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OCTASUBSTITUTED FLAVONES FROM *AGERATUM HOUSTONIANUM**

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Key Word Index—*Ageratum houstonianum*, Asteraceae, Eupatorieae, octasubstituted flavones, 3'-hydroxy-5,6,7,8,2',4',5'-heptamethoxyflavone, 5,3'-dihydroxy-6,7,8,2',4',5'-hexamethoxyflavone

Abstract—Two new highly oxygenated flavones, were isolated from aerial parts of *Ageratum houstonianum* Their structures were established as 3'-hydroxy-5,6,7,8,2',4',5'-heptamethoxyflavone and 5,3'-dihydroxy-6,7,8,2',4',5'-hexamethoxyflavone on the basis of spectral data and chemical degradation The structure of the latter compound was confirmed by X-ray analysis

INTRODUCTION

In a previous paper we described the isolation and structure elucidation of agehoustins A (**1c**) and B (**2**), two highly oxygenated flavones from the less polar extract of *Ageratum houstonianum* [1] Now we have undertaken a study of the chloroform extract of *A houstonianum* which resulted in isolation of the flavonoids previously isolated from the petrol extract [1] as well as two new members of the rare group of octasubstituted flavones with a 2',3',4',5'-oxygenation pattern, which we have named agehoustins C (**1a**) and D (**1b**)

RESULTS AND DISCUSSION

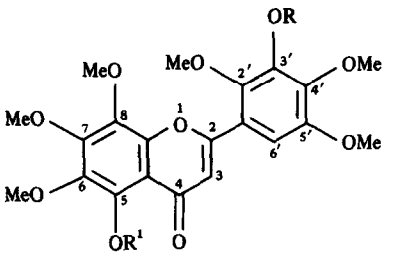
The chloroform extract of the aerial parts of *A houstonianum* afforded a mixture of several flavones previously isolated from the petrol extract [1], as well as two new octasubstituted flavones, agehoustin C (**1a**) and D (**1b**)

Agehoustin C (**1a**), $C_{22}H_{24}O_{10}$ ($[M]^+$ at *m/z* 448), mp 145–146°, was isolated as a pale yellow crystalline compound Both the UV (268, 318 nm) and the IR (3240, 1635 cm^{-1}) absorptions were typical of flavones [2] The ^1H NMR spectrum (Table 1) was similar to that of agehoustin A (**1c**), except that one methoxyl signal was missing and instead it showed a non-chelated hydroxyl signal at δ6 0 Methylation of **1a** with diazomethane afforded agehoustin A (**1c**) indicating that **1a** has the same substitution pattern as **1c** The mass spectral peaks at *m/z* 225 $[A_1 - Me]^+$ and 197 $[A_1 - Me - CO]^+$ indicated that the A-ring was fully methoxylated [1], therefore, the hydroxyl group must be at the B-ring Consistent with this, alkaline hydrolysis of **1a** afforded 2-hydroxy-3,4,5,6-tetramethoxyacetophenone (**3a**) and 2-hydroxy-3,4,5,6-tetramethoxybenzoic acid (**3b**) [1] These fragments resulted from the A-ring In addition, a monohydroxytrimethoxybenzoic acid and a monohydroxytrimethoxyacetophenone were isolated These fragments must have arisen from the B-ring and they are products expected from fission of the β-diketone, **4**, which was also isolated, mainly as the keto–enol form, **5** The remaining problem was the relative position of the hydroxyl group in the B-ring The ^1H NMR data showed the absence of a weak hydrogen-bonded hydroxyl group at the 2'-position, since the hydroxyl proton signal in the ^1H NMR spectrum of **1a** appeared at δ6 0 In contrast, a hydroxyl group at C-2' would give rise to a proton signal at *ca* δ8 0 [3] The 4'-position was ruled out, since the UV spectrum of **1a** on addition of sodium methoxide, indicated the presence of a blocked hydroxyl at C-4' (bathochromic shift of band I of only 14 nm) These facts indicated that the lone hydroxyl was not on C-2' or C-4' On the other hand, benzene-induced shift data suggested the presence of a methoxy group at C-5', since a large upfield shift was observed for one of the seven methoxyl signals [4] similar to that observed for the methoxyl group at C-5' in **1c** and **2** (Table 1) Hence, the hydroxyl group must be attached at C-3' and **1a** is 3'-hydroxy-5,6,7,8,2',4',5'-heptamethoxyflavone and, therefore, the fragments from the B-ring in the alkaline hydrolysis must be 3-hydroxy-2,4,5-trimethoxyacetophenone (**6a**) and 3-hydroxy-2,4,5-trimethoxybenzoic acid (**6b**)

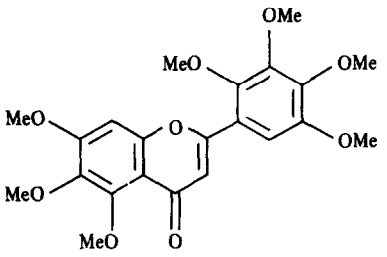
Agehoustin D (**1b**), $C_{21}H_{22}O_{10}$ ($[M]^+$ at *m/z* 434), mp 168–169°, was a bright yellow crystalline dihydroxyhexamethoxyflavone The ^1H NMR data (Table 1), which was very similar to that of agehoustin C (**1a**), showed the following differences from **1a** One methoxy signal was missing and, instead, an extra hydroxyl proton signal appeared at δ12 46, indicating that an additional hydroxyl group must be at C-5 Acetylation of **1b** afforded the corresponding diacetate, **1e**, which showed a C-5 acetyl signal at δ2 46 [4] Hence, **1b** must be 5-*O*-demethylagehoustin C Selective demethylation of **1a** with aqueous hydrochloric acid [5], confirmed the above assumption and established the structural relationship between agehoustins C and D Therefore, agehoustin D must be 3',5-dihydroxy-6,7,8,2',4',5'-hexamethoxyflavone (**1b**)

Alkaline hydrolysis of **1b** furnished the same com-

*Part 4 in the series "Flavonoids from *Ageratum* Species" For Part 3 see ref [1] This is contribution No 713 of the Instituto de Química, UNAM



1a R = H, R^1 = Me
1b R = R^1 = H
1c R = R^1 = Me
1d R = Ac, R^1 = Me
1e R = R^1 = Ac



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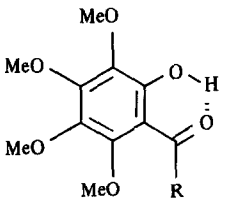
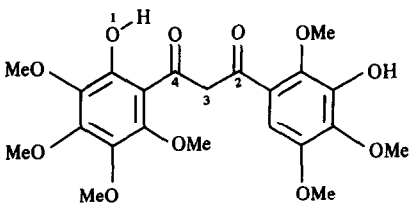


3a R = Me
3b R = OH



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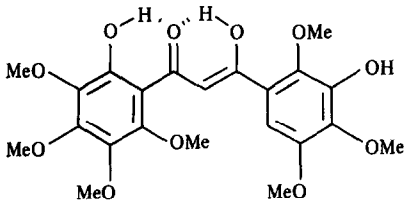

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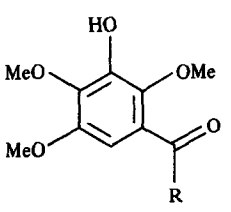
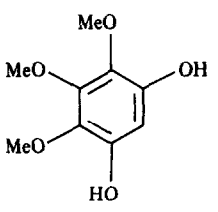


6a R = Me
6b R = OH



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pounds, **6a** and **6b**, from the B-ring as given by **1a** In addition, 4,5,6-trimethoxyresorcinol (**7**) was obtained Compound **7** can be derived from the A-ring by decarboxylation of the corresponding acid

Final confirmation of the structures of **1a** and **1b** was obtained by single crystal X-ray diffraction studies performed on **1b** The molecular structure of **1b**, as determined by the X-ray data, is illustrated in Fig 1

Finally, our results of the alkaline hydrolysis require a comment Kostanecki pointed out that four possible products are expected from fission of the intermediary β-diketone, which can seldom be isolated in the alkaline hydrolysis of flavones [6] Nevertheless, the literature reports reveal that the carbonyl group adjacent to the B-ring (C-2) is, in general, the one chiefly attacked, favouring formation of an *ortho*-hydroacetophenone from the A-ring and a benzoic acid from the B-ring Now, in contrast with the above generality, we have observed that methoxy substitution at C-2′ favours the isolation of the β-diketone (mainly as the keto–enol form) as well as its four possible further degradation products, the two different acetophenones and two different benzoic acids from both rings A and B [1, 7]

EXPERIMENTAL

Aerial parts of *Ageratum houstonianum* Mill were collected *ca* 60 km south of Mexico City at the end of the road to Cuernavaca

Table 1 ^{1}H NMR data of agehoustins A (1c), B (2), C (1a), D (1b) and their acetates 1d and 1e*

	1a		1b		1c	2	1d		1e	
	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6	C_6D_6	C_6D_6	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6
H-3	6 89†	7 20	6 95	7 08	7 13	7 00	6 86	7 07	6 84	6 98
H-8	—	—	—	—	—	6 40	—	—	—	—
H-6'	6 93†	6 84	6 95	6 81	7 06	6 88	7 27	7 07	7 26	6 98
OMe	3 86	3 38	3 85	3 38	3 40	3 17	3 77	3 32	3 75	3 33
	3 90	3 62	3 88	3 60	3 62	3 40	3 91	3 53	3 87	3 46
	3 94	3 65	3 93	3 63	3 65	3 60	3 91	3 66	3 91	3 58
	3 95	3 67	3 93	3 66	3 65	3 67	3 94	3 75	3 91	3 70
	3 98	3 71	3 98	3 82	3 71	3 75	3 94	3 77	3 99	3 75
	3 99	3 74	4 07	3 85	3 72	3 75	3 97	3 82	4 07	3 80
	4 08	4 00			3 74	4 07	4 07	4 05		
					4 00					
OH	6 05	6 43	6 05	5 97	—	—	—	—	—	—
			12 46	13 24						
OAc	—	—	—	—	—	—	2 40	1 90	2 39	1 90
									2 46	2 37

Values are in δ-values all signals are singlets
*At 80 MHz with TMS as int standard
†Values may be reversed

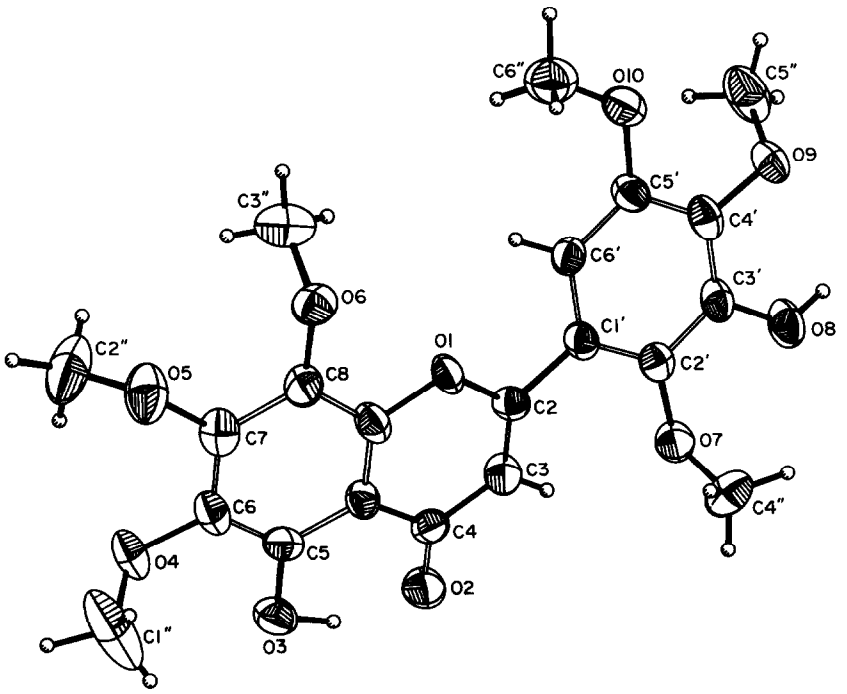

Fig 1 Molecular structure of agehoustin D (1b)

(Edo Morelos, Mexico) The plant material was extracted with $CHCl_3$ as described previously [1, 7] The crude syrup (80 g) obtained was pre-adsorbed on 120 g silica gel (Merck 35–70 mesh) and chromatographed on 500 g silica gel (Merck 70–230 mesh), using petrol and petrol–EtOAc as eluants

Agehoustin C (1a) Fractions 166–180 eluted with petrol–EtOAc mixtures (7 3 and 3 2) were combined and purified by TLC ($CHCl_3$–Me_2CO, 9 1, ×3), yielding 115 mg 1a which was crystallized from $CHCl_3$–Et_2O, mp 145° UV λ_{max}^{MeOH} nm (ε) 268 (25 000), 318 (30 000) IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3520, 1637, 1590, 1510, 1465 EIMS (probe) 70 eV *m/z* (rel int) 448 $[M]^+$ (23), 433 $[M-Me]^+$ (100), 418 $[M-CH_2O]^+$ (6 1), 403 $[M-Me-CH_2O]^+$ (20), 225 $[A_1-Me]^+$ (9 5), 197 $[A_1-Me-CO]^+$ (20), 193 $[B_1-Me]^+$ (6)

Agehoustin D (1b) Purification of fractions 101–110 (eluted with petrol–EtOAc, 17 3) by TLC (Et_2O–petrol, 4 1, ×3) afforded 90 mg 1b, which was crystallized from $CHCl_3$–Et_2O, mp 168–169° UV λ_{max}^{MeOH} nm (ε) 279 (23 600), 324 (19 500)

IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3520, 1655, 1610, 1590, 1576, 1465 EIMS (probe) 70 eV *m/z* (rel int) 434 $[M]^+$ (52), 419 $[M-Me]^+$ (100), 211 $[A_1-Me]^+$ (13), 183 $[A_1-Me-CO]^+$ (13), 193 $[B_1-Me]^+$ (4)

Agehoustin C acetate (**1d**) Acetylation of 27 mg **1a**, with Ac_2O–pyridine, as usual, gave the acetate, **1d**, mp 109–111° ($CHCl_3$–Et_2O) UV λ_{max}^{MeOH} nm (ε) 270 (19300), 324 (16850) IR ν_{max}^{film} cm^{-1} 1770, 1640, 1582, 1498, 1460 EIMS (probe) 70 eV *m/z* (rel int) 490 $[M]^+$ (28), 475 $[M-Me]^+$ (100), 433 $[M-Me-CH_2CO]^+$ (23), 225 $[A_1-Me]^+$ (14), 197 $[A_1-Me-CO]^+$ (32), 43 $[MeCO]^+$ (45)

Methylation of agehoustin C (**1a**) Compound **1a** (23 mg) in dry Me_2CO (25 ml), dry K_2CO_3 (200 mg) and 0 5 ml MeI were refluxed for 4 hr and worked-up as usual Crystallization from $CHCl_3$–Et_2O gave a compound identical in all respects with agehoustin A (**1c**)

Alkaline degradation of agehoustin C (**1a**) A 55 mg sample of **1a** was refluxed with 50% KOH (15 ml) in EtOH (15 ml) under N_2 for 15 hr The reaction mixture was worked-up as described before [1] The neutral fraction after TLC separation (Et_2O–petrol, 7 3, twice) yielded three products The less polar compound was the oily 2-hydroxy-3,4,5,6-tetramethoxyacetophenone (**3a**), identified by NMR and comparison with an authentic sample [1]

The second neutral product crystallized from $CHCl_3$–petrol, mp 98–100° Its spectral data were in accord with 3-hydroxy-2,4,5-trimethoxyacetophenone (**6a**) UV λ_{max}^{MeOH} nm (ε) 218 (24500), 274 (11300), 318 (4000) IR ν_{max}^{film} cm^{-1} 3250, 1655, 1594, 1495, 1466, 1425 EIMS (probe) 70 eV *m/z* (rel int) 226 $[M]^+$ (100), 211 $[M-Me]^+$ (78), 183 $[M-MeCO]^+$ (36), 193 $[M-Me-H_2O]^+$ (17), 179 $[M-Me-MeOH]^+$ (29), 196 $[M-30]^+$ (10), 43 $[MeCO]^+$ (17) ^{1}H NMR (80 MHz, $CDCl_3$) δ2 64 (COMe), 3 86, 3 90, 3 97 (OMe), 5 84 (OH), 6 84 (H-6)

The third neutral product was an oil identified as the tautomeric mixture of **4** and **5** UV λ_{max}^{MeOH} nm (ε) 204 (38000), 285 (9750), 365 (12850) IR ν_{max}^{film} cm^{-1} 3380, 1585, 1560, 1495, 1402 EIMS (probe) 70 eV *m/z* (rel int) 466 $[M]^+$ (10), 435 $[M-OMe]^+$ (17), 241 $[C_{11}H_{13}O_6]^+$ (12), 240 $[C_{12}H_{15}O_6-Me]^+$ (27), 225 $[C_{11}H_{13}O_5]^+$ (18), 211 $[C_{10}H_{11}O_5]^+$ (100) ^{1}H NMR (80 MHz, $CDCl_3$) δ3 80–4 05 (OMe), 5 85 (OH-3'), 7 0 (H-3), 7 65 (H-6'), 12 4 (ϕ_A – OH), 15 74 (OH-2) The following signals at δ4 65 (OC–CH_2–CO), 7 01 (H-6'), 12 90 (ϕ_A – OH), indicated the presence of the β-diketone, **4**

From the acidic fraction after TLC separation (Et_2O–petrol, 17 3, × 3), two benzoic acids were isolated The less polar one was crystallized from $CHCl_3$–petrol, mp 125° and identified as 3-hydroxy-2,4,5-trimethoxybenzoic acid (**6b**) UV λ_{max}^{MeOH} nm (ε) 208 (34200), 256 (8250), 300 (3000) IR ν_{max}^{film} cm^{-1} 3240, 1730, 1605, 1585, 1492, 1431 EIMS (probe) 70 eV *m/z* (rel int) 228 $[M]^+$ (86), 210 $[M-H_2O]^+$ (22), 195 (65), 167 (100), 152 (24), 181 (14) ^{1}H NMR (80 MHz, $CDCl_3$) δ3 88, 4 01, 4 08 (OMe), 5 9 (*br*, COOH), 7 24 (H-6), (C_6D_6) δ3 16, 3 43, 3 44 (OMe), 7 35 (H-6) The more polar acidic product was identified as 2-hydroxy-3,4,5,6-tetramethoxybenzoic acid (**3b**) by ^{1}H NMR and comparison with an authentic sample [1]

Agehoustin D diacetate (**1e**) Acetylation of **1b** (29 mg) with Ac_2O and a drop of $HClO_4$, followed by usual work-up and TLC purification ($CHCl_3$–Me_2CO, 9 1), gave the diacetate **1e**, mp 140–142° UV λ_{max}^{MeOH} nm (ε) 265 (38700), 322 (38300) IR ν_{max}^{film} cm^{-1} 1775, 1645, 1607, 1570, 1503, 1465 EIMS (probe) 70 eV *m/z* (rel int) 518 $[M]^+$ (8), 476 $[M-CH_2CO]^+$ (100), 461 $[M-Me-CH_2CO]^+$ (84), 419 $[M-Me-2CH_2CO]^+$ (41), 211 $[A_1-Me]^+$ (16), 183 $[A_1-Me-CO]^+$ (19), 193 $[B_1-Me]^+$ (7)

Alkaline degradation of agehoustin D (**1b**) A 50 mg sample of **1b**, treated under the same degradative conditions as described above, afforded the same acetophenone (**6a**) and benzoic acid (**6b**) as obtained from **1a** In addition, 4,5,6-trimethoxyresorcinol (**7**) was also isolated UV λ_{max}^{MeOH} nm (ε) 202 (31100), 233 (3180) IR ν_{max}^{film} cm^{-1} 3400, 1606, 1485, 1425 EIMS (probe) 70 eV *m/z* (rel int) 200 $[M]^+$ (63), 185 $[M-Me]^+$ (100), 157 $[M-43]^+$ (20), 142 $[M-68]^+$ (37) ^{1}H NMR (80 MHz, $CDCl_3$) δ 3 85 (6H), 3 93 (3H) (OMe), 5 49 (2H) (OH), 6 32 (H-2)

Selective demethylation of agehoustin C (**1a**) A mixture of **1a** (5 mg), conc HCl (0 5 ml) and conc HOAc (0 5 ml) was heated on a steam bath for 12 hr, diluted with H_2O and extracted with EtOAc The EtOAc soln was washed with H_2O, dried and evaporated The residue was purified by TLC ($CHCl_3$–Me_2CO, 9 1, twice) giving a crystalline compound identified as agehoustin D (**1b**) by ^{1}H NMR and comparison with an authentic sample

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