A Novel 2-Hydroxyflavanone from *Collinsonia canadensis*

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A new flavonoid, 2,5-dihydroxy-6,7-dimethoxyflavanone (**1**), was isolated from the leaf and stem exudates of *Collinsonia canadensis* along with three known flavones, baicalein-6,7-dimethyl ether (**2**), norwogenin-7,8-dimethyl ether (**3**), and tectochrysin (5-hydroxy-7-methoxyflavone).

Stoneroot (*Collinsonia canadensis* L., Lamiaceae) is a perennial herb with a fresh lemon scent, occurring from eastern Canada to Florida. The dried rhizomes and roots are used as a diaphoretic and diuretic. They have also been used for the treatment of infantile and biliary colic as well as for dropsy.[1] Many species of the Lamiaceae are known to accumulate flavonoid aglycone and terpenoids that are secreted by glandular trichomes.[2] In the present study, a new 2-hydroxyflavanone (**1**) was isolated from the leaf and stem exudates of *C. canadensis*. Baicalein-6,7-dimethyl ether (**2**),[3] norwogenin-7,8-dimethyl ether (**3**),[4] and tectochrysin[5] were also obtained from the exudate fraction. *C. canadensis* has not been studied previously for the presence of exudate flavonoids.

The leaf-surface flavonoids were isolated from the acetone leaf washings by column chromatography on Sephadex LH-20 and polyamide SC-6. The flavonoids were separated from each other by preparative TLC on Si gel. Compounds **1** and **2** were further purified by preparative HPLC on RP-18. The mass of the M^+ ion of **1** found by HRFABMS was consistent with $C_{17}H_{16}O_6$. ^1H and ^{13}C NMR analysis yielded more signals than was expected for a homogeneous substance. In an attempt to purify compound **1** by preparative HPLC, a small amount of flavone **2** was obtained in addition to compound **1**. The mass difference between **1** and **2** (18 amu) indicated that flavone **2** was the anhydro derivative of compound **1**, apparently formed during purification. Because compound **1** exhibited two nonequivalent methylene H-3 protons in the ^1H NMR spectrum recorded in DMSO, it was concluded that the 2,3-bond of flavone **2** was hydrated in compound **1** with the hydroxyl substituent at C-2. The signal at δ_H 7.74 was attributed to the OH-2 proton, which showed a small transdiaxial coupling with H-3*ax* (δ_H 3.33,[6] $J = 2$ Hz) indicating that the configuration at C-2 is *S*.[7] A coupling between OH-2 and H-3*ax* was also observed in the $^1H-^1H$ COSY spectrum.

2-Hydroxyflavanones exist together with dibenzoylmethanes as tautomeric pairs in solvents such as DMSO[8] and Me_2CO.[7,9] In DMSO, the dibenzoylmethane or oxo form (**1a**) of compound **1** gave rise to its own set of ^1H and ^{13}C resonances that partially overlapped with those of the cyclic hemiacetal form **1** (Table 1). The presence of the oxo form (**1a**) was most conspicuous by the appearance of two additional C=O signals at δ_C 195.1 and 200.1, which showed interactions with a singlet at δ_H 4.67 ($-CH_2-$) in the HMBC spectrum. Most of the other carbon resonances appeared as signal pairs. Many of these signals could not be assigned to one or the other tautomer with certainty because both tautomers were present in roughly equal amounts. Additional ^1H and ^{13}C resonances pointed to the presence of yet other minor forms, possibly the enolic tautomers of the oxo form, but no attempt was made to assign these signals.

Table 1. NMR Data (DMSO-d_6) of Compound **1** [δ in ppm, mult., (J in Hz)]

atom no.	**1** δ_C (cyclo)[a]	**1** δ_H (cyclo)	**1a** δ_C (oxo)[a]	**1a** δ_H (oxo)
2	102.2		200.1	
3	48.5	2.84 d (17) 3.33 dd (17,2)	54.5	4.67 s
4	196.0		195.1	
5	153.7		158.4	
6	128.6		128.7	
7	160.5		161.1	
8	92.9	6.30 s	92.9	6.26 s
9	155.7		150.9	
10	101.9		102.0	
1′	142.2		142.4	
2′/6′	125.47	7.69−7.66 m	125.50	7.69−7.66 m
3′/5′	128.1	7.48−7.39 m	128.1	7.48−7.39 m
4′	128.2	7.48−7.39 m	128.2	7.48−7.39 m
OH-2		7.74 d (2)		
OH-5		11.80 s		11.88 s
OMe-6	60.1	3.67 s	60.5	3.67 s
OMe-7	56.26	3.86 s	56.28	3.86 s

[a] Many of the carbon signals could not be assigned to the cyclo or oxo form with certainty and may be interchanged (both tautomers were present in roughly equal amounts).

The substitution pattern on the A-ring was determined to be 5-hydroxy-6,7-dimethoxy by $^1H-^{13}C$ HMQC and HMBC. In the HMBC spectrum, the aromatic A-ring proton of **1** (δ_H 6.30) showed interactions with C-6, C-7, C-9, and C-10, but not with C-5; this indicates that the aromatic A-ring proton is located *para* to OH-5. Assignment of the singlet at δ_H 6.30 to H-8 was confirmed by a cross peak with δ_C 92.9 in the HMQC spectrum; this chemical shift is consistent only with the A ring. The identity of C-5 followed from a cross peak with the low field hydrogen-bonded hydroxyl proton at δ_H 11.80. These assignments leave the two methoxyls (δ_H 3.67 and 3.86) to be placed at carbons 6 and 7, which was confirmed by correlations between these carbons (δ_{C-6} 128.6 and δ_{C-7} 160.5) and the methoxyl protons in the HMBC spectrum. Rather unexpectedly, the H-8 resonance of compound **1** is shifted 0.7 ppm upfield relative to the corresponding signal in flavone **2**. A similar large upfield shift is noted when the 2,3 unsaturated bond of tectochrysin (δ_{H-8} 6.76 and δ_{H-6} 6.37) is hydrated (δ_{H-8} 6.14 and δ_{H-6} 6.12 in 2,5-dihydroxy-7-methoxyflavanone).[6] These examples demonstrate that C−H correlation is

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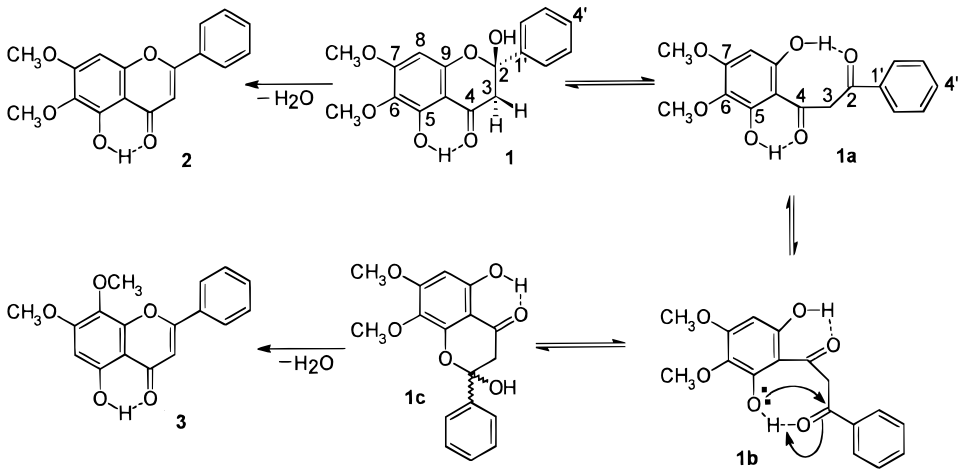

necessary to distinguish between H-6 and H-8 protons of 2-hydroxyflavones.

In the Lamiaceae, flavones are dominant among the exudate flavonoid aglycons. Furthermore, additional oxygenation of the phloroglucinol ring A is most common at C-6 followed by dioxygenation at C-6 and C-8. Because oxygenation seldom starts at C-8 in the Lamiaceae,[2] it is conceivable that flavone **3** is formed from 2-hydroxyflavanone **1** in the plant as depicted: **1** → **1a** → **1b** → **1c** → **3**. The dibenzoylmethane **1a** may exist in equilibrium with solution conformer **1b**, which may subsequently cyclize to form the hemiacetal **1c**. Elimination of water from **1c** is the last step in the formation of the stable 7,8-dimethoxyflavone **3**. It is unlikely, however, that flavone **3** is an artifact of compound **1**, because 'purification' of **1** gave only the anhydro derivative **2** as a minor byproduct.

2-Hydroxyflavanones are extremely rare natural products. Previous reports include 2,5-dihydroxy-7-methoxyflavanone from *Populus nigra*[10] and *Uvaria rufus*;[11] 2,5,7,3′,4′-pentahydroxyflavanone-5-glucoside from *Galega officinalis*;[12] and 2β,5,7-trihydroxyflavanone from *Baccharis bigelovii*.[13] It has been suggested that 2-hydroxyflavanones are intermediates in the conversion of β-hydroxychalcones to flavones.[14]

Baicalein-6,7-dimethyl ether (**2**) has previously been reported from four species (none from the Lamiaceae).[15,16] Norwogonin-7,8-dimethyl ether (**3**) is known from four families, including several *Scutellaria* species (Lamiaceae). Tectochrysin is a less rare compound than earlier thought. It has been found in species belonging to various families,[15] including one species of the Lamiaceae, *Hoslundia opposita*.[17]

Experimental Section

General Experimental Procedures. UV spectra were obtained in MeOH on a Cecil 3021 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 at room temperature on a Bruker DRX 600 spectrometer at 600 and 150.9 MHz, respectively. The DMSO resonances at 2.50 and 39.51 ppm were used as internal shift references. The ^{1}H−^{1}H COSY, ^{1}H−^{13}C HMQC, and HMBC spectra were recorded using standard pulse sequences. Low resolution atmospheric pressure chemical ionization (APCI) MS were recorded on a PE Sciex API III+ triple quadrupole instrument. Samples were introduced into the mass spectrometer by direct injection via a heated nebulizer interface kept at 480 °C, which heats the transfer solvent (MeCN−1% HCOOH, 1:1) to ca. 120 °C. Ionization of the analyte vapor mixture was initiated by a corona discharge needle at ca. 6 kV and a discharge current of ca. 3 μA. The orifice plate voltage was set at 55 V in the positive ion mode. Collision-induced dissociation (CID) experiments were performed with Ar−N_2 (9:1) as target gas at a thickness of ca. 1.9×10^{14} atoms/cm^2. The collision energy was 30 V. Other operating conditions were standard. HRFABMS were recorded on a Kratos MS-50 instrument using polyethylene glycol as the matrix. TLCs were run on Si gel with toluene−dioxane−HOAc (18:5:1) and on polyamide DC-11 with toluene−petroleum (bp 100−140 °C)−MeCOEt−MeOH (12:6:2:1). Developed plates were examined under UV (350 nm) before and after spraying with diphenyl−boric acid−ethanolamine complex (1%) in MeOH (Naturstoffreagenz A).

Plant Material. Plants of *C. canadensis* were cultivated in the Botanischer Garten der Technischen Universität Darmstadt. The aerial parts were collected after flowering. A voucher (acc. no. 1144) is kept in the Herbarium of the Botanischer Garten der TU Darmstadt.

Extraction and Isolation. The leaves of one plant of *C. canadensis* were briefly rinsed with Me_2CO to dissolve the exudate material. The concentrated solution was defatted by precipitation of lipophilic materials from MeOH. The supernatant was passed over Sephadex LH-20 (eluted with MeOH) to separate terpenoids from flavonoid aglycones. The combined flavonoid fractions were passed over a small column filled with Polyamide SC-6, eluted with mixtures of toluene−MeCOEt−MeOH in which the proportions of MeCOEt and MeOH were gradually increased. These chromatographic steps yielded two flavonoid fractions that were subjected to preparative TLC on Si gel using toluene−MeCOEt (9:1) as the eluting solvent. Four flavonoids were recovered from the plates in milligram quantities. Flavone **3** and tectochrysin were of sufficient purity to allow identification by UV, MS, and co-TLC with markers. Compounds **1** and **2** were further purified by preparative HPLC on a 10-μm Econosil (250 × 22 mm) RP-18 column using a linear gradient from 40% to 100% MeCN in 1% aqueous HCOOH over 30 min at 11.2 mL/min. The UV trace was monitored at 280 nm. Peak fractions were collected manually and taken to dryness by rotary evaporation followed by lyophilization.

2,5-Dihydroxy-6,7-dimethoxyflavanone (1): pale yellow powder (8 mg); UV (MeOH) λ_{max} (log ϵ) 232 sh (4.12), 290 (4.21), and 345 (3.70) nm; ^{1}H and ^{13}C NMR data, see Table 1; HRFABMS, *m/z* 317 $[MH]^+$ (100), 316.0950 $[M]^+$ ($C_{17}H_{16}O_6$ requires 316.0947) (48), 299.0923 $[MH - H_2O]^+$ ($C_{17}H_{15}O_5$ requires 299.0920) (52); APCIMS, *m/z* 317 $[MH]^+$ (92), 299 $[MH - H_2O]^+$ (100); MS−MS, *m/z* 197 $[^{1,3}A]^+$ (70), 182 $[^{1,3}A-CH_3]^+$ (40), 164 (20), 136 (19), 105 $[ArC_2H_4]^+$ (100), 77 $[C_6H_5]^+$ (20).

Baicalein-6,7-dimethyl ether (2): yellow powder (6 mg); UV and NMR are in agreement with published data;[3] APCIMS, *m/z* 299 $[MH]^+$ (100); MS−MS, *m/z* 284 $[MH - CH_3]^+$ (14), 266 $[MH - CH_3 - H_2O]^+$ (73), 238 $[266 - CO]^+$ (100), 210 $[266 - 2CO]^+$ (22), 153 (4), 136 (16); identity confirmed by co-TLC with an authentic marker[3] on Si gel and on polyamide.

Norwogonin-7,8-dimethyl ether (3): yellow powder; UV and NMR in agreement with published data;[4] APCIMS, *m/z* 299 $[MH]^+$ (100); MS−MS, *m/z* 284 $[MH - CH_3]^+$ (64), 283 $[MH - CH_4]^+$ (100), 267 (30), 266 $[MH - CH_3 - H_2O]^+$ (35), 255 $[283 - CO]^+$ (98), 238 $[266 - CO]^+$ (76), 210 $[266 - 2CO]^+$ (12), 152 (15), 136 (21); identity confirmed by co-TLC with an authentic marker[4] on Si gel and on polyamide.

Tectochrysin: yellow powder; UV in agreement with published data;[18] APCIMS, *m/z* 269 $[MH]^+$ (100); MS−MS, *m/z* 254 $[MH - CH_3]^+$ (31), 226 $[MH - CH_3 - CO]^+$ (100), 167 $[^{1,3}A]^+$ (27); identity confirmed by co-TLC with an authentic marker[5] on Si gel and on polyamide.

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