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QUERCITRIN 3′-SULPHATE FROM LEAVES OF *LEEA GUINENSIS*

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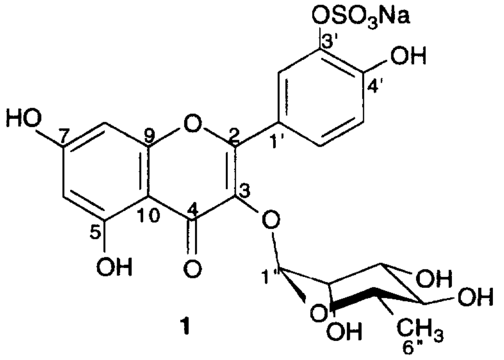
Key Word Index—*Leea guinensis*; Leeaceae; leaves; quercitrin 3′-sulphate.

Abstract—A new natural product was isolated together with known flavonoids from the leaves of *Leea guinensis* (Leeaceae). It was characterized as quercetrin 3-*O*-α-L-rhamnopyranoside-3′-sulphate by means of spectroscopic analysis, especially 2D NMR experiments. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Sulphated flavonoids have been found in over 160 plant species from 16 families [1, 2] but have not been reported previously from the Leeaceae. Pharmacological evaluation of this type of compound showed an enhancement of the known flavonoid antioxidant activity and an aldose reductase inhibition [3, 4]. During our survey for bioactive compounds from plants used in folk medicine for cardiovascular diseases, significant activity was detected in both aqueous and butanolic extracts of *Leea guinensis* G. Don (Leeaceae). This genus is a widespread shrub in tropical Africa where the leaves are used for their cardiac activity [5]. We report here the isolation and structural determination of a new sulphated flavonoid (**1**).



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RESULTS AND DISCUSSION

The molecular formula $C_{21}H_{19}O_{14}SNa$ for **1** was determined by HRFAB mass spectrometry (*m/z* 551.051 $[M+H]^+$, Δ −3.9 mmu). Negative FAB mass spectrometry exhibited peaks at *m/z* 527 $[M-Na]^+$ and 447 $[ROH]^+$. The EIMS of **1** showed a peak at *m/z* 301 corresponding to the loss of a SO_3Na group and a deoxyhexose unit in a terminal position. UV spectral maxima in methanol at 265 and 342 nm indicated that **1** was a flavonol [6]. Addition of HCl resulted in a weak bathochromic shift of 13 nm for band I, revealing a 3′-*O*-substitution [7]. A bathochromic shift of 50 nm was observed for band I after addition of NaOMe indicative of a free hydroxyl group in the 4′ position, whereas the absence of a shift after the addition of H_3BO_3 confirmed that the 3′ position was substituted. On the basis of the mass and UV spectral data, **1** was assigned to be a sodium salt of a monoglycosyl sulphated flavonol. The ^{1}H NMR spectrum showed five aromatic signals at δ 6.20 (*d*, 1H), 6.39 (*d*, 1H), 6.96 (*d*, 1H), 7.52 (*dd*, 1H) and 7.76 (*d*, 1H) which were assigned, respectively, to H-6, H-8, H-5′, H-6′, and H-2′ in a tetrasubstituted flavonol unit (Table 1). These data were confirmed by ^{1}H-^{1}H COSY correlations. Along with the aromatic signals, sugar signals (0.8–5.2 ppm) could be assigned to a deoxyhexose moiety. The methyl group at δ 0.8 ppm (3H, *d*, 5.7) and the anomeric proton H-1″ at δ 5.2 ppm (1H, *br s*) suggested an α-rhamnopyranosyl unit. The ^{1}H-^{1}H COSY experiment enabled the total assignment of the sugar protons.

The deoxyhexose moiety was confirmed by ^{13}C spectral analysis, by the presence of an anomeric carbon at δ 102 ppm, four sugar carbons at δ 70.1–7.14 ppm and a methyl group at δ 17.4 ppm. The assignment was in total concordance with the sugar unit in quercitrin (Table 1). Beside the sugar signals, 15 carbons from 93.6 to 177.6 ppm were assigned to the tetrahydroxylated flavonol unit (Table 1). The chemical shifts of the flavonol unit were in good agreement with that published for quercitrin, except those of ring B

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Table 1. NMR Data for **1**, Quercitrin and Quercetin in DMSO-d_6

	1		Quercitrin*		Quercetin†	
Position	^{1}H‡	^{13}C§	^{1}H‡	^{13}C§	^{1}H‡	^{13}C§
2		156.4		156.4		146.7
3		134.5		134.2		138.6
4		177.6		177.7		175.8
5		161.2		161.2		160.7
6	6.20 *d* (2.1)	98.7	6.20 *d* (2.0)	98.6	6.17 *d* (1.6)	98.1
7		164.2		164.1		163.6
8	6.39 *d* (2.0)	93.6	6.38 *d* (2.0)	93.6	6.40 *d* (1.7)	93.3
9		156.7		157.2		156.1
10		104.1		104.0		102.9
1′		123.0		120.7		121.9
2′	7.76 *d* (2.0)	120.8	7.29 *d* (1.9)	115.4	7.66 *d* (1.8)	114.9
3′		140.7		145.1		145.0
4′		151.7		148.4		147.8
5′	6.96 *d* (8.1)	117.0	6.86 *d* (8.3)	115.8	6.87 *d* (8.5)	115.5
6′	7.52 *dd* (2.3–8.5)	125.6	7.24 *dd* (2.2–8.4)	121.0	7.53 *dd* (8.4)	119.9
1″	5.2 *br s*	102.0	5.24 *d* (1.1)	101.8		
2″	3.98 *br s*	70.1	3.96 *br s*	70.3		
3″	3.55 *m*	70.6	3.50 *dd* (3.1–8.6)	70.5		
4″	3.1 nd¶	71.4	3.21–3.29 nd¶	71.1		
5″	3.0–3.3 nd¶	70.1	3.08–3.21 nd¶	70.0		
6″	0.8 *d* (5.7)	17.4	0.81 *d* (5.5)	17.4		
OH-5	12.59 *s*		12.64 *s*		12.46 *s*	
OH-7	10.83 *s*		10.83 *s*		10.76 *s*	
OH-3′			9.29 *s*		9.26–9.55	
OH-4′	9.62 *s*		9.66 *s*		9.26–9.55	
OH-3					9.26–9.55	

* Authentic sample.

† Isolated compound.

‡ Recorded at 200 MHz. Coupling constants (Hz) are between parentheses.

§ Recorded at 50 MHz.

¶ Not defined.

[8]. An upfield shielding for the substituted C-3′ was observed, while both the *ortho* and *para* carbons underwent a significant downfield shielding. The calculation of the shifts were indicative of a 3′-*O*-sulphate [9]. On the other hand, the shifts of carbons 2, 3, 4, 1′ were in complete agreement with this type of substitution [8, 10]. The ^{13}C and ^{1}H spectra were completely assigned by means of 2D NMR experiments (^{1}H-^{1}H COSY, XHCORR and COLOC). The long range ^{1}H-^{13}C coupling enabled the precise location of the α-rhamnopyranosyl unit, by a cross-peak between H-1″ and C-3.

After a complete assignment, **1** was identified as a sodium salt of quercetin 3-*O*-α-L-rhamnopyranoside-3′-sulphate. Since *Leea guinensis* grows in swampy areas the presence of a flavonoid sulphate is not unexpected and **1** is unlikely to be an artefact of the extraction process. Compound **1** was found to co-occur with quercetin and kaempferol, which were identified by comparison with authentic samples.

EXPERIMENTAL

General. Chemical shifts are expressed in ppm (δ) relative to TMS as int. standard. All NMR experiments were run on a BRUKER AC 200 instrument.

Extraction and isolation. Leea guinensis G. Don was collected from Bella territory, Cameroon. A voucher specimen is deposited in The National Herbarium (3115), Museum of Yaounde. Leaves of *L. guinensis* (200 g) were ground to a fine powder then extracted successively with hexane (Soxhlet, 11 hr), and with 50% aq. EtOH (reflux 3 hr) The concd EtOH extract was partitioned [see 11] using successively hexane and CH_2Cl_2. The aq. layer was concd *in vacuo* and further partitioned with EtOAc. Traces of EtOAc were removed *in vacuo*. The aq. content was finally partitioned with BuOH. The BuOH extract (3 g) was first fractionated by LC on Sephadex LH 20^{R} using a gradient of MeOH in H_2O to yield 10 frs. The fr. containing **1** (61.9 mg) was subsequently subjected to MPLC on a RP-18^{R} column using a linear gradient from H_2O to MeOH to afford 30 mg of **1**.

Compound **1**. Yellow paste. HRFABMS *m/z* (rel. int.): 551.051 $[M+H]^+$ $\Delta = -3.9$ mmu for $C_{21}H_{20}O_{14}SNa$). Negative FABMS *m/z* 527 $[M-Na]^+$, 447 $[ROH]^-$. EIMS *m/z* 301 [ROH-deoxyhexose] (51). UV λ_{max}^{MeOH} nm: 265, 342; +HCl: 256, 355; +NaOMe: 272, 324, 392; +NaOAc: 268, 344; +NaOAc+H_3BO_3: 269, 342; +$AlCl_3$: 273, 306, 357, 395; +$AlCl_3$+HCl: 274, 341, 389.

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