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6-HYDROXY- AND 6-METHOXYFLAVONOIDS FROM *NEUROLAENA LOBATA* AND *N. MACROCEPHALA*

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Key Word Index—*Neurolaena lobata*; *N. macrocephala*; Asteraceae; Heliantheae; Galinsogineae; 6-hydroxykaempferol methyl ethers, glucosides and sulfate; quercetagetin methyl ethers, glucosides and sulfate; 6-hydroxyflavone methyl ethers and glucoside.

Abstract—Twelve flavonoids including one new sulfate were isolated from *Neurolaena lobata*, and six known flavonoids were obtained from *N. macrocephala*. The new compound isolated from *N. lobata* is 6-hydroxykaempferol 3-methyl ether 7-sulfate, and the known flavonoids are 6-hydroxykaempferol 3,7-dimethyl ether, 6-hydroxykaempferol, 3-methyl ether 7-glucoside, 6-hydroxykaempferol 7-glucoside, quercetagetin and its 7-glucoside, quercetagetin 3,6- and 3,7-dimethyl ethers, quercetagetin 3-methyl ether 7-glucoside and 7-sulfate, 6-hydroxyluteolin 3′-methyl ether and 6-hydroxyluteolin 7-glucoside. The known flavonoids identified from *N. macrocephala* are quercetagetin 3,6- and 3,7-dimethyl ethers, quercetagetin 6-methyl ether 7-glucoside, quercetagetin 3,6-dimethyl ether 7-glucoside, quercetagetin 7-glucoside and quercetagetin 3-methyl ether 7-sulfate.

INTRODUCTION

In a continuation of our biochemical systematic investigation of the genus *Neurolaena* (Asteraceae–Heliantheae) [1, 2], we report the isolation and characterization of twelve flavonoids from *N. lobata* (L.) R. Br., a widespread tropical weed in southern Mexico and South America, and six from *N. macrocephala* Sch.-Bip. ex Hemsl., a taxon endemic to Veracruz, Mexico. One of the flavonoids from *N. lobata*, 6-hydroxykaempferol 3-methyl ether 7-sulfate, is a new compound. We previously described fifteen flavonoids from *N. oaxacana* B. L. Turner [1], and seven from *N. venturana* B. L. Turner [2]. Two sesquiterpene lactones were previously reported from *N. lobata* [3], and the thymol derivatives of *N. lobata*, *N. oaxacana* and *N. venturana* have been described [4].

RESULTS

Leaves of *N. lobata* and *N. macrocephala* were both extracted with aqueous methanol and the syrup obtained after concentrating the extract was partitioned between water and three organic solvents: *n*-hexane, dichloromethane and ethyl acetate. The hexane extract was discarded. The dichloromethane extract of *N. lobata* yielded 6-hydroxykaempferol 3,7-dimethyl ether (**1**) [5], quercetagetin 3,7-dimethyl ether (**2**) [5], quercetagetin 3,6-dimethyl ether (**3**) [6], 6-hydroxyluteolin 3′-methyl ether (**4**) [7], and 6-hydroxykaempferol 7-glucoside (**5**) [8]. The ethyl acetate extract yielded additional 6-hydroxykaempferol 7-glucoside plus quercetagetin (**6**) [9], and 6-hydroxyluteolin 7-glucoside (**7**) [10]. The water extract yielded the remaining compounds: quercetagetin 3-methyl ether 7-sulfate (**8**) [1], 6-hydroxykaempferol 3-methyl ether 7-sulfate (**9**), 6-hydroxykaempferol 3-methyl ether 7-glucoside (**10**) [1], quercetagetin 3-methyl ether 7-glucoside (**11**) [1] and quercetagetin 7-glucoside (**12**) [11].

The identities of all the known flavonoids were determined by direct comparison (TLC, UV, NMR) with authentic samples previously obtained from *N. oaxacana* [1]. The structure assignment of the new compound is discussed separately, and all previously unreported color, TLC, UV, NMR and MS data for all the flavonoids are recorded in Tables 1 and 2 or in the Experimental.

6-*Hydroxykaempferol* 3-*methyl ether* 7-*sulfate* (**9**)

The presence of a 6-hydroxyl group in **9** was first suspected from the purple-brown color of the spot on a paper chromatogram under UV light which was unchanged when exposed to ammonia vapor or sprayed with NA. The UV band I shift of +24 nm in $AlCl_3$/HCl relative to band I in MeOH indicated a flavonoid with C-6 oxygenation. The absence of a band III, band I at 390 nm in NaOMe vs 400 nm in NaOAc, and no shift of band II in NaOAc suggested an -OR substituent at C-7 [12]. The lack of a shift of band I with NaOAc/H_3BO_3 established that there was no *ortho*-dihydroxyl group in the B-ring. The electrophoretic mobility to the anode (4.5 cm) of this compound under standard conditions [13] at pH 1.9 indicated that it was a monosulfated compound. This was confirmed when sulfatase hydrolysis yielded 6-hydroxykaempferol 3-methyl ether (TLC comparison with an authentic sample). The UV spectral findings require that the sulfate moiety is at C-7. Thus, the new compound is 6-hydroxykaempferol 3-methyl ether 7-sulfate.

The flavonoids isolated from the dichloromethane extract of *N. macrocephala* were quercetagetin 3,6-dimethyl ether (**3**) [6] and quercetagetin 3,7-dimethyl ether (**2**) [5]. The ethyl acetate extract yielded quercetagetin 6-methyl ether 7-glucoside (**13**) [14], quercetagetin

Table 1. Chromatographic data (R_fs × 100 and colors) for flavonoids of *N. lobata**

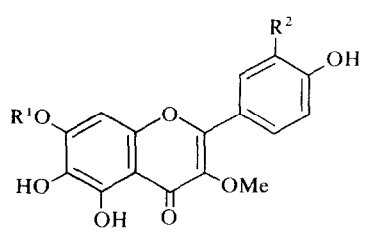
Compound	Paper		Cellulose				Polyamide		Colors†		
			HOAc		TBA	n-BAW					
	TBA	HOAc	15%	40%			BMM	BPMM	UV	UV/NH_3	UV/NA
6-Hydroxykaempferol 7-glucoside (**5**)	25	9	5	28	19	24	29	0	PBr	PBr	PBr
Quercetagetin (**6**)	20	0.2	2	10	19	37	19	2	P	PBr	Or
6-Hydroxykaempferol 3-methyl ether 7-sulfate (**9**)	39	68	56	79	45	48	—	—	P	PBr	PBr
Quercetagetin 3,6-dimethyl ether 7-glucoside (**14**)	44	36	34	57	36	40	0	0	P	YBr	Or

* See ref. [1] for chromatographic data of other flavonoids from *N. lobata* and solvent key.
† P = purple, PBr = purplish-brown, Or = orange, YBr = yellowish brown; NA = Naturstoff reagenz A in MeOH.

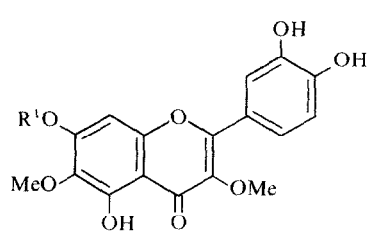
3,6-dimethyl ether 7-glucoside (**14**) [8], and quercetagetin 7-glucoside (**12**) [11]. Quercetagetin 3-methyl ether 7-sulfate(**8**) [1] was obtained from the water fraction. The identities of all these flavonoids were determined by direct comparison (TLC, UV) with authentic samples previously obtained from *N. oaxacana* [1]. The UV and chromatographic data for quercetagetin 3,6-dimethyl ether 7-glucoside are reported in Tables 1 and 2.

EXPERIMENTAL

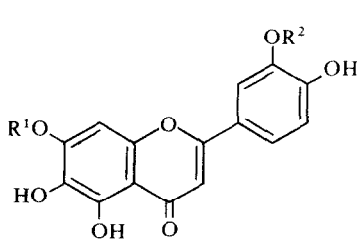
Plant material. Leaves and vouchers of *N. lobata* were collected from along the roadside several km N. of Atzalan at La Calavera, Vercruz, Mexico, on 9 March 1979 (voucher specimen *K. M. Kerr* 124 is deposited in the Lundell Herbarium, The University of Texas at Austin). Leaves and vouchers of *N. macrocephala* were collected 300 m from the entrance to Los Tuxtlas Biological



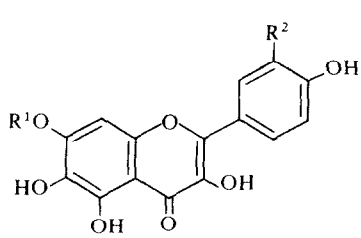
1 R^1 = Me, R^2 = H
2 R^1 = Me, R^2 = OH
8 R^1 = SO_3^-, R^2 = OH
9 R^1 = SO_3^-, R^2 = H
10 R^1 = glc, R^2 = H
11 R^1 = glc, R^2 = OH



3 R^1 = H
14 R^1 = glc



4 R^1 = H, R^2 = Me
7 R^1 = glc, R^2 = H



5 R^1 = glc, R^2 = H
6 R^1 = H, R^2 = OH
12 R^1 = glc, R^2 = OH

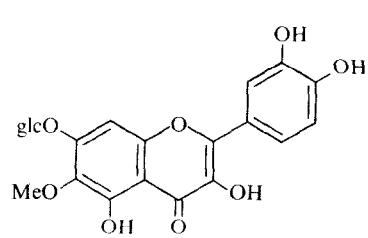


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Table 2. UV data (λ_{max}, nm) for flavonoids of *N. lobata**

Compound	MeOH	NaOMe	$AlCl_3$	$AlCl_3$/HCl	NaOAc	NaOAc/H_3BO_3
5	374 (sh), 346 (1),† 276 (0.8), 256 (0.7), 234 (0.7)	400 (1), 290 (0.5), 254 (0.7)	436 (sh), 388 (1), 287 (0.9)	428 (1), 382 (1.2), 267 (1.5)	384 (sh), 348 (1), 272 (0.9), 254 (0.8)	380 (sh), 386 (1), 278 (1.5)
6	360 (1), 272 (sh), 255 (0.7)	390 dec. (1), 304 (3), 244 (4)	444 (1), 280 (0.7)	436 (sh), 396 (1), 270 (1)	388 (1), 260 (0.7)	376 (1), 292 (sh), 266 (0.7)
9	366 (1), 294 (sh), 270 (0.8)‡	390 (1), 300 (sh), 270 (0.6)	400 (sh), 364 (1), 304 (sh), 278 (1)	398 (sh), 358 (1), 302 (sh), 280 (1.5)	400 (sh), 344 (1), 268 (0.8)	366 (1), 270 (0.5)
14	350 (1), 292 (sh), 260 (1.5)	400 (1), 276 (sh), 252 (1.3)	434 (1), 364 (sh), 326 (sh), 304 (sh), 280 (2)	400 (sh), 370 (1), 298 (sh), 268 (1)	370 (1), 290 (8), 260 (1.4)	370 (1), 290 (0.8), 264 (1.4)

* See ref. [1] for the UV data of other flavonoids from *N. lobata*.
† Relative absorptivities are given for each λ_{max} relative to the most intense wavelength peak as (1).
‡ MeOH/HCl: 338 (1), 280 (0.8).

Station on 13 March 1979 (voucher specimen *K. M. Kerr* 136 is deposited in the Lundell Herbarium, The University of Texas at Austin).

Extraction, purification and identification of flavonoids from N. lobata *and* N. macrocephala. General chromatographic and electrophoretic techniques have been previously described [1]. Ground leaves of *N. lobata* (200 g) were extracted 3 × with aq. MeOH. The combined extracts were concd *in vacuo* to 100 ml, and the aq. concentrate successively extracted with CH_2Cl_2 and EtOAc. A. CH_2Cl_2 *extract*. The syrup from this extract (5 g) was passed over Sephadex LH-20, and the flavonoid mixture (0.7 g) obtained from this column was chromatographed over a Polyclar column (4 × 17 cm; 50 g). Elution was initiated with Egger's solvent (CH_2Cl_2–MeOH–MeCOEt–Me_2CO, 20:10:5:1) and the polarity of the solvent was gradually increased by the elimination of CH_2Cl_2 (0:10:5:1). The compounds eluted in the following order: 6-hydroxykaempferol 3,7-dimethyl ether (**1**), 1.5 mg; quercetagetin 3,7-dimethyl ether (**2**), 3 mg; quercetagetin 3,6-dimethyl ether (**3**), 1 mg; 6-hydroxyluteolin 3′-methyl ether (**4**), 1 mg; and 6-hydroxykaempferol 7-glucoside (**5**), 14 mg. B. EtOAc *extract*. The syrup from this extract (9.2 g) was passed over a Sephadex LH-20 column and the flavonoids were obtained as a mixture (3 g) which was chromatographed over Polyclar (4 × 32 cm; 90 g). The column was first eluted with H_2O–MeOH–MeCOEt–Me_2CO (13:3:3:1), and the polarity was decreased by the gradual elimination of H_2O. The compounds obtained were quercetagetin (**6**), 5 mg, and 6-hydroxyluteolin 7-glucoside (**7**), 8 mg. C. H_2O *extract*. The aq. extract concentrate (11.7 g) was chromatographed over Polyclar (5 × 35 cm; 150 g) in the same manner as for the EtOAc extract. Compounds eluted in the following order: quercetagetin 3-methyl ether 7-sulfate (**8**), 1 mg; 6-hydroxykaempferol 3-methyl ether 7-sulfate (**9**), 4 mg; 6-hydroxykaempferol 3-methyl ether 7-glucoside (**10**), 14 mg; quercetagetin 3-methyl ether 7-glucoside (**11**), 11 mg; and quercetagetin 7-glucoside (**12**), 30 mg.

NMR and MS data for N. lobata *flavonoids*. 6-Hydroxykaempferol 7-glucoside (**5**) and quercetagetin (**6**); MS of **5** derivatized *m/z* (rel. int.), $M^{+\cdot}$ 302 (100), M − H 301 (40), M − H_2O 286 (50), M − CHO 274 (60), M − COMe 259 (20), A_1 168 (50), A_1 − 16 152 (60), B_2 121 (100). ^{1}H NMR (90 MHz, CCl_4, TMS): δ 3.3–3.8 (5 H, *m*, 6 glucosyl protons), 4.95 (1-H, *d*, J = 8 Hz, for H″-1), 6.5 (1 H, *s*, H-8), 6.8 (2 H, *d*, J = 9 Hz, H-3′ and H-5′), 7.93 (2 H, *d*, J = 9 Hz, H-2′ and H-6′). Compound **6**: ^{1}H NMR (90 MHz, CCl_4, TMS): δ 6.5 (1 H, *s*, H-8), 6.85 (1 H, *d*, J = 9 Hz, H-5′), 7.65 (1 H, *dd*, J = 9 and 2 Hz, H-6′), 7.6 (1 H, *d*, J = 2 Hz, H-2′).

The extraction techniques used for the isolation of flavonoids from 50 g of dry leaf material of *N. macrocephala* were similar to those already described for *N. lobata*. A. CH_2Cl_2 *extract*. The syrup from the CH_2Cl_2 extract was chromatographed by 1 D PC in TBA (*t*-butanol–HOAc–H_2O, 3:1:1) on Whatman 3MM paper. The flavonoids were eluted with MeOH and purified over Sephadex LH-20. The following compounds were obtained: quercetagetin 3,6-dimethyl ether (**3**), 1 mg, and quercetagetin 3,7-dimethyl ether (**2**), 2 mg. B. EtOAc *extract*. After the same purification procedure of the conc. EtOAc extract the following compounds were isolated: quercetagetin 6-methyl ether 7-glucoside (**13**), 2 mg; quercetagetin 3,6-dimethyl ether 7-glucoside (**14**), 4 mg, and quercetagetin 7-glucoside (**12**), 5 mg. C. H_2O *extract*. On purification as above the conc H_2O extract gave quercetagetin 6-methyl ether 7-glucoside (**13**), quercetagetin 7-glucoside (**12**) and a trace of quercetagetin 3-methyl ether 7-sulfate (**8**).

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