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FURTHER POLYSUBSTITUTED FLAVONES FROM *AGERATUM HOUSTONIANUM**

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Key Word Index—*Ageratum houstonianum*; Asteraceae; Eupatorieae; heptasubstituted and hexasubstituted flavones; structure determination.

Abstract—The aerial parts of *Ageratum houstonianum* afforded three new flavones, 5-hydroxy-6,7,8,2′,4′,5′-hexamethoxyflavone; 5,2′-dihydroxy-6,7,8,4′,5′-pentamethoxyflavone and 5-hydroxy-6,7,2′,4′,5′-pentamethoxyflavone. The known 5,6,7,3′,4′,5′-hexamethoxyflavone was also isolated. Structural determinations involved spectral methods and chemical degradations.

INTRODUCTION

In earlier papers we have published the isolation of three octasubstituted flavones from *Ageratum houstonianum*, agehoustins A (**3a**), C (**3b**) and D (**3c**), which represent the highest oxygenated flavones and the first flavonoids with a tetrasubstituted B-ring yet reported from nature [1, 2]. To our knowledge, 10 other octasubstituted compounds have been isolated so far, all flavonol 3-*O*-methyl ethers, including exoticin (3,5,6,7,8,3′,4′,5′-octa methoxyflavone) from *Murraya exotica* (Rutaceae) [3] and purpurascenin (3,5,6,7,8,2′,4′,5′-octamethoxyflavone) from *Pogostemon purpurascens* (Labiatae) [4]. The other eight compounds were isolated from *Gutierrezia dracunculoides* and *G. grandis* (Asteraceae) and all of them have the same substitution pattern as exoticin and purpurascenin [5, 6]. Now we report the isolation of three new highly oxygenated flavones from *A. houstonianum*, which we name agehoustins E (**1a**), F (**1b**) and G (**2a**), besides the known 5,6,7,3′,4′,5′-hexamethoxyflavone (**4**).

RESULTS AND DISCUSSION

Agehoustin E (**1a**) was isolated as a yellow crystalline compound, mp 140–143°. The mass spectrum of **1a** showed a $[M]^+$ at m/z 418 ($C_{21}H_{22}O_9$), indicating a heptasubstituted flavone with one hydroxyl and six methoxyl groups.

The ^1H NMR spectrum of **1a** (Table 1) was very similar to that of agecorynin C (**1d**) [7], isolated from *A. corymbosum*, suggesting the same substitution pattern. It showed three singlets (1H each) at δ 6.55, 7.05 and 7.52 due to three flavone-nucleus protons and resonances between 3.89 and 4.06 due to six methoxyl groups, which were clearly observed when the ^1H NMR spectrum (Table 1) was determined in C_6D_6. The presence of a hydroxyl proton signal at δ 12.58 indicated that the hydroxyl group must be at C-5. Acetylation of **1a** gave the corresponding monoacetate **1c**, mp 173–174°, which showed the C-5 acetyl signal at δ 2.47 [8]. Hence, agehoustin E must be 5-*O*-demethylagecorynin C (**1a**). Selective demethylation of **1d** with hydrochloric acid [9] confirmed the above assumption and established the structural relationship between agecorynin C (**1d**) and agehoustin E (**1a**).

Agehoustin F (**1b**), $C_{20}H_{20}O_9$ ($[M]^+$ at m/z 404), mp. 237–238°, was a deep yellow crystalline compound. The ^1H NMR spectrum of **1b** (Table 1) clearly established the presence of five methoxyl groups and two hydrogen-bonded hydroxyl groups with resonances at δ 12.23 and 9.5, indicating the presence of hydroxyl groups at C-5 and very likely at C-2′. The one-proton singlets at δ 6.55, 7.18 and 7.21 suggested the same substitution pattern as in **1a** and **1d**. The mass spectral peaks at m/z 389 $[M - Me]^+$, 211 $[A - Me]^+$ and 183 $[M - Me - CO]^+$ confirmed the A-ring substitution of **1b** as in agehoustins D (**3c**) and E (**1a**). Acetylation of **1b** afforded the corresponding diacetate, **1e**, mp 187–189°, which showed the C-5 and C-2′ acetyl signals at δ 2.47 and 2.33. Selective methylation of **1b** with diazomethane afforded the monomethylated derivative, which was identical in all respects with agehoustin E (**1a**), establishing the substitution pattern and the structural relationship between **1a** and **1b**. Therefore, agehoustin F is very likely 5,2′-dihydroxy-6,7,8,4′,5′-pentamethoxyflavone (5-*O*-demethylagehoustin E) (**1b**).

Alkaline hydrolysis of **1b** furnished two degradation products, which were spectroscopically identified as 2-hydroxy-4,5-dimethoxyacetophenone (**5a**), which must have been derived from the B-ring, and 2,3,4-trimethoxyresorcinol (**6**). As in case of agehoustin D (**3c**) [1], **6** can be derived from the A-ring by decarboxylation of the corresponding acid.

Agehoustin G (**2a**), mp 199–203°, was a yellow crystalline flavone with one hydroxyl and five methoxyl groups. Accordingly the mass spectrum showed a $[M]^+$ at m/z 388 and the ^1H NMR spectrum (Table 1), determined in C_6D_6,

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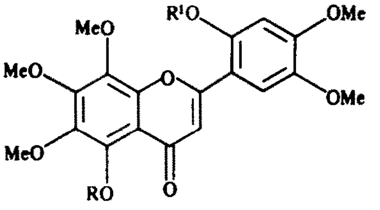
*Dedicated to Professor Tirso Rios upon completing 30 years in teaching and research.

Part 5 in the series "Flavonoids from *Ageratum* species". For part 4, see ref. [1]. This is contribution No. 817 of the Instituto de Química, UNAM.



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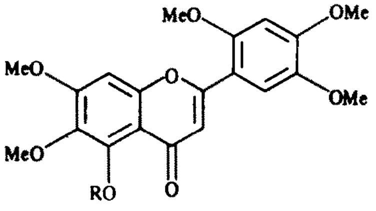
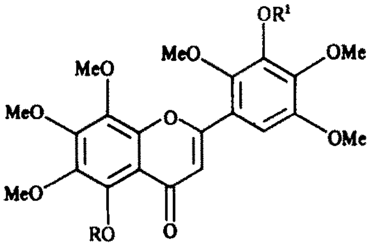
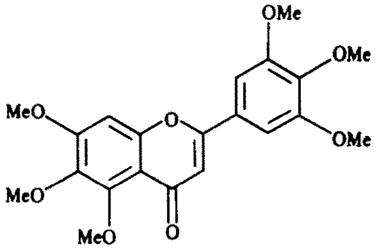
1b R = R¹ = H
1c R = Ac R¹ = Me
1d R = R¹ = Me
1e R = R¹ = Ac



2a R = H
2b R = Ac
2c R = Me



3a R = R¹ = Me
3b R = Me, R¹ = H
3c R = R¹ = H



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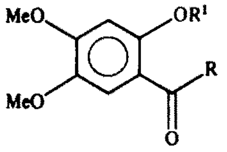
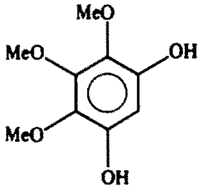


5a R = Me, R¹ = H
5b R = OH, R¹ = Me
5c R = R¹ = Me



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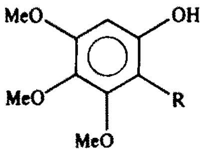

7a R = COMe
7b R = COOH
7c R = H

five sharp methoxyl signals. The ^1H NMR spectrum of **2a**, which was similar to that of a agehoustin E (**1a**), showed the following differences from **1a**. One methoxyl signal was missing and, instead, an extra one-proton singlet appeared at δ6.46, indicating the absence of substitution at C-8 (or C-6).

Acetylation of **2a** afforded the monoacetate derivative **2b**, mp 200–204°, which showed the acetyl signal at δ2.45 confirming the presence of a C-5 hydroxyl group in the molecule. Confirmation of the substitution pattern was achieved by methylation of **2a** with methyl iodide, giving the methyl ether derivative **2c**, which was identical in all respects with 5,6,7,2',4',5'-hexamethoxyflavone [10].

Alkaline hydrolysis of **2c** furnished the expected ortho-hydroxyacetophenone **7a** and the benzoic acid **5b** derived from the A- and B-ring, respectively. As in case of other 2'-substituted flavones [1, 2], further degradation products from fission of the intermediary β-diketone were also isolated and identified as 1,4,5-trimethoxyacetophenone (**5c**) derived from the B-ring and 6-hydroxy-2,3,4-trimethoxybenzoic (**7b**) acid derived from the A-ring, as well as its corresponding decarboxylation product 3,4,5-trimethoxyphenol (**7c**).

Synthesis of 5-hydroxy-6,7,2',4',5'-pentamethoxyflavone has been recently reported. The physical and spectroscopic data of **2a** are similar to those reported for the synthetic product [11].

5,6,7,3',4',5'-Hexamethoxyflavone (**4**), mp 142–144°, recently isolated from *Bauhinia championii* [12], was also isolated from *A. houstonianum*. It was identified by chemical degradation and comparison with the reported data of the permethylated derivative of 5,7,4'-trihydroxy-6,3',5'-trimethoxyflavone [13] and the synthetic product [14].

EXPERIMENTAL

Plant material. A. houstonianum Mill. was collected from the state of Morelos, México, *ca* 60 km south of Mexico City, on the road to Cuernavaca. The plant material (leaves and flowers) was

Table 1. *^{1}H NMR data of agehoustins E (**1a**), F (**1b**), G (**2a**) and their acetates **1c**, **1e** and **2b**

	1a		**1b**		**2a**		**1c**		**1e**		**2b**	
	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6
H-3	6.55	6.07	6.55	6.41	6.56	6.06	6.56	6.09	6.46	6.47	6.54	6.12
H-8					6.46	6.13					6.81	6.32
H-3′	7.05	7.28	7.18	6.89	6.98	7.24	6.98	7.31	6.66	6.65	6.86	7.20
H-6′	7.52	7.52	7.21	6.92	7.35	7.38	7.50	7.49	7.20	6.98	7.30	7.32
OMe	3.89	3.20	3.88	3.22	3.91	3.14	3.87	3.15	3.87	3.22	3.82	3.09
	3.92	3.35	3.93	3.38	3.91	3.22	3.90	3.33	3.91	3.38	3.85	3.12
	3.92	3.55	3.95	3.62	3.91	3.30	3.92	3.56	3.92	3.54	3.88	3.27
	3.92	3.66	3.97	3.75	3.95	3.53	3.95	3.57	3.95	3.69	3.92	3.54
	3.94	3.85	4.01	3.82	3.95	3.88	4.02	3.72	4.08	3.74	3.95	3.74
	4.06	3.85					4.08	3.75				
OH	12.58	13.42	9.50	—	12.83	13.70						
			12.23	—								
OAc							2.47	2.38	2.47	2.37	2.45	2.37
									2.33	1.95		

*Run at 80 MHz with TMS as internal standard; values are in δ. All signals are singlets.

extracted with $CHCl_3$ and the crude residue chromatographed as described in ref. [2].

Fractions eluted with petrol–EtOAc (3:1) were combined and crystallized yielding a mixture of agehoustin B and eupalestin [2, 7]. Further prep. TLC ($CHCl_3$–Me_2CO, 19:1, 3×) of the mother liquor yielded pure **1a**. Fractions eluted with petrol–EtOAc (7:3 and 3:2) were combined and chromatographed by prep. TLC ($CHCl_3$–Me_2CO, 9:1, 3×) to yield 4 bands. Bands 1, 2 and 3, after crystallization ($CHCl_3$–Et_2O) afforded **2a**, **4** and **1b**, respectively. Band 4 contained **3b** [1].

Agehoustin E (**1a**). $C_{21}H_{22}O_9$, yellow crystals, mp 140–143° ($CHCl_3$–Et_2O). UV λ_{max}^{MeOH} nm (ε): 260 sh (12200), 278 (13400), 365 (14500). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3200–2500, 1655, 1620, 1580, 1565. EIMS (probe) 70 eV *m/z* (rel. int.): 418 $[M]^+$ (50), 403 $[M-Me]^+$ (100), 211 $[A_1-Me]^+$ (20), 183 $[A_1-Me-CO]^+$ (6), 177 $[B_1-Me]^+$ (6).

Agehoustin F (**1b**). $C_{20}H_{20}O_9$, deep yellow crystals, mp 237–239° ($CHCl_3$–Et_2O). UV λ_{max}^{MeOH} nm (ε): 247 (22350), 370 (24600). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 1645, 1610, 1595, 1565. EIMS (probe) 70 eV *m/z* (rel. int.): 404 $[M]^+$ (43), 389 $[M-Me]^+$ (100), 211 $[A_1-Me]^+$ (24), 183 $[A_1-Me-CO]^+$ (17), 178 $[B_1]^+$ (5), 163 $[B_1-Me]^+$ (5).

Agehoustin G (**2a**). $C_{20}H_{20}O_8$, yellow crystals, mp 199–203° ($CHCl_3$–Et_2O), UV λ_{max}^{MeOH} nm (ε): 256 (19700), 270 sh (18100), 358 (22800). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2700–3200, 1660, 1620, 1585, 1565, 1520. EIMS (probe) 70 eV *m/z* (rel. int.): 388 $[M]^+$ (100), 373 $[M-Me]^+$ (97), 359 $[M-Me-CH_2]^+$ (24), 342 $[M-Me-OMe]^+$ (24), 192 $[B_1]^+$ (5), 177 $[B_1-Me]$ (7).

Agehoustin E acetate (**1c**). Acetylation of **1a** (25 mg) (Ac_2O–pyridine) at 100° for 48 hr followed by TLC purification ($CHCl_3$–Me_2CO, 9:1) gave the monoacetate **1c**, as a yellow crystalline compound, mp 173–174°. UV λ_{max}^{MeOH} nm (ε): 255 (17500), 265 sh (15900), 301 sh (8500), 358 (16500). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1635, 1568, 1517. EIMS (probe) 70 eV *m/z* (rel. int.): 460 $[M]^+$ (10), 418 $[M-CH_2CO]^+$ (57), 403 $[M-CH_2CO-Me]^+$ (100), 388 $[M-CH_2CO-30]^+$ (3), 43 $[MeCO]^+$ (6).

Demethylation of agecorynin C (**1d**). **1d** (10 mg) was heated at 100° with conc. HCl (0.5 ml) and HOAc (0.5 ml) for 24 hr and worked-up as described in ref. [1]. TLC purification of the residue gave a crystalline compound identical with agehoustin E (**1a**).

Agehoustin F diacetate (**1e**). Acetylation of **1c** (25 mg) with Ac_2O–$HClO_4$, followed by usual work-up, purification of the residue by TLC ($CHCl_3$–Me_2CO, 4:1) and crystallization gave the diacetate **1e**, mp 187–189° ($CHCl_3$–Et_2O). UV λ_{max}^{MeOH} nm (ε): 246 (21900), 322 (18000), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1645, 1615, 1570. EIMS (probe) 70 eV *m/z* (rel. int.): 488 $[M]^+$ (5), 446 $[M-CH_2CO]^+$ (60), 431 $[M-CH_2CO-Me]^+$ (26), 404 $[M-2CH_2CO]^+$ (36), 389 $[M-2CH_2CO-Me]^+$ (100), 211 $[A_1-Me]^+$ (4), 183 $[A_1-Me-CO]^+$ (8).

Methylation of agehoustin F (**1b**). Methylation of **1b** (15 mg) with CH_2N_2–Et_2O followed by purification of the residue by TLC ($CHCl_3$–Me_2CO, 19:1, 3×) gave a yellow crystalline compound identical with agehoustin E (**1a**).

Alkaline degradation of agehoustin F (**1b**). **1b** (38 mg) was refluxed with 50% KOH (10 ml) in EtOH (10 ml) under N_2 for 5 days. The reaction mixture was cooled acidified with dil. HCl, extracted with EtOAc and the residue purified by prep. TLC ($CHCl_3$–Me_2CO, 9:1, 2×). The less polar band furnished after crystallization 2-hydroxy-4,5-dimethoxy acetophenone (**5a**), mp 103–105° ($CHCl_3$–petrol). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2500–3300, 1638, 1568, 1515. EIMS (probe) 70 eV *m/z* (rel. int.): 196 $[M]^+$ (81), 181 $[M-Me]^+$ (100), 153 $[M-MeCO]^+$ (10), 135 $[M-MeCO-H_2O]^+$ (29), 43 $[MeCO]^+$ (17). ^{1}H NMR (80 MHz, $CDCl_3$): δ2.54 (3H, *s*, COMe); 3.84, 3.89 (3H, 2 × OMe), 6.42 (H-3, *s*), 7.03 (H-6, *s*), 12.55 (OH, *s*). The more polar compound was the oily 2,3,4-trimethoxyresorcinol (**6**) [1].

Agehoustin G monoacetate (**2b**). Acetylation of **2a** (10 mg) (Ac_2O–$HClO_4$), gave after purification by TLC ($CHCl_3$–Me_2CO, 9:1, 2×), the monoacetate **2b**, mp 200–204° ($CHCl_3$–Et_2O). UV λ_{max}^{MeOH} nm (ε): 237 (17000), 250 (17000), 303 sh (11300), 354 (17100). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1768, 1634, 1517, 1495. EIMS (probe) 70 eV *m/z* (rel. int.): 430 $[M]^+$ (20), 388 $[M-CH_2CO]^+$ (100), 373 $[M-CH_2CO-Me]^+$ (86), 359 $[M-CH_2CO-29]^+$ (19), 342 $[M-CH_2CO-Me-OMe]^+$ (23), 181 $[A_1-Me]^+$ (13), 153 $[A_1-Me-CO]^+$ (31), 43 (32).

Methylation of agehoustin G (**2a**). A soln of **2a** (55 mg) in dry Me_2CO (25 ml), dry K_2CO_3 (250 mg) and 0.5 ml of MeI was refluxed for 10 hr and worked up as usual, to give a crystalline compound identical in all respects with an authentic sample of 5,6,7,2′,4′,5′-hexamethoxyflavone (**2c**) kindly supplied by Dr. J. D. Connolly, University of Glasgow [10].

Alkaline degradation of **2c**. **2c** (30 mg) was treated under the same degradative conditions as described above, followed by usual work-up. The neutral fraction after TLC sepn (Et_2O–petrol, 1:1, 2×), afforded **7a** [7] and a mixture of **5b** [7] and **7c** which were further separated by TLC ($CHCl_3$–Me_2CO, 9:1). 3,4,5-trimethoxyphenol (**7c**). Colourless crystals, mp 124–126°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3280, 1615, 1512, 1498. EIMS (probe) 70 eV *m/z* (rel. int.): 184 $[M]^+$ (76), 169 $[M-Me]^+$ (100), 141 $[M-Me-CO]^+$ (85), 126 $[M-2Me-CO]^+$ (59), 111 $[M-3Me-CO]^+$ (48), 69 (51). The acidic fraction after TLC purification (Et_2O–petrol, 4:1, 3 ×) yielded **5b** [7] and **7b** [2].

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