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MYRICETIN AND QUERCETIN METHYL ETHERS FROM *HAPLOPAPPUS INTEGERRIMUS* VAR. *PUNCTATUS*

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Key Word Index—*Haplopappus integerrimus* var. *punctatus*; Compositae; myricetin 3,3′,4′-trimethyl ether; myricetin 3′,4′-dimethyl ether; quercetin methyl ethers.

Abstract—Nine flavonoids including two new myricetin derivatives, myricetin 3′,4′-dimethyl ether and myricetin 3,3′,4′-trimethyl ether, were obtained from *Haplopappus integerrimus* var. *punctatus*. The known compounds are quercetin 7,3′-dimethyl ether, quercetin 3,3′-dimethyl ether, isorhamnetin, quercetin 3,7-dimethyl ether, quercetin 3-methyl ether, quercetin and quercetin 3-β-D-glucoside.

INTRODUCTION

Previous reports of flavonoids from South American *Haplopappus* concerned species of sections *Haplopappus* [1, 2] and *Polyphylla* [3, 4]. As a part of our continuing chemical investigation of this genus, we report here the flavonoids of *Haplopappus integerrimus* (Hook and Arn.) Hall var. *punctatus* (Willd.) Brown and Clark. This taxon has recently been treated in *Haplopappus* section *Steriphe* [5].

RESULTS AND DISCUSSION

Leaves of *H. integerrimus* collected in Chile were extracted with aqueous ethanol and the syrup obtained after concentrating the extract was partitioned between *n*-hexane, chloroform and ethyl acetate. Two-dimensional chromatography showed the flavonoids to be primarily in the chloroform and ethyl acetate concentrates. The combined chloroform–ethyl acetate concentrate yielded quercetin 7,3′-dimethyl ether (**1**) [6], quercetin 3,3′-dimethyl ether (**2**) [7], myricetin 3,3′,4′-trimethyl ether (**3**), myricetin 3′,4′-dimethyl ether (**4**), isorhamnetin, quercetin 3,7-dimethyl ether (**5**) [8], quercetin 3-methyl ether (**6**) [7], quercetin and its 3-β-D-glucoside.

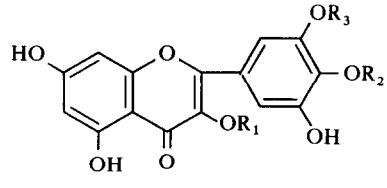
The known compounds were identified by UV, MS and with the exception of the new 3′,4′-dimethyl and 3,3′,4′-trimethyl ethers of myricetin, direct TLC comparisons. The colors, TLC, UV and MS data for all the flavonoids are recorded in Tables 1 and 2. The structural assignments of the new compounds are discussed separately.

Myricetin 3,3′,4′-trimethyl ether (**3**)

The MS of this new natural product gave M^+ at *m/z* 360 in accord with a flavonoid containing three hydroxyl and three methoxyl groups, a result confirmed by the MS of the PDM derivative which exhibited M^+ at *m/z* 411 in accord with three perdeuteriomethoxyl and three methoxyl groups. Furthermore, the NMR in $CDCl_3$ of the underivatized compound established a myricetin skeleton with three methyl ethers δ 3.88 (2 × OMe) and 3.92 (1 × OMe), and four doublets (J = 2.5 Hz) for H-6 and H-8 at δ 6.18 and 6.45, and H-2′ and H-6′ at 7.25 and 7.28, respectively. Since the new flavonoid appeared purple with and without ammonia when viewed as a spot on paper over UV light (366 nm), two of the methoxyl groups should be at the 3- and 4′-positions and a C-5 hydroxyl should be present. Thus, the third methoxyl could only be at either the 7- or 3′-position, and since the NMR data indicated an unsymmetrical B-ring (two doublets for H-2′ and H-6′), it must be at 3′. The UV as well as additional MS data (Table 2) confirmed the structure assignment. Band I in the UV spectrum in NaOMe exhibited a bathochromic shift of only 32 nm and with a lower intensity relative to band I (345 nm) in MeOH indicating substitution of the 4′-hydroxyl group. The presence of band III at 312 nm in the NaOMe spectrum and a 10 nm bathochromic shift in band II in the NaOAc spectrum relative to band II in the MeOH spectrum indicated an unsubstituted 7-hydroxyl group. Furthermore, the $AlCl_3$/HCl spectrum was typical for a 5,7-dihydroxy A-ring and the lack of a shift of band I with NaOAc/H_3BO_3 showed that there was no *ortho*-dihydroxyl group in the B-ring. Both the MS of **3** and its PDM derivative gave fragments for M − 15 (*m/z* 345, 25% and 396, 50%, respectively) for loss of the 4′-methyl ether group while the PDM of myricetin gave a fragment for M − 18 (*m/z* 402, 95%) (M − CD_3).

Myricetin 3′,4′-dimethyl ether (**4**)

A second new flavonol with a free 3-hydroxy (yellow color on paper over UV light and band I in MeOH at 362



3 $R_1 = R_3 = R_2 = Me$
4 $R_1 = H$, $R_3 = R_2 = Me$

Table 1. Chromatographic data (R_fs × 100 and colors) for flavonoids of *H. integerrimus* var. *punctatus*

Compound	Cellulose					Colors*		
	15% HOAc	40% HOAc	TBA†	*n*-BAW†	Polyamide BMM†	UV	UV/NH_3	UV/NA
Quercetin 7,3′-dimethyl ether (**1**)	1	22	74	90	67	y	y	y
Quercetin 3,3′-dimethyl ether (**2**)	1	51	78	90	73	p	y	y
Myricetin 3,3′,4′-trimethyl ether (**3**)	3	62	75	96	83	p	p	br-y
Myricetin 3′,4′-dimethyl ether (**4**)	10	30	75	90	62	y	y	y

* 1D TLC on cellulose and polyamide NM (Polygram). Colors were observed on paper for UV and UV/NH_3 and on TLC plates for NA: p = purple; y = yellow; or = orange; br = brown. NA refers to Naturstoffreagenz A.

† The TLC solvents were: TBA = *t*-BuOH–HOAc–H_2O, 3:1:1; *n*-BAW = *n*-BuOH–HOAc–H_2O, 4:1:5; BMM = C_6H_6–MeCOEt–MeOH, 4:3:3.

nm) was isolated in amounts sufficient only for UV and MS. The MS of the compound indicated a flavonol with two methoxyl and four hydroxyl groups (M^+ at *m/z* 346, 100%). An M − 15 peak at *m/z* 331 (55%) suggested one of the methoxyl groups could be at the 4′ position, a conclusion confirmed by the NaOMe UV spectrum: band I exhibited a bathochromic shift of 53 nm with a lower intensity relative to band I in the MeOH. Since the MS also gave A_1 and B_2 fragments at *m/z* 152 (for an A-ring with two hydroxyl groups) (20%) and 181 (32%) (for a B-ring with one hydroxyl and two methoxyl groups), the second methoxyl group must be at the 3′-position.

EXPERIMENTAL

Plant material. Leaves of *Haplopappus integerrimus* var. *punctatus* were collected 30 km east of Los Angeles, Prov. Biobio, Chile in February 1979. A voucher specimen (Clark and Brown 1389) is deposited in the Herbarium of Arizona State University.

Extraction, purification and identification of flavonoids. The general chromatographic techniques have been described previously [3]. Ground leaves of *H. integerrimus* (200 g) were extracted with aq. EtOH (× 5), and the combined extracts concd *in vacuo* to 250 ml. This aq. concentrate was successively extracted with *n*-hexane, $CHCl_3$ and EtOAc. The $CHCl_3$ and EtOAc concentrates were combined and chromatographed over a Polyclar column (4 × 50 cm). Elution of the column was initiated with Egger's solvent (CH_2Cl_2–MeOH–MeCOEt–Me_2CO, 20:10:5:1) and the polarity gradually increased by reducing the amount of CH_2Cl_2. The compounds eluted in the following order: quercetin 7,3′-dimethyl ether (**1**) (5 mg), quercetin 3,3′-dimethyl ether (**2**) (5 mg), myricetin 3,3′,4′-trimethyl ether (**3**) (12 mg), myricetin 3′,4′-dimethyl ether (**4**) (3 mg), isorhamnetin (3 mg), quercetin 3,7-dimethyl ether (**5**) (6 mg), quercetin 3-methyl ether (**6**) (6 mg), quercetin (2 mg) and its 3-glucoside (4 mg).

Myricetin 3,3′,4′-trimethyl ether (**3**). UV λ_{max}^{MeOH} nm: 345 (1), 304 (sh), 264 (1.2); + NaOMe 377 (1), 312 (0.3), 273 (2); + $AlCl_3$ 400 (1), 348 (1.25), 305 (0.3), 276 (2); + $AlCl_3$/HCl 398 (1), 346 (1.3), 304 (0.8), 278 (3); + NaOAc 358 (1), 305 (0.9), 274 (1.8) and NaOAc/H_3BO_3 348 (1), 306 (sh), 264 (1.15).

Myricetin 3′,4′-dimethyl ether (**4**). UV λ_{max}^{MeOH} nm: 362 (1), 305 (sh), 264 (1), 250 (sh); + NaOMe 415 (1), 324 (sh), 278 (1.25); + $AlCl_3$ 424 (1), 352 (0.25), 310 (sh), 270 (1.2); + $AlCl_3$/HCl 420 (1), 352 (0.2), 310 (sh), 270 (1.20); + NaOAc 380 (1), 300 (sh), 276 (1.5) and NaOAc/H_3BO_3 430 (sh), 367 (1), 306 (sh), 266 (1.2).

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Table 2. MS data for flavonoids of *H. integerrimus* var. *punctatus**

Compound	M^+	(M − 1)	(M + 1)	(M − 15)	(M − 43)	A_1	B_2
1	330(100)	329(40)	331(42)	315(15)	287(20)	167(10)†	151(15)
2‡	330	—	—	315	—	153†	151
3	360(85)	359(100)	361(47)	345(25)	317(15)	153(13)†	181(8)
3 PDM	411(100)	410(75)	412(30)	396(50)	—	187(10)†	198(10)
4	346(100)	345(20)	347(32)	331(55)	303(10)	152(20)	181(32)
5	330(100)	329(80)	331(25)	—	287(25)	167(20)†	137(20)
6	316(100)	315(80)	317(20)	—	273(35)	153(20)†	137(15)

* MS were recorded at 70 eV, source temperature 200° and probe temperature from 250° to 425°. Values are given in *m/z*; in parentheses the % abundance relative to the base peak.

† These values are for (A_1 + H) fragments.

‡ Relative intensities are not given because of the poor quality of the spectrum.

REFERENCES

1. Horhammer, L., Wagner, H., Wilkomirsky, M. T. and Iyenger, M. A. (1973) *Phytochemistry* **12**, 2068.
2. Oksuz, S., Ulubelen, A., Clark, W. D., Brown, G. K. and Mabry, T. J. *Rev. Latinoam. Quím.* (in press).
3. Ulubelen, A., Clark, W. D., Brown, G. K. and Mabry, T. J. *J. Nat. Prod.* (in press).
4. Ulubelen, A., Ayanoglu, E., Clark, W. D., Brown, G. K. and Mabry, T. J. *J. Nat. Prod.* (in press).
5. Brown, G. K. and Clark, W. D. *Syst. Botany* (submitted).
6. Wollenweber, E., Lebreton, P. and Chadenson, M. (1972) *Z. Naturforsch.* **27**, 567.
7. Yang, C. H., Braymer, H. D., Murphy, E. L., Chorney, W., Scully, N. and Wender, S. H. (1960) *J. Org. Chem.* **25**, 2063.
8. Castillo, J. B., Gonzales, A. G. and Eglinton, G. (1968) *Ann. Quim.* (*Spain*) **64**, 193.