhamnetin and quercetin 3-sulphates [1]; isorhamnetin 3,7- [2], quercetin 3,4′- [1] and 3,7- [3] disulphates; quercetin 3,7,4′- [1] and 3,7,3′- [4] trisulphates, as well as quercetin 3-acetyl-7,3′,4′-trisulphate [5] and 3,7,3′,4′-tetrasulphate [6]. Leaves of *F. chloraefolia*, on the other hand, contain quercetin 3-sulphate [7], 3,4′- and 3,3′-disulphates [8]; patuletin 3-sulphate [7] and 3,3′-disulphate [8], together with 6-methoxy kaempferol, spinacetin, eupalitin, eupatolitin and eupatin 3-monosulphates [9]. In continuation of our phytochemical studies of *Flaveria* flavonoids, we wish to report the identification of three additional flavonoid compounds from the leaves of *F. chloraefolia*.

RESULTS AND DISCUSSION

Fresh leaves of *F. chloraefolia* were extracted with 50% aqueous methanol. After concentration under reduced pressure, the aqueous extract was partitioned successively against hexane, chloroform, ethyl acetate and butanol. The ethyl acetate extract contained the non-sulphated flavonoids, which after column chromatography yielded two major compounds, patuletin **1** and its 3-glucoside **2**. Compound **1** was identified by co-chromatography with a reference compound, while **2** was characterized on the basis of its UV, ^{1}H NMR, ^{13}C NMR and FABMS spectroscopic data. The butanolic extract after gel filtration on Sephadex LH-20, followed by preparative TLC on polyamide plates, afforded the new sulphated flavonoid **3**.

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Identification of ombuin 3-sulphate (**3**)

UV spectral analysis of **3** exhibited no shifts in the presence of aryl-sulphatase reagent [10] and gave a bathochromic shift of 22 nm only after the addition of HCl, both of which are indicative of a flavonol 3-monosulphate ester [10]. On the other hand, the absence of a shift for band II after the addition of sodium acetate as well as the bathochromic shift of 22 nm only in presence of sodium methoxide, demonstrated the substitution of the 7- and 4′-hydroxyl groups, respectively. Acid hydrolysis of **3** gave an aglycone **3a** whose chromatographic and UV spectral properties, as well as its EIMS data were identical to a reference sample of ombuin (quercetin 7,4′-dimethylether). Furthermore, the enzymatic synthesis of ombuin 3-sulphate was demonstrated using cell-free extracts of *F. chloraefolia* and [^{35}S] 3′-phosphoadenosine-5′-phosphosulphate (PAPS) as the sulphate group donor. This enzyme preparation is known to catalyse the sulphation of flavonols at position 3 [11]. When ombuin was incubated with the enzyme preparation and the sulphate donor, the labelled reaction product was found to co-chromatograph with **3**, on polyamide TLC plates, using various solvent systems.

Patuletin 3-glucoside appears to be a characteristic constituent of the genus *Flaveria*, since it has previously been identified in *F. bidentis* [3], *F. linearis* and *F. trinervia* [12]. However, although ombuin 3,3′-disulphate has been identified in *Acrotema uniflorum* (Dilleniaceae) [13] this is the first report of ombuin 3-sulphate as a natural product.

EXPERIMENTAL

Plant material. Seeds of *F. chloraefolia* A. Gray were a generous gift from Professor A. M. Powell (Sul Ross State University, Alpine, TX) and were raised to fully grown plants under greenhouse conditions.

Source of reference compounds. Patuletin was a gift from Dr M. H. Moubasher (University of Texas, Austin, TX). Ombuin was from our laboratory collection. [^{35}S] PAPS was purchased from New England Nuclear, Boston, MA.

General methods. Acid hydrolysis, ^{1}H NMR, ^{13}C NMR, FABMS and EIMS analyses were carried out according to refs [7, 9]. UV spectra were obtained following standard procedures [14, 15], except for the spectra in presence of HCl and aryl-sulphatase which were recorded as in [9] and [10]. Prep. reverse-phase HPLC of the non-sulphated flavonoids was performed on a μ-Bondapack C_{18} semi-prep. column (300 × 7.8 mm), using a UV detector (340 nm), a flow rate of 3 ml/min, and the following solvents: A, 90% aq. MeOH + 0.1% HOAc; B, 20% aq. MeOH + 0.1% HOAc. Preparation of cell-free extracts of *F. chloraefolia* was carried out as in [16], and the enzymatic sulphation of ombuin was demonstrated using the standard flavonoid sulphotransferase assay [16]. Co-chromatography of the labelled ombuin 3-sulphate product with **3** was performed on polyamide-DC 6.6 TLC plates (Macherey–Nagel) using the following solvents, (a) 0.1% aq. tetrabutylammonium hydrogen sulphate–pyridine (4:1), 2 migrations; followed by (b) 0.1% aq. tetrabutylammonium hydrogen sulphate–pyridine (7:3), 2 migrations. The radioactive spots were located on the plate after autoradiography on X-ray film.

Extraction and isolation of the flavonoids. Extraction and liquid–liquid partition of the extracts were carried out as in ref. [7]. Chromatography of the EtOAc extract was performed on a polyamide SC-6 column, using a gradient of C_6H_6–MeCOEt–MeOH (9 : 5 : 5) to (3 : 1 : 1), and yielded patuletin (**1**) and its 3-glucoside (**2**). Both compounds were purified by HPLC using 60% A + 40% B (**1**; R_t = 7.5 mn) or 40% A + 60% B (**2**; R_t = 10.5 mn). Ombuin 3-sulphate (**3**) was isolated from the BuOH extract after chromatography on a Sephadex LH-20 column as reported in ref. [7]. It was converted to its tetrabutylammonium salt, and purified by prep. TLC on polyamide plates, according to ref. [9].

Patuletin (**1**) was identified by co-chromatography with a reference compound on polyamide TLC using C_6H_6–MeCOEt–MeOH (3:1:1), and cellulose TLC using *n*-BuOH–HOAc–H_2O (3:1:1); ^{1}H NMR (400.13 MHz, DMSO-d_6): 6.50 (1H, H-8), 6.87 (1H, *d*, *J* = 8.3 Hz, H-5′), 7.53 (1H, d, *J* = 8.3 Hz, H-6′), 7.66 (1H, H-2′). The UV spectral data of **1** was similar to reference patuletin.

Patuletin 3-O-β-glucoside (**2**). UV λ_{max}^{MeOH} nm: 255, 270 sh, 350; + NaOMe: 249, 305 sh, 382; + $AlCl_3$: 290, 325 sh, 383; + $AlCl_3$ + HCl: 258, 280 sh, 300 sh, 363; + NaOAc: 270, 410; + NaOAc + H_3BO_3: 268, 385; ^{1}H NMR (80 MHz, DMSO-d_6): *ca* 3.00–3.65 (5H, m, H-2″, H-3″, H-4″, H-5″ and H-6″), 3.75 (3H, s, Ar-OMe), *ca* 4.50–5.30 (OH gluc.), 5.45 (1H, d, *J* = 5.5 Hz, H-1″), 6.50 (1H, *s*, H-8), 6.83 (1H, d, *J* = 6.8 Hz, H-5′), *ca* 7.57 (2H, *m*, H-2′ and H-6′); ^{13}C NMR (100.13 MHz, DMSO-d_6): 60.07 (Ar-OMe), 61.10 (C-6″), 70.06 (C-4″), 74.22 (C-2″), 76.61 (C-3″), 77.61 (C-5″), 93.87 (C-8), 101.04 (C-1″), 104.25 (C-10), 115.32 (C-5′), 116.27 (C-2′), 121.32 (C-6′), 121.69 (C-1′), 131.47 (C-6), 133.07 (C-3), 144.90 (C-3′), 148.54 (C-4′), 151.70 (C-9), 152.40 (C-5), 156.30 (C-2), 158.01 (C-7), 177.65 (C-4); Negative FABMS (glycerol): 494 [M − H], 331 [M − glucose − H]. Acid hydrolysis of **2** gave **1** and glucose. The sugar was identified by co-chromatography with reference glucose on silica TLC plate according to [17].

Ombuin 3-sulphate (**3**). UV $\lambda_{max}^{H_2O}$ nm: 330; + Aryl-sulphatase: 330; λ_{max}^{MeOH} nm: 255, 338; + HCl: 360; + NaOMe: 260, 360; + $AlCl_3$: 260, 300 sh, 362, 390 sh; + $AlCl_3$ + HCl: 257, 300 sh, 360, 390 sh; + NaOAc: 255, 345; + NaOAc + H_3BO_3: 255, 340. Acid hydrolysis of **3** gave an aglycone **3a**; UV λ_{max}^{MeOH} nm: 252, 360; + NaOMe: 270, 425; + $AlCl_3$: 257, 300 sh, 420; + $AlCl_3$ + HCl: 257, 300 sh, 415; + NaOAc: 252, 290 sh, 360, 420 sh; + NaOAc + H_3BO_3: 252, 360; EIMS 70 eV, m/z: 330 $[M]^+$, 315 [M − Me], 167 [A_1 + H], 151 [A_1 − Me] and [B_2], 123 [B_2 − CO]. Chromatographic and UV spectral properties for **3a** were similar to reference 7,4′-dimethylquercetin (ombuin).

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