Polyoxygenated Flavones from the Leaves of *Comptonella microcarpa*

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Received February 26, 1999

The leaves of *Comptonella microcarpa* have yielded one alkaloid, dictamnine, and four known polyoxygenated flavonoids, meliternatin, 3,5,8-trimethoxy-6,7-3′,4′-dimethylenedioxyflavone, 7-(3-methylbut-2-enyloxy)-3,5,8-trimethoxy-3′,4′-methylenedioxyflavone (**3**), 7-hydroxy-3,5,8-trimethoxy-3′,4′methylenedioxyflavone. In addition, two new flavonoids were found whose structures were established on the basis of their spectral data as 7-hydroxy-3,5,6,8-tetramethoxy-3′,4′-methylenedioxyflavone (**1**) and 7-(3-methylbut-2-enyloxy)-3,5,6,8-tetramethoxy-3′,4′-methylenedioxyflavone (**2**).

The genus *Comptonella* Baker f., recently revised by Hartley,[1] consists of eight species in the family Rutaceae endemic to New Caledonia. Previous chemical studies in this genus are limited to reports of alkaloids from *C. sessilifoliola*[2] (Guillaumin) Hartley, *C.* (*Dutaillyea*) *drupacea*[3] (Labill.) Guillaumin, *C.* (*Dutaillyea*) *oreophila*[3] (Guillaumin) Hartley, and *C.* (*Dutaillyea*) *baudouinii* [4] (Baillon) Hartley.

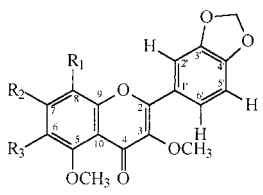
The recent isolation from several species in the Rutaceae of polyalkoxylated flavonoids able to inhibit tubulin assembly into microtubules[5,6] prompted us to study the less polar flavonoids of the leaves of *C. microcarpa* (Perkins) Hartley. Accordingly, as a continuation of our systematic screening of the rutaceous plants from the Pacific region, we report here the structure determination of two novel polyoxygenated flavones (**1** and **2**) from *C. microcarpa*, along with four known flavones and the furoquinoline alkaloid, dictamnine.

The chloroform extract of the leaves of *Comptonella microcarpa* yielded the new compounds **1** and **2**, together with the four known flavones, meliternatin,[7] 3,5,8-trimethoxy-6,7-3′,4′-dimethylenedioxyflavone,[7] 7-(3-methylbut-2-enyloxy)-3,5,8-trimethoxy-3′,4′-methylenedioxyflavone,[8,9] 7-hydroxy-3,5,8-trimethoxy-3′,4′-methylenedioxyflavone,[8,9] and the known furoquinoline alkaloid, dictamnine,[10] which were identified by comparison of their spectral data with those of the literature. Previous ^{13}C NMR assignments for 7-(3-methylbut-2-enyloxy)-3,5,8-trimethoxy-3′,4′-methylenedioxyflavone[8] were revised as described in the Experimental Section.

7-Hydroxy-3,5,6,8-tetramethoxy-3′,4′-methylenedioxyflavone (**1**) was the main flavonoid constituent of *C. microcarpa* leaves and was obtained as yellow octahedral crystals from MeOH (mp, 210−212 °C). The UV spectrum was typical of a polyoxygenated flavone.[11] Strong bathochromic shift, induced upon addition of NaOAc, indicated the presence of a free phenolic group at C-7.[11] The empirical formula was established by HREIMS as $C_{20}H_{18}O_9$. The ^1H NMR spectrum exhibited, in the aromatic area, a series of signals typical of a 3′,4′-disubstituted flavone B ring. Additional signals accounted for one methylenedioxy group, four methoxyl groups, and one free phenolic hydroxyl group. The position of the methylenedioxy group at 3′,4′ on the flavone B ring was deduced from strong correlations observed on the 2D COLOC spectrum between the methylenedioxy protons (δ 6.06) and the carbons C-3′ and C-4′ resonating at 147.9 and 149.5 ppm, respectively. Observation of a strong EIMS fragment ion at *m/z* 149 was consistent with this proposal.[12,13] The structure of compound **1** was therefore proposed as 7-hydroxy-3,5,6,8-tetramethoxy-3′,4′-methylenedioxyflavone.

7-(3-Methyl-but-2-enyloxy)-3,5,6,8-tetramethoxy-3′,4′-methylenedioxyflavone (**2**) was obtained as a yellow amorphous solid. The empirical formula was established by HREIMS as $C_{25}H_{26}O_9$. Its spectral data were closely comparable to those of compound **1**. However the UV spectrum was unchanged upon addition of NaOAc, revealing the lack of a free hydroxyl group at C-7. The ^1H NMR spectrum differed from that of **1** only by the presence of signals that could be assigned to a 3-methylbut-2-enyloxy side chain: two methyl singlets resonating at δ 1.73 and 1.78, one two-proton doublet resonating at δ 4.76 (J = 7.4 Hz), and a broad triplet integrating for one proton at δ 5.58 (J = 7.4 Hz). This was substantiated by a fragment ion at *m/z* 401 [M − C_5H_9]$^+$ in the EIMS. Those spectral data allowed us to propose the structure of 7-(3-methyl-but-2-enyloxy)-3,5,6,8-tetramethoxy-3′,4′-methylenedioxyflavone for **2**. The structure was confirmed by means of chemical correlation, in which condensation of **1** with 4-bromo-2-methylbut-2-ene in the presence of K_2CO_3 and KI in acetone[14] led to compound **2** in 50% yield.

The flavones of *Comptonella microcarpa* exhibited no cytotoxic activity against (KB) human nasopharyngeal carcinoma cells.[15] They have also been screened for a possible inhibition of the tubulin assembly[16] by comparison to the activity of 3-methoxy flavones isolated from other Rutaceae, but they do not exhibit any significant activity.



	R_1	R_2	R_3
1	OCH_3	OH	OCH_3
2	OCH_3	$OCH_2CHC(CH_3)_2$	OCH_3
3	OCH_3	$OCH_2CHC(CH_3)_2$	H

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10.1021/np9900730 CCC: $18.00
Published on Web 07/27/1999

Experimental Section

General Experimental Procedures. Melting points were measured on a Totoli melting point apparatus. UV spectra were recorded in MeOH on a Shimadzu UV 160A UV spectrometer and IR spectra in KBr on a Shimadzu FTIR-8201PC IR spectrometer. ^{1}H and ^{13}C NMR spectra were obtained in $CDCl_3$ on a Bruker AC 300 (300 MHz) NMR spectrometer. COSY, HETCORR, and NOESY experiments were performed using the standard Bruker microprograms. MS were recorded using a Nermag R-10-10-H instrument in the Cl mode (NH_3). Extractions were carried out using a Soxhlet apparatus.

Plant Material. The plant material used in this study was collected in May 1996, by one of us (M.L.) in Frouin Forest, Mandjélia, New Caledonia, at medium altitude (720 m). A voucher specimen has been deposited at the herbarium of the Centre ORSTOM de Nouméa under the reference number LIT 0105.

Extraction and Isolation. Dried powdered leaves of *C. microcarpa* (630 g) were defatted by extraction with petroleum ether (bp 40−60 °C), then extracted sequentially with $CHCl_3$ and MeOH. The $CHCl_3$ extract (10 g) was subjected to column chromatography using Si gel 60 (Merck; 0.063−0.200 mm) packed in hexane. Elution was performed with hexane containing increasing amounts of $CHCl_3$, then $CHCl_3$ containing increasing amounts of EtOAc, and finally with MeOH. Each fraction was monitored by TLC; those containing comparable mixtures were combined and purified by repeated preparative TLC fractions ($CHCl_3$−MeOH 19:1). Fractions eluted with $CHCl_3$ gave compound **1** (60 mg). Fractions eluted with $CHCl_3$−EtOAc (10:1) gave **2** (6.9 mg). Fractions eluted with $CHCl_3$−EtOAc (5:1) gave compound **3** (31 mg), and a mixture of meliternatin (12 mg) and 3,5,8-trimethoxy-6,7-3′,4′-dimethylenedioxyflavone (9 mg) separated after recrystallization (MeOH). Finally, fractions eluted with $CHCl_3$−EtOAc (1:1) led to the isolation of 7-hydroxy-3,5,8-trimethoxy-3′,4′methylenedioxyflavone (12 mg).

7-Hydroxy-3,5,6,8-tetramethoxy-3′,4′-methylenedioxyflavone (1): yellow octahedral crystals ($CHCl_3$−MeOH); mp 210−212 °C; UV (MeOH) λ_{max} (log ϵ) 254 (5.02), 267 (4.92), 340 (5.05) nm; UV (MeOH + NaOAc) λ_{max} (log ϵ) 216, 279, 326, 371 nm; IR (KBr) ν_{max} 3200 (OH), 1593 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.87 (3H, s, CH_3O-3), 3.95 (3H, s, CH_3O-5), 4.01 (3H, s, CH_3O-6), 4.01 (3H, s, CH_3O-8), 6.06 (2H, s, $-OCH_2O-$), 6.54 (1H, s, HO-7), 6.94 (1H, d, J = 8.3 Hz, H-5′), 7.64 (1H, d, J = 1.8 Hz, H-2′), 7.73 (1H, dd, J = 1.8, 8.3 Hz, H-6′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 59.9 (CH_3O-3), 61.6 (CH_3O-6 or CH_3O-8) and 61.8 (CH_3O-6 or CH_3O-8), 62.2 (CH_3O-5), 101.6 ($-OCH_2O-$), 108.4 (C-2′), 108.5 (C-5′), 118.2 (C-10), 123.2 (C-2′), 124.6 (C-1′), 131.6 (C-8), 138.7 (C-6), 140.5 (C-3), 146.6 (C-9), 147.1 (C-7 and C-5), 147.9 (C-3′), 149.5 (C-4′), 152.8 (C-2), 173.8 (C=O); EIMS m/z 402 [M]$^+$ (48), 387 [M − CH_3]$^+$ (67), 149 [$C_8H_5O_3$] (30); HREIMS m/z 402.0973 (calcd for $C_{20}H_{18}O_9$, 402.0951).

7-(3-Methylbut-2-enyloxy)-3,5,6,8-tetramethoxy-3′,4′-methylenedioxyflavone (2): yellow amorphous solid; UV (MeOH) λ_{max} 256, 271 sh, 342 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.73 (1H, s, CH_3-4″), 1.78 (1H, s, CH_3-4″), 3.87 (3H, s, CH_3O-3), 3.95 (3H, s, CH_3O-5 or CH_3O-6 or CH_3O-8), 3.97 (3H, s, CH_3O-5 or CH_3O-6 or CH_3O-8), 4.00 (3H, s, CH_3O-5 or CH_3O-6 or CH_3O-8), 4.76 (2H, d, J = 7.4 Hz, H_2-2″), 5.58 (1H, br t, J = 7.4 Hz, H-3″), 6.08 (2H, s, OCH_2O), 6.94 (1H, d, J = 8.7 Hz, H-5′), 7.69 (1H, d, J = 1.5 Hz, H-2′), 7.78 (1H, dd, J = 1.5, 8.7 Hz, H-6′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 18.7 (CH_3-4″), 26.3 (CH_3-4″), 60.0 (CH_3O-3), 62.4 (CH_3O-5 or CH_3O-6), 62.0 CH_3O-8), 62.7 (CH_3O-5 or CH_3O-6), 67.3 (C-2″), 102.3 ($-OCH_2O-$), 109.0 (C-2′ and C-5′), 113.5 (C-10), 119.8 (C-3″), 124.1 (C-6′), 124.9 (C-1′), 139.3 (C-8), 139.7 (C-4″), 141.0 (C-3), 145.2 (C-6 or C-5), 145.8 (C-9), 148.3 (C-5 or C-6), 148.7 (C-3′), 149.5 (C-4′), 151.1 (C-7), 153.3 (C-2), 174.2 (C-4); EIMS m/z 471 [M + H]$^+$ (100), 401 [M − C_5H_9]$^+$ (41), 149 [$C_8H_5O_3$] (56); HREIMS m/z 470.1595 (calcd for $C_{25}H_{26}O_9$, 470.1577).

Condensation of 1 with 4-Bromo-2-methylbut-2-ene. To a solution of **1** (10 mg) in dry Me_2CO (4 mL) containing K_2CO_3 (12.5 mg) and KI (12.5 mg) was added 4-bromo-2-methylbut-2-ene (5 mg). The mixture was stirred for 2 h at 20 °C, and 5.8 mg of **2** (50% yield) were obtained after purification by preparative TLC. The ^{1}H NMR data of the obtained compound were identical with those found with the natural product **2**.

7-(3-Methylbut-2-enyloxy)-3,5,8-trimethoxy-3′,4′-methylenedioxyflavone (3): ^{13}C NMR ($CDCl_3$, 75 MHz) δ 18.0 (CH_3-3″), 27.0 (CH_3-3″), 56.5 (CH_3O-5), 59.8 (CH_3O-3), 61.4 (CH_3O-5), 66.2 (C-1′), 93.9 (C-6), 102.5 ($-OCH_2O-$), 108.0 (C-2′ and C-5′), 109.0 (C-9), 119.0 (C-2″), 123.2 (C-2′), 124.8 (C-1′), 131.0 (C-8), 138.0 (C-3″), 140.8 (C-3), 147.8 (C-4′), 149.3 (C-9), 150.9 (C-3′), 152.1 (C-2), 155.6 (C-7), 156.0 (C-5), 174.2 (C-4).

Acknowledgment. The authors wish to thank Mrs. Any Regnier and Mr. Fabien Lagier for technical assistance.

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NP9900730