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TWO POLYMETHOXYFLAVONES FROM *AGERATUM HOUSTONIANUM**

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Key Word Index—*Ageratum houstonianum*; Asteraceae; Eupatorieae; new octasubstituted and heptasubstituted flavones.

Abstract—Besides agecorynin C, eupalestin and lucidin dimethyl ether, two new highly oxygenated flavones were isolated from *Ageratum houstonianum*. Their structures were established by spectroscopic and degradative evidence as 5, 6, 7, 8, 2′, 3′, 4′, 5′-octamethoxyflavone and 5, 6, 7, 2′, 3′, 4′, 5′-heptamethoxyflavone.

INTRODUCTION

In previous papers we have published the isolation and structure elucidation of several flavonoids from *Ageratum corymbosum* and *A. strictum* [1, 2]. Now we report the results of our study of *Ageratum houstonianum* Mill. which resulted in the isolation of the already known lucidin dimethyl ether (**2a**) [3] eupalestin (**2b**) and agecorynin C (**3**) [1] as well as two new highly oxygenated flavones with a novel substitution pattern in the B-ring, named agehoustin A (**1a**) and B (**1b**). To our knowledge this is the first report of flavones with the 2′, 3′, 4′, 5′-oxygenation pattern and the second of flavones with a tetra-*O*-substituted B-ring [4]. Agehoustin A (**1a**) is the first octamethoxyflavone from the Asteraceae and the third compound of this type yet reported from nature. Exoticin (3,5,6,7,8,3′,4′,5′-octamethoxyflavone) [5] and purpurascenin (3,5,6,7,8,2′,4′,5′-octamethoxyflavone) [6] are the other two octamethoxyflavonoids isolated so far. Some other octasubstituted flavonoids, with the same substitution pattern as exoticin have been also isolated [7].

RESULTS AND DISCUSSION

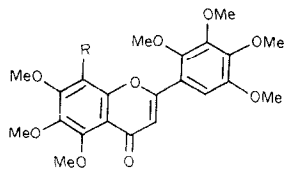
The petrol extract of the aerial parts of *Ageratum houstonianum* afforded a mixture of several flavones. Three have been isolated before: lucidin dimethyl ether (**2a**) from *Lindera lucida* [3], eupalestin (**2b**) and agecorynin C (**3**) from *Ageratum corymbosum* [1]. In addition two new fully methylated and highly oxygenated flavones, agehoustin A (**1a**) and B (**1b**), were isolated.

Agehoustin A (**1a**), $C_{23}H_{26}O_{10}$, was the less polar flavone isolated from the petrol extract, mp 116–118°. Both the UV (268, 318 nm) and the IR (1636, 1580 cm^{-1}) absorptions were typical of non-phenolic flavones [8]. The ^1H NMR spectrum of **1a** clearly indicated it to be an octasubstituted flavonoid, since it showed two singlets (1H each) at δ 6.88 and 7.13 indicating the presence of only two flavone-nucleus protons. Six sharp singlets at 3.89 (3H), 3.91 (3H), 3.95 (9H), 3.98 (3H), 3.99 (3H), 4.09 (3H), indicated the presence of eight methoxy groups. The mass spectrum of agehoustin A (**1a**) confirmed the assumption that it is an octamethoxyflavone, since the molecular ion peak was observed at *m/z* 462 and a base peak at 447 due to $[M-Me]^+$. Other spectral peaks at *m/z* 225 $[A_1-Me]^+$, 197 $[A_1-Me-CO]^+$ due to A-ring fragmentation [9], suggested that, as in exoticin [5] and purpurascenin [6], agehoustin A (**1a**) must have all A-ring positions substituted. Therefore the remaining four methoxyl groups can be placed on the B-ring since the ^1H NMR displayed two singlet flavone-nucleus protons. In addition, since the ^1H NMR spectrum of **1a** is different from that of purpurascenin (3, 5, 6, 7, 8, 2′, 4′, 5′-octamethoxyflavone) [6], it must have the proposed structure **1a**. Final confirmation of structure **1a** was achieved by alkaline degradation which furnished 2-hydroxy-3, 4, 5, 6-tetramethoxyacetophenone (**4a**) [1] and 2, 3, 4, 5-tetramethoxybenzoic acid (**5a**) identified by its mp 82–84° [10], IR, NMR and mass spectra. Hence agehoustin A is 5, 6, 7, 8, 2′, 3′, 4′, 5′-octamethoxyflavone (**1a**).

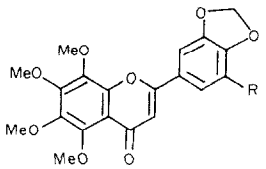
As in the case of agecorynin C (**3**) [1], additional degradation products were isolated and identified as the keto-enol **6a** and 2, 3, 4, 5-tetramethoxyacetophenone (**4b**) and 2-hydroxy-3, 4, 5, 6-tetramethoxybenzoic acid (**5b**). **4b** and **5b** can be explained as further degradation products of **6a** (or its corresponding β-diketone).

A second new flavone, agehoustin B (**1b**), $C_{22}H_{24}O_9$, mp 85–86°, was isolated from the petrol extract. The ^1H NMR of **1b** was very similar to that of agecorynin C (**3**) [1]. It showed three singlets (1H each) at δ 6.70, 6.74 and 6.94 due to three flavone nucleus protons and five sharp singlets between 3.8 and 4.0 due to seven methoxy groups. Hence **1b** must be a new heptamethoxylated flavone whose structure can be

*Part 3 in the series "Flavonoids from *Ageratum* Species". For Part 2 see ref. [2]. This is contribution No. 604 of the Instituto de Química, U.N.A.M.



1a R = OMe
1b R = H



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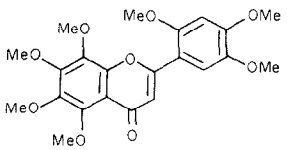

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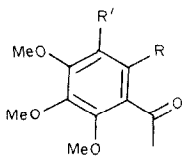
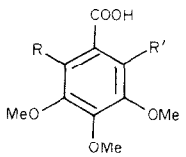
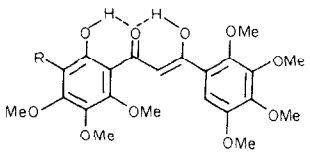


4a R = OH, R′ = OMe
4b R = H, R′ = OMe
4c R = OH, R′ = H



5a R = H, R′ = OMe
5b R = OH, R′ = OMe



6a R = OMe
6b R = H

represented as 5,6,7,2′,3′,4′,5′-heptamethoxyflavone (**1b**). The mass spectrum was in agreement with this assumption, the molecular ion peak being observed at *m/z* 432 and the base peak at 417 due to $[M-Me]^+$ and other significant fragmentation peaks at *m/z* 195 $[A_1-Me]^+$, 167 $[A_1-Me-CO]^+$ which suggested the trisubstitution of the A-ring [9]. Alkaline degradation of agehoustin B (**1b**) gave the same 2,3,4,5-tetramethoxybenzoic acid (**5a**) as in the case of **1a**. The neutral product was identified as 6-hydroxy-2,3,4-trimethoxyacetophenone (**4c**) by comparison with an authentic sample previously obtained from agecorynin A [1]. Therefore the structure of agehoustin B corresponds to 5,6,7,2′,3′,4′,5′-heptamethoxyflavone (**1b**).

As in the case of **1a** three additional degradation products were also isolated and identified as the same acetophenone **4b** obtained from **1a** and 6-hydroxy-2,3,4-trimethoxybenzoic acid [11], both further degradation products of **6b** (or its corresponding β-diketone) which was also identified.

EXPERIMENTAL

Ageratum houstonianum Mill. was collected in October 1980, in México: Morelos, road to Cuernavaca, *ca* 60 km south of México City. A voucher, Quijano 40, is on deposit at the Herbarium of Instituto de Biología (UNAM), México.

The air-dried plant material, leaves and flowers (2.9 kg), were extracted with petrol as described before [1], giving 45 g crude syrup which was chromatographed over 300 g Si gel, using petrol–$CHCl_3$ and $CHCl_3$–Me_2CO mixtures as eluants. Fractions eluted with $CHCl_3$–Me_2CO (9 : 1) gave a mixture of several flavonoids. Crystallization from $CHCl_3$–Et_2O gave a mixture of lucidin dimethyl ether (**2a**) and a new octamethoxyflavone (**1a**). The mother liquor residue (14 g) was rechromatographed over Si gel (200 g) and gave on elution with petrol–EtOAc (4 : 1) further quantities of **1a** and lucidin dimethyl ether (**2a**), mp 166–168° (lit. [3] 163–168°). Further elution with petrol–EtOAc (7 : 3) gave eupalestin (**2b**) and a new heptamethoxyflavone (**1b**). Later fractions eluted with petrol–EtOAc (3 : 7) afforded agecorynin C (**3**). Eupalestin (**2a**) and agecorynin C (**3**) were identified by comparison with authentic samples previously isolated from *Ageratum corymbosum* [1].

Agehoustin A (**1a**). Earlier fractions eluted with petrol–EtOAc (4 : 1) from the rechromatography gave 600 mg **1a** as needles from $CHCl_3$–Et_2O, mp 116–117°. UV λ_{max}^{MeOH} nm (ε): 268 (20 970), 318 (18 980). IR ν_{max}^{film} cm^{-1}: 1636, 1580, 1560, 1490, 1460, 1410, 1400. ^1H NMR (80 MHz, $CDCl_3$): δ 3.89 (3H, *s*), 3.91 (3H, *s*), 3.95 (9H, *s*), 3.98 (3H, *s*), 3.99 (3H, *s*), 4.09 (3H, *s*) (8 × OMe); 6.88 (1H, *s*, H-3), 7.13 (1H, *s*, H-6′). EIMS (probe) 70 eV *m/z* (rel. int.): 462 $[M]^+$ (19.5), 447 $[M-Me]^+$ (100), 417 $[M-Me-CH_2O]^+$ (19.9), 389 $[M-Me-CH_2O-CO]^+$ (6.09), 225 $[A_1-Me]^+$ (4.8), 197 $[A_1-Me-CO]^+$ (9.7), 222 $[B_1]^+$ (2.4), 207 $[B_1-Me]^+$ (3.6). (Found: C, 59.61; H, 5.71; O, 34.60. $C_{23}H_{26}O_{10}$ requires: C, 59.73; H, 5.67; O, 34.60%.)

Agehoustin B (**1b**). Rechromatography fractions eluted with petrol–EtOAc (13 : 7) gave 300 mg **1b** as crystals from $CHCl_3$–Et_2O, mp 85–86°. UV λ_{max}^{MeOH} nm (ε): 237 sh (22 500), 265 sh (13 050), 313 (17 500). IR ν_{max}^{film} cm^{-1}: 1732, 1595, 1485, 1410, 1400. ^1H NMR (80 MHz, $CDCl_3$): δ 3.86 (3H, *s*), 3.91

(6H, *s*), 3.96 (3H, *s*), 3.97 (6H, *s*), 3.99 (3H, *s*) (7×OMe), 6.70 (1H, *s*, H-3 or H-8), 6.75 (1H, *s*, H-3 or H-8), 6.94 (1H, *s*, H-6'). EIMS (probe) 70 eV *m/z* (rel. int.): 432 $[M]^+$ (18.5), 417 $[M-Me]^+$ (100), 401 $[M-MeO]^+$ (48.4), 387 $[M-Me-CH_2O]^+$ (33.8), 195$[A_1-Me]^+$ (6.5), 167 $[A_1-Me-CO]^+$ (14.6), 207 $[B_1-Me]^+$ (3.6). (Found: C, 60.64; H, 5.68; O, 33.50. $C_{22}H_{24}O_9$ requires: C, 61.10; H, 5.59; O, 33.30.)

Alkaline degradation of agehoustin A (**1a**). A 200 mg sample of **1a** was refluxed with 50% KOH (25 ml) in EtOH (10 ml) under N_2 for 72 hr. The reaction mixture was cooled, acidified with dil. HCl and extracted with EtOAc. The EtOAc extract was washed with 10% $NaHCO_3$, then H_2O, dried, concd *in vacuo* and the residue purified by prep. TLC to afford the oily 2-hydroxy-3, 4, 5, 6-tetramethoxyacetophenone (**4a**) identified by comparison with a sample previously obtained from eupalestin [1]. The more polar compound was spectroscopically identified as 2, 3, 4, 5-tetramethoxyacetophenone (**4b**). UV λ_{max}^{MeOH} nm (ϵ): 215 (15600), 265 (6717), 310 (2934). IR ν_{max}^{film} cm^{-1}: 1670, 1585, 1480, 1460, 1400. EIMS (probe) 70 eV *m/z* (rel. int.): 240 $[M]^+$ (20.1), 225 $[M-Me]^+$ (41.6), 197 $[M-MeCO]^+$ (8.5), 43 $[MeCO]^+$ (100). ^1H NMR (80 MHz, $CDCl_3$): δ 2.58 (3H, *s*, COMe), 3.83, 3.84, 3.86, 3.93 (4×3H, *s*, 4×OMe), 7.02 (1H, *s*, H-6).

The $NaHCO_3$ soln was acidified with HCl, extracted with EtOAc, washed with H_2O, dried, concd *in vacuo* and purified by prep. TLC to give 2, 3, 4, 5-tetramethoxybenzoic acid (**5a**), mp 82–84° (lit. [10], 87–88°). UV λ_{max}^{MeOH} nm (ϵ): 206 (35417), 246 sh (4600), 285 (2016). IR ν_{max}^{KBr} cm^{-1}: 2600–3300, 1695, 1675, 1595, 1570. EIMS (probe) 70 eV *m/z* (rel. int.): 242 $[M]^+$ (100), 227 $[M-Me]^+$ (53.9), 184 $[M-58]^+$ (60.1). ^1H NMR (80 MHz, $CDCl_3$): δ 3.87, 3.90, 3.95, 4.05 (4×3H, *s*, 4×OMe), 7.37 (1H, *s*, H-6), 11.9 (1H, *br*, COOH).

A second acidic product was isolated and identified as 2-hydroxy-3, 4, 5, 6-tetramethoxybenzoic acid (**5b**). IR ν_{max}^{film} cm^{-1}: 2600–3400, 1700, 1450, 1420, 1380. EIMS (probe) 70 eV *m/z* (rel. int.): 258 $[M]^+$ (32.4), 240 $[M-H_2O]^+$ (100), 225 $[240-Me]^+$ (96.8), 197 $[225-CO]^+$ (86.9), 182 $[197-Me]^+$ (31.7). ^1H NMR (80 MHz, $CDCl_3$): δ 3.80, 3.86, 4.04, 4.09 (4×3H, *s*, 4×OMe).

Keto-enol **6a**. A 100 mg sample of **1a**, refluxed with 50% KOH (20 ml) in EtOH (10 ml) under N_2 for 4 hr gave the keto-enol **6a** as main product. Mp 100–102°. UV λ_{max}^{MeOH} nm (ϵ): 205 (37600), 282 (10266), 373 (15600). IR ν_{max}^{film} cm^{-1}: 2500–3500, 1600 sh, 1580, 1555. EIMS (probe) 70 eV *m/z* (rel. int.): 480 $[M]^+$ (7.3), 449 $[M-31]^+$ (20.6), 241 $[C_{11}H_{13}O_6]^+$ (6.3), 240 $[C_{11}H_{12}O_6]^+$ (9.7), 225 $[C_{11}H_{13}O_5]^+$ (100). ^1H NMR (80 MHz, $CDCl_3$): δ 3.82 (3H, *s*), 3.88 (9H, *s*), 3.90 (3H, *s*), 3.93 (3H, *s*), 3.95 (3H, *s*), 4.10 (3H, *s*, 8×OMe); 7.18 (1H, *s*, H-3), 7.66 (1H, *s*, H-6), 12.35 (1H, *s*, ϕ_A–OH), 15.81 (1H, *s*, C_2–OH).

Alkaline degradation of agehoustin B (**1b**). A 200 mg sample of **1b** was treated and worked-up as described above. The neutral material after TLC purification afforded 6-hydroxy-3, 4, 5-trimethoxyacetophenone (**4c**), identical with an authentic sample obtained from agecorynin A [1] and the same acetophenone **4b** obtained from **1a**. A third neutral compound was isolated and identified as the keto-enol **6b**, mp 93–94°. UV λ_{max}^{MeOH} nm (ϵ): 206 (33750), 281 (12421), 333 (10078), 385 (13664). IR ν_{max}^{film} cm^{-1}: 2500–3400, 1600, 1560. EIMS (probe) 70 eV *m/z* (rel. int.): 450 $[M]^+$ (17.5), 419 $[M-MeO]^+$ (26.1), 225 $[C_{11}H_{13}O_5]^+$ (100), 211 $[C_{10}H_{11}O_5]^+$ (11.0). ^1H NMR (80 MHz, $CDCl_3$): δ 3.78 (3H, *s*), 3.87 (12H, *s*), 3.94 (3H, *s*), 3.95 (3H, *s*) (7×OMe), 6.25 (1H, *s*, H-8), 7.17 (1H, *s*, H-3), 7.65 (1H, *s*, H-6'), 12.74 (1H, *s*, ϕ_A–OH), 15.75 (1H, *s*, C_2–OH).

The acidic material after TLC purification gave the same tetramethoxybenzoic acid **5a** obtained from **1a** and 6-hydroxy-2, 3, 4-trimethoxybenzoic acid, mp 107–110° (lit. [11], 113–115°). IR ν_{max}^{film} cm^{-1}: 2500–3500, 1630, 1460, 1420, 1370, 1260, 1090. EIMS (probe) 70 eV *m/z* (rel. int.): 228 $[M]^+$ (26.8), 210 $[M-H_2O]^+$ (87.8), 195 [210–Me]$^+$ (86.9), 167 [195–CO]$^+$ (100), 94 (29.2), 69 (40.7). ^1H NMR (80 MHz, $CDCl_3$): δ 3.78, 3.87, 4.14 (3×3H, *s*, 3×OMe), 6.32 (1H, *s*, H-3), 11.94 (2H, *s*, COOH and OH).

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