Phytochemistry, Vol. 31, No. 8, pp. 2859–2862, 1992
Printed in Great Britain.

0031-9422/92 $5.00+0.00

FLAVONOIDS FROM *AGERATUM CORYMBOSUM**

LEOVIGILDO QUIJANO, FEDERICO GÓMEZ-GARIBAY, GUILLERMINA GARCIA-PEREZ and TIRSO RIOS

Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, México, D.F., México

(*Received* 28 *October* 1991)

Key Word Index—*Ageratum corymbosum*; Asteraceae; Eupatoriae; flavones; flavanones; heptasubstituted and hexasubstituted flavones.

Abstract—Investigation of a new collection of *Ageratum corymbosum* resulted in the isolation of three new flavonoids, 4′-hydroxy-5,6,7,3′-tetramethoxyflavanone, 5-hydroxy-6,7,2′,3′,4′,5′-hexamethoxyflavone and 3′-hydroxy-5,6,7,2′,4′,5′-hexamethoxyflavone, named agecorynins E, F and G, respectively. Their structures were established by spectral methods and chemical transformations. 5-Hydroxy-7,8,2′,3′,4′,5′-hexamethoxyflavone (isoagecorynin F), obtained from agehoustin B, is also reported.

INTRODUCTION

Ageratum is a genus which has been shown to produce highly oxygenated flavonoids. So far the most highly substituted flavones, agehoustins A (**6a**), B (**1f**), C (**6b**) and D (**6c**), have been isolated from *Ageratum houstonianum* [1–3]. However, 5,3′-*O*-demethylagehoustin B (psiadiarabin) (**1a**) has been identified in *Psiadia arabica* [4]. As part of our studies on *Ageratum*, we have reported previously the isolation of several flavonoids from *A. corymbosum* collected at the University campus in México City [5]. We now report the results of our study on a different collection from the state of Puebla, México. This study resulted in the isolation of three new flavonoids, one flavanone which we named agecorynin E (**2a**) and two new members of the rare group of flavones with a 2′,3′,4′,5′-tetraoxygenated B-ring, agecorynins F (**1b**) and G (**1c**), together with the known agehoustin B (**1f**) [3] and psiadiarabin (**1a**) [4] of the same novel group. Agehoustin G (**4a**), its methyl ether (**4b**) [1], ageconyflavone A (**5a**) [6], lucidin dimethyl ether (**5b**) [3] and agestricin B methyl ether (**2b**) were also isolated. To our knowledge this is the first time that **2b** has been observed as a natural product.

RESULTS AND DISCUSSION

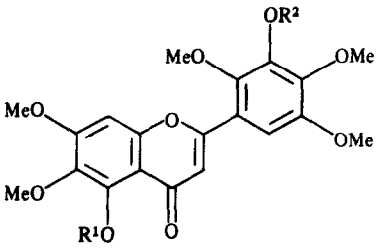
From the aerial parts of *Ageratum corymbosum* f. *corymbosum* collected in Puebla, México, ten flavonoids were isolated by CC and TLC on silica gel. Four of them were heptasubstituted flavones with a tetraoxygenated B-ring, the known agehoustin B (**1f**) and three new derivatives of **1f**.

Compound **1a**, isolated from the medium polar fractions of the petrol extract, was a yellow crystalline dihydroxypentamethoxyflavone, $C_{20}H_{20}O_9$ ([M]$^+$ at *m/z* 404). Both the UV (270, 332 nm) and IR (3500–3000, 1655 cm^{-1}) absorptions were typical of a hydroxyflavone [7]. The ^{1}H NMR data (Table 1), which was similar to that of **1f**, clearly established the presence of five methoxyl groups, when determined in C_6D_6. The presence of a proton singlet at δ13.45 indicated that a hydroxyl group must be present at C-5. A non-chelated hydroxyl signal at δ5.80 indicated the presence of an extra hydroxyl group at C-3′ as in agehoustins C (**6b**) and D (**6c**). Methylation of **1a** with MeI–K_2CO_3 yielded **1f**, establishing the structural relationship between **1a** and **1f**. Acetylation of **1a** gave the corresponding diacetate **1d**, confirming the presence of two hydroxyl groups in **1a**, one of them at C-5 (acetyl signal at δ2.49). Confirmation of the hydroxyl group at C-3′ was achieved by alkaline hydrolysis of **1a**, which furnished 3-hydroxy-2,4,5-trimethoxyacetophenone, as given by agehoustin D (**6c**) with proven structure by X-ray diffraction [2]. Hence **1a** must be 3′,5-dihydroxy-6,7,2′,4′,5′-pentamethoxyflavone (psiadiarabin), a flavone recently isolated from *Psiadia arabica*, whose structure was established by X-ray analysis [4].

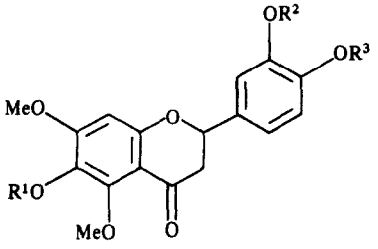
Agecorynin F (**1b**) was a monohydroxyflavone as indicated by the UV (272, 325 nm) and IR (2800–3300, 1655 cm^{-1}) absorptions. The ^{1}H NMR data (Table 1), which was almost identical to that of agehoustin B (**1f**) [3], indicated that **1b** must be 5-*O*-demethylagehoustin B. Accordingly the ^{1}H NMR lacked one methoxyl signal and showed an extra hydrogen-bonded hydroxyl group signal at δ12.69. Acetylation of **1b** afforded the monoacetate **1e**, confirming the presence of a hydroxyl group at C-5 (acetyl signal at δ2.48). Methylation of psiadiarabin (**1a**) with CH_2N_2 afforded **1b**, confirming the structure of agecorynin F. Furthermore, selective demethylation of **1f** with HCl–HOAc [8] yielded **1b**. The last two reactions established the structural relationship between **1a**, **1b** and **1f**.

In addition to **1b**, demethylation of **1f** afforded another crystalline compound (**3a**). All the spectral data indicated a compound isomeric with **1b**. Thus, the EIMS showed the same molecular ion peak ([M]$^+$ at *m/z* 418), as well as

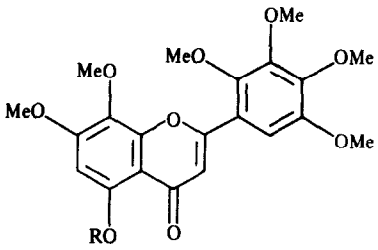
* Part 6 in the series 'Flavonoids from *Ageratum* species'. For Part 5 see ref. [1]. This is contribution No. 1110 of the Instituto de Química, UNAM. In commemoration of its 50th aniversary.



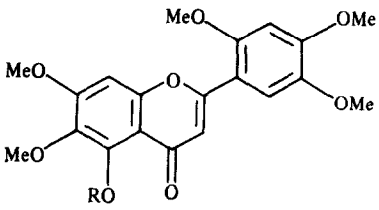
	R^1	R^2
1a	H	H
1b	H	Me
1c	Me	H
1d	Ac	Ac
1e	Ac	Me
1f	Me	Me



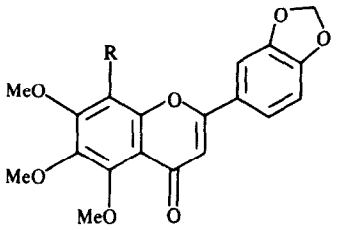
	R^1	R^2	R^3
2a	Me	Me	H
2b	Me	O – CH_2 – O	
2c	H	Me	Me
2d	H	Me	H



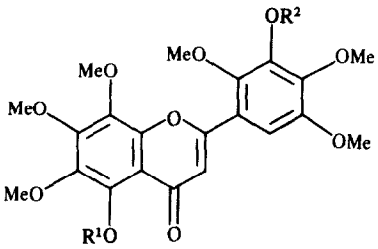
	R
3a	H
3b	Me
3c	Ac



	R
4a	H
4b	Me



	R
5a	H
5b	OMe



	R^1	R^2
6a	Me	Me
6b	Me	H
6c	H	H

other fragmentation peaks at *m/z* 195 $[A_1]^+$ and 180 $[A_1 - Me]^+$. As in **1b**, the ^1H NMR spectrum of **3a** (Table 1) displayed signals due to six methoxyl groups, three flavone-nucleus protons and two hydroxyl groups, but with different chemical shifts. All the above data indicated that the structure of **3a** must correspond to the isomeric compound 5-hydroxy-7,8,2',3',4',5'-hexamethoxyflavone, derived from the opening and reclosing of the γ-pyrone ring in a different position [8]. Methylation of **3a** gave the corresponding 5,7,8,2',3',4',5'-heptamethoxyflavone (**3b**). Acetylation of **3a** afforded the monoacetate **3c**.

Agecorynin G (**1c**), $[M]^+$ at *m/z* 418, was another hydroxy-hexamethoxyflavone. Its structure followed from the ^1H NMR and mass spectra, which suggested an isomeric relationship between **1c** and **1b**. Accordingly the ^1H NMR spectrum displayed signals for six methoxyl groups, but no hydrogen-bonded hydroxyl group at C-5. Instead a signal at δ5.80, interchangeable with D_2O, indicated the presence of a non-chelated hydroxyl group. The mass spectral fragment peaks at *m/z* 195 $[A_1 - Me]^+$ and 167 $[A_1 - Me - CO]^+$ indicated that the hydroxyl group must be on the B-ring, probably at C-3'. Confirmation of the structure of **1c** was achieved by chemical correlation with agehoustin B (**1f**) and psiadiarabin (**1a**). Methylation of agecorynin G (**1c**) with MeI–K_2CO_3 and selective demethylation with HCl–HOAc afforded **1f** and **1a**, respectively. Hence agecorynin G, is 3'-hydroxy-5,6,7,2',4',5'-hexamethoxyflavone (**1c**).

Agecorynin E (**2a**), $[M]^+$ at *m/z* 360, was isolated as a yellow crystalline compound. Its ^1H NMR spectrum (Table 1) suggested a 5,6,7,3',4',-pentasubstituted flavanone containing one hydroxyl and four methoxyl groups

Table 1. ^{1}H NMR data of psiadiarabin (**1a**), agecorynins F (**1b**) and G (**1c**), isoagecorynin F (**3a**) and derivatives*

H	1a	1b	1c	1d	1e	3a	3c
3	6.80 (6.66)	6.84 (6.87)	6.72 (6.87)	6.85 (6.83)	6.85 (6.83)	6.93 (7.06)	6.85
6						6.42 (6.28)	6.63
8	6.48 (6.16)	6.47 (6.16)	6.76 (6.70)	6.74 (6.39)	6.76 (6.39)		
6′	6.86 (6.98)	6.99 (6.96)	6.76 (7.04)	7.11 (6.85)	6.96 (6.96)	7.17 (†)	7.13
OMe	3.82 (3.22)	3.84 (3.24)	3.82 (3.45)	3.71 (3.14)	3.82 (3.13)	3.89 (3.17)	3.85
	3.90 (3.37)	3.90 (3.43)	3.90 (3.61)	3.84 (3.34)	3.85 (3.44)	3.89 (3.40)	3.88
	3.91 (3.54)	3.90 (3.57)	3.90 (3.76)	3.88 (3.42)	3.89 (3.54)	3.89 (3.58)	3.92
	3.95 (3.61)	3.94 (3.66)	3.95 (3.80)	3.89 (3.74)	3.94 (3.67)	3.94 (3.64)	3.96
	3.99 (3.88)	3.94 (3.74)	3.98 (3.89)	3.97 (3.78)	3.96 (3.75)	3.94 (3.69)	3.96
		3.97 (3.86)	3.98 (4.00)		3.98 (3.75)	3.97 (3.74)	3.96
OH	5.95 (5.80)		5.87				
	12.65 (13.45)	12.69 (13.42)				12.57 (13.40)	
AcO				2.39 (1.88)			2.43
				2.49 (2.37)	2.49 (2.38)		

*Run at 80 MHz in $CDCl_3$ or C_6D_6 with TMS as internal standard; values are in δ. All signals are singlets.
†Obscured by solvent signal (benzene).
Values in parentheses are chemical shifts in C_6D_6.

indicated by the typical ABX system with signals centred at δ2.78 and 5.30 due to H-2 and H-3, an aromatic proton singlet at δ6.32 and four sharp methoxyl singlets between δ3.75 and 3.95. Both the UV (275, 320 nm) and IR (3542, 1675 cm^{-1}) absorptions were in agreement with a hydroxyflavanone structure. The above data were similar to those of agestricin C (**2c**), isolated from *Ageratum strictum* [9], suggesting an isomeric structure. The EIMS confirmed this assumption, since it showed the same molecular ion peak at *m/z* 360, but different fragmentation peaks at *m/z* 211 $[A_1H]^+$, 210 $[A_1]^+$ and 150 $[B_1]^+$, indicating the presence of three methoxyl groups on the A-ring and a hydroxyl group on the B-ring. The ^{1}H NMR data suggested that the hydroxyl group must be at C-4′ as in agestricin D (**2d**) [10]. Therefore, the most probable structure for agecorynin E is 4′-hydroxy-5,6,7,3′-tetramethoxyflavanone (**2a**).

EXPERIMENTAL

Aerial parts of *Ageratum corymbosum* Zuccag. ex Pers. *forma corymbosum* were collected in grounds of the Observatory in Tonanzintla, Puebla (México) on 24 September 1987. Plant material was identified by J. L. Villaseñor and O. Tellez and a voucher specimen (No. 522947) deposited at the herbarium of the Instituto de Biología, UNAM (MEXU). Air-dried plant material (leaves and flowers; 362 g) was extracted successively with petrol and CH_2Cl_2. Both extracts, after elimination of the solvent, were chromatographed separately on silica gel (Merck 70–230 mesh), using petrol and petrol–EtOAc mixtures of increasing polarity as eluants. Known compounds were identified by comparison of spectral data with those of authentic materials and/or those reported in the lit.

The petrol extract (4.42 g) was separated by CC over silica gel (100 g). The less polar fractions provided sitosterol, taraxasteryl acetate, taraxasterol, coumarin and lutein. Fractions 59–64 (256 mg), after further TLC purification (0.5 mm × 10 × 20 cm; CH_2Cl_2–Me_2CO, 19:1, ×4), afforded **2a** (16.6 mg), **1b** (53.7 mg) and **1f** (30.7 mg). Rechromatography of fractions 91 and 92 (2 g) over silica gel (30 g) yielded 156 mg of **1a**, mp 197–199° (lit. 228° [4]) and 1.0 g of **5a**, mp 187–189° (lit. 189–190° [6]). Fraction 93 (2.0 g) afforded further amounts of **5a** (570 mg) and **1f** (1.4 g). Fractions 94–99 (161 mg) gave 52 mg of **4b**, mp 182–184° (lit. 184° [1]) and 29 mg of **4a**, mp 192–194° (lit. 199–203° [1]). The CH_2Cl_2 extract (15.9 g) was chromatographed over 350 g of silica gel. Rechromatography of fractions 76–95 (181.5 mg) yielded 10 mg of **2a** and further amounts of **1f** (60 mg) and **5a** (57 mg). Fractions 96–100 (187 mg) gave, after further CC and TLC (2 mm × 10 × 20 cm; CH_2Cl_2–Me_2CO, 19:1, ×4) purification, 56 mg of **1b**, 36 mg of **5b** [5] and 56 mg of **1f**. Fractions 101–160 (285 mg) provided further quantities of **1a** (92 mg), **1f** (250 mg) and **5a** (216 mg).

Agecorynin E (**2a**). $C_{19}H_{20}O_7$, yellow crystals, mp 161–162° (Et_2O). UV λ_{max}^{MeOH} nm (ε): 275 (18720), 320 (5400). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3542, 1675, 1602, 1567, 1518, 1485. EIMS (probe) 70 eV, *m/z* (rel. int.): 360 $[M]^+$ (54), 345 $[M-Me]^+$ (4), 210 $[A_1]^+$ (68), 211 $[A_1H]^+$ (60), 195 $[A_1-Me]^+$ (81), 167 $[A_1-Me-CO]^+$ (100), 150 $[B_1]^+$ (22), 135 $[B_1-Me]^+$ (26). ^{1}H NMR (80 MHz, $CDCl_3$): δ2.85 (2H, ABm, H-3), 3.80, 3.85, 3.90, 3.92 (4 × 3H, *s*, 4 × OMe), 5.30 (*dd*, J = 12.0 Hz, J = 4.0 Hz, H-2), 5.66 (*s*, OH), 6.32 (*s*, H-8), 6.91 (3H, *br s*, H-2′, H-5′, H-6′).

Agecorynin F (**1b**). $C_{21}H_{22}O_9$, yellow crystals, mp 69–71° (Et_2O). UV λ_{max}^{MeOH} nm (ε): 272 (12283), 325 (12925). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300–2800, 1655, 1611, 1491, 1460. EIMS (probe) 70 eV, *m/z* (rel. int.): 418 $[M]^+$ (100), 403 $[M-Me]^+$ (73), 373 $[M-Me-CH_2O]^+$ (22), 181 $[A_1-Me]^+$ (18).

Agecorynin G (**1c**). $C_{20}H_{20}O_9$, yellow crystals, mp 199–200° (Et_2O). UV λ_{max}^{MeOH} nm (ε): 276 (13520), 358 (8325). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3516, 1637, 1602, 1509, 1464. EIMS (probe) 70 eV, *m/z* (rel. int.): 418 $[M]^+$ (24), 403 $[M-Me]^+$ (100), 388 $[M-CH_2O]^+$, 373 $[M-Me-CH_2O]^+$ (12), 208 $[B_1]^+$ (4), 195 $[A_1-Me]^+$ (15), 193 $[B_1-Me]^+$ (9), 165 $[A_1-Me-CH_2O]^+$ (48).

Psiadiarabin diacetate (**1d**). Acetylation of 22 mg of **1a** with Ac_2O–$HClO_4$ afforded, after usual work-up, the diacetate **1d** as colourless crystals, mp 148–150°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1734, 1630, 1600, 1516, 1436, 1421. EIMS (probe) 70 eV, *m/z* (rel. int.): 488 $[M]^+$ (18), 446 $[M-CH_2CO]^+$ (100), 431 $[M-CH_2CO-Me]^+$ (24), 404 $[M-2CH_2CO]^+$ (12), 389 $[M-2CH_2CO-Me]^+$ (18), 167 $[A_1H-CH_2CO-CO]^+$ (15), 43 $[MeCO]^+$ (28).

Agecorynin F acetate (**1e**). Acetylation of **1b** (25 mg) with Ac_2O–pyridine as usual, gave the monoacetate **1e** (18 mg), mp 127–129° (Et_2O). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760, 1566, 1443, 1461, 1423.

EIMS (probe) 70 eV, *m/z* (rel. int.): 460 $[M]^+$ (23), 418 $[M-CH_2CO]^+$(100), 403 $[M-CH_2CO-Me]^+$ (56), 43 $[MeCO]^+$ (24).

Demethylation of agehoustin B (**1f**). A mixture of 50 mg of **1f**, conc. HCl (0.5 ml) and conc. HOAc (0.5 ml) was heated for 24 hr and worked up as described in ref. [2]. Purification of the residue by CC gave a crystalline compound identical with agecorynin F (**1b**).

Isoagecorynin F (**3a**). Mp 185–197° (Et_2O). UV λ_{max}^{MeOH} nm (ε): 272 (23 028), 334 (17 916). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3002, 1653, 1607, 1490, 1461, 1409. EIMS (probe) 70 eV, *m/z* (rel. int.): 418 $[M]^+$ (55), 403 $[M-Me]^+$ (100).

Isoagecorynin F acetate (**3c**). Acetylation of 35 mg of **3a** with Ac_2O–pyridine followed by usual work-up gave the monoacetate **3c** (10.7 mg), mp 181–184° (Et_2O). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1739, 1651, 1606, 1492, 1463. EIMS (probe) 70 eV, *m/z* (rel. int.): 460 $[M]^+$ (3), 418 $[M-CH_2CO]^+$ (51), 403 $[M-CH_2CO-Me]^+$ (100).

Methylation of isoagecorynin F (**3a**). Methylation of **3a** with MeI in Me_2CO with dry K_2CO_3 gave the yellow crystalline **3b** (29 mg), mp 154–157° (Et_2O) (lit. 159–161° [91]). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1640, 1554, 1474. EIMS (probe) 70 eV, *m/z* (rel. int.): 432 $[M]^+$ (88), 417 $[M-Me]^+$ (100), 195 $[A_1-Me]^+$ (14), 167 $[A_1-Me-CO]^+$ (38).

Methylation of agecorynin G (**1c**). Similar methylation of **1c** gave a crystalline compound identical with agehoustin B (**1f**).

Demethylation of agecorynin G (**1c**). Compound **1c** (250 mg) was selectively demethylated as described above to give 17 mg of a crystalline compound identical in all respects with psiadiarabin (**1a**).

Alkaline degradation of psiadiarabin (**1a**). Psiadiarabin (**1a**; 55 mg) was refluxed with 50% KOH (15 ml) in EtOH (15 ml) under N_2 for 15 hr. The reaction mixture, worked-up as described in ref. [1], afforded 18 mg of 3-hydroxy-2,4,5-trimethoxyacetophenone, mp 97–100° (Et_2O) (lit. 98–100° [2]).

Acknowledgements—The authors wish to thank Messrs Misael Torres, Ruben Gabiño and Luis Velasco for IR, UV, NMR and mass spectra and Mr Oswaldo Tellez (Instituto de Biología, UNAM) for botanical assistance.

REFERENCES

1. Quijano, L., Calderón, J. S., Gómez-G., F., Escobar, E. and Rios, T. (1987) *Phytochemistry* **26**, 2075.
2. Quijano, L., Calderón, J. S., Gómez-G., F., Escobar, E. and Rios, T. (1985) *Phytochemistry* **24**, 1085.
3. Quijano, L., Calderón, J. S., Gómez-G., F. and Rios, T. (1982) *Phytochemistry* **21**, 2695.
4. Al-Yahya, M. A., Hifnawy, M. S., Mossa, J. S., El-Feraly, F. S., McPhail, D. R. and McPhail, A. T. (1987) *Phytochemistry* **26**, 2648.
5. Quijano, L., Calderón, J. S., Gomez-G., F., Soria, I. E. and Rios, T. (1980) *Phytochemistry* **19**, 2439.
6. Vyas, A. V. and Mulchandani, N. B. (1986) *Phytochemistry* **25**, 2625.
7. Mabry, T. A., Markham, K. B. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*, p. 41. Springer, New York.
8. Geissman, T. A. (1962) *The Chemistry of Flavonoid Compounds*, p. 184. Mcmillan, New York.
9. Iinuma, M., Tanaka, T. and Matsuura, S. (1984) *Chem. Pharm. Bull.* **32**, 3354.
10. Quijano, L., Calderón, J. S., Gómez-G., F. and Rios, T. (1982) *Phytochemistry* **21**, 2575.