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HIGHLY OXYGENATED FLAVONOIDS FROM *AGERATUM CORYMBOSUM**

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Key Word Index—*Ageratum corymbosum*; Compositae; Eupatorieae; new flavanones; new flavones.

Abstract—The investigation of *Ageratum corymbosum* resulted in the isolation of four new highly oxygenated flavonoids, and their structures established by spectroscopic and degradative evidence as 5,6,7,5′-tetramethoxy-3′,4′-methylenedioxyflavanone; 5,6,7,8,5′-pentamethoxy-3′,4′-methylenedioxyflavanone; 5,6,7,8,2′,4′,5′-heptamethoxyflavone and 5,2′,4′-trihydroxy-6,7,8,5′-tetramethoxyflavone. The recently reported gardenin A monomethyl ether and 5′-methoxylucidin dimethyl ether (eupalestin) were also isolated.

INTRODUCTION

Ageratum is a genus botanically and chemically closely related to *Eupatorium*, a genus which has been shown to produce sesquiterpene lactones and flavonoids [1], a number of them with cytotoxic and antitumor activity [2]. In continuation of our chemical systematic study of plants of the tribe Eupatorieae (Compositae), we have undertaken the study of *Ageratum corymbosum* Zucc. and isolated coumarin, taraxasterol, sitosterol, stigmasterol and several new, highly oxygenated flavonoids whose structures were established by spectroscopic and chemical evidence.

RESULTS AND DISCUSSION

From the aerial parts of *Ageratum corymbosum*, six flavonoids were isolated by column chromatography on Si gel. Compound **1a**, which had been obtained as a precipitate on initial concentration of the petrol extract, was also isolated by chromatography and its structure determined by spectroscopic methods and by chemical degradation to the corresponding 2-hydroxy-3,4,5,6-tetramethoxyacetophenone (**5**) and 3-methoxy-4,5-methylenedioxybenzoic acid. Compound **1b** was obtained in a mixture of three compounds which could be separated only after repeated TLC. It was shown to be the methyl ether of gardenin A (**1c**) [3], based on spectroscopic data and alkaline hydrolysis. As in the case of **1a**, the same acetophenone **5** and 3,4,5-trimethoxybenzoic acid were obtained.

During the preparation of this paper, the isolation of the above-described flavones **1a** and **1b** from *Ageratum conyzoides* and *Eupatorium coelestinum* [4, 5] was published. All IR, UV, NMR, MS data for the flavones and the degradation products were identical with the reported data.

In addition to the above-mentioned flavones **1a** and **1b**, four new flavonoids which we named agecorynin A, B, C and D were isolated. Agecorynin A (**2a**), $C_{20}H_{20}O_8$, was the less polar flavonoid isolated from the petrol extract, mp 174–176°, $[\alpha]_D$ − 11.2°. Both the UV (276, 323 nm) and the IR (1680, 1520 cm^{-1}) absorptions were typical of non-phenolic flavanones [6]. The ^1H NMR spectrum (Table 1) was similar to that of **1a**, but in addition agecorynin A (**2a**) showed an ABX system with signals centred at δ 2.70, 2.98 and 5.26 due to H-2 and H-3 protons, which confirmed the flavanone nucleus. The ^1H NMR spectrum also indicated the presence of four methoxy groups with resonances at 3.80 (3 H), 3.86 (3 H) and 3.92 (6 H). Two further singlets at 6.32 (1 H) and 5.96 (2 H) could be assigned either to H-6 or H-8 and the 3′,4′-methylenedioxy protons, respectively. Finally a broad two-proton singlet at 6.62 was assigned to H-2′ and H-6′. The MS of agecorynin A (**2a**) was in agreement with the proposed structure, the molecular ion peak was observed at *m/e* 388 ($C_{20}H_{20}O_8$) and other significant fragmentation peaks were at *m/e* 210 ($C_{10}H_{10}O_5$), 195 ($C_{10}H_{10}O_5$ − Me) and 167 ($C_{10}H_{10}O_5$ − Me − CO) due to the A-ring and at *m/e* 178 ($C_{10}H_{10}O_3$) due to the B-ring.

Final confirmation of structure **2a** was achieved by alkaline hydrolysis which furnished two neutral products. The more polar one was identified as 3-methoxy-4,5-methylene-dioxybenzaldehyde, mp 128–130° [7]. The less polar degradation product was the 2-hydroxy-4,5,6-trimethoxyacetophenone (**6**) [8]. Therefore, the structure of agecorynin A corresponds to 5,6,7,5′-tetramethoxy-3′,4′-methylenedioxyflavanone (**2a**).

Purification of the mother liquors of **2a** by TLC afforded a second new flavanone, agecorynin B (**2b**), mp 135–137°. Although this compound was a flavanone, it showed an optical rotation value of zero. The UV and IR spectra were very similar to those of **2a**. The ^1H NMR spectrum (Table 1) was almost identical, but lacked the H-8 singlet and showed an extra methoxy group signal indicating that the A-ring in agecorynin B was fully

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Table 1. ^{1}H NMR spectral data of agecorynin A (**2a**), B (**2b**), C (**3a**), and D (**3b**)*

	2a	2b	3a	3b
H-2	5.26 *dd*† (4.83)	5.30 *dd*† (4.75)		
H-3	2.70 *dd*‡ (2.4–2.8 *m*)	2.7–3.2 *m* (2.4–2.7)	6.58 (6.17)	6.59
	2.98 *dd*§			
H-8	6.32 (6.08)			
H-2′	6.62 *br* (6.40 *d*‖)	6.61 *br* (6.37 *d*‖)		
H-3′			7.04 (7.38)	7.15
H-6′	6.62 *br* (6.51 *d*‖)	6.61 *br* (6.50 *d*‖)	7.54 (7.62)	7.42
OMe	3.80 (3.18)	3.82 (3.45)	3.96 (3.27)	3.82
	3.86 (3.47)	(6 H) (3.64)	3.97 (3.40)	3.84
	3.92 (3.70)	3.86 (3.70)	(9 H) (3.60)	3.94
	(6 H) (3.98)	3.90 (3.76)	3.98 (3.70)	4.03
		4.03 (3.98)	4.03 (3.77)	
			4.11 (3.81)	
			(4.04)	
O–CH_2–O	5.96 (5.31)	5.95 (5.28)		

*Run at 100 MHz in $CDCl_3$ or C_6D_6 with TMS as internal standard; **3b** was run in polysol. Values are in ppm (δ). Values in parentheses are chemical shifts in C_6D_6.
†J = 4, 12 Hz.
‡J = 4, 16 Hz.
§J = 12, 16 Hz.
‖J = 2 Hz.

substituted. The MS supported structure **2b** since it showed a molecular ion peak at *m/e* 418 ($C_{21}H_{22}O_9$) and fragmentation peaks due to the the A-ring at *m/e* 240 ($C_{11}H_{12}O_6$), 225 ($C_{11}H_{12}O_6$ – Me), 197 ($C_{11}H_{12}O_6$ – Me – CO) and due to the B-ring at *m/e* 178 ($C_{10}H_{10}O_3$).

A third yellow compound, agecorynin C (**3a**), was isolated from later fractions of the column chromatography only after TLC, mp 158–160°. The UV spectrum showed absorption at 256, 267, 361 nm and IR absorption bands at 1640, 1570, 1520 cm^{-1} typical of flavones [6]. The MS of agecorynin C (**3a**) was very similar to that of gardenin A monomethyl ether (**1b**) with a molecular ion peak at *m/e* 432 ($C_{22}H_{24}O_9$) and a base peak at 417 (M – Me) characteristic of 6-methoxyflavones [9], and other spectral peaks at *m/e* 225, 197, 192, which suggested that as in **1b**, agecorynin C should have the same substitution of the A-ring. Therefore the difference should be in the substitution of the B-ring of the flavone nucleus. Since the ^{1}H NMR spectrum of **3a** exhibited the presence of seven methoxy groups as in **1b**, but three different singlet proton absorptions at δ 6.58, 7.04 and 7.54, the structure of agecorynin C can be represented by **3a**.

Confirmation of the structure was achieved by alkaline hydrolysis to give the same acetophenone (**5**) as given by **1a** and **1b**, and 2,4,5-trimethoxybenzoic acid or asaronic acid, identified by mp, IR and ^{1}H NMR [10, 11]. In addition to the above-mentioned acetophenone and asaronic acid, two additional degradation products were isolated and identified, the β-diketone **4**, mainly as the keto–enol form and the 2,4,5-trimethoxyacetophenone (**7**). The latter compound can be explained as a further degradation product of the β-diketone **4**.

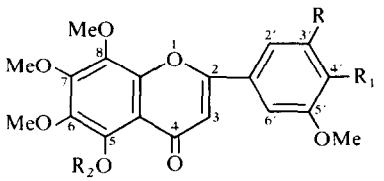
Chromatography of the chloroform extract afforded another new flavone as a crystalline yellow compound which we named agecorynin D (**3b**), $C_{19}H_{18}O_9$, mp 258–260°. Both the UV (273, 380 nm) and the IR (2500–3600, 1650 cm^{-1}) absorptions were typical of phenolic flavones [6]. The ^{1}H NMR spectrum of agecorynin D (**3b**) indicated the presence of a C-5 hydrogen-bonded OH group at δ 13.8 and three flavone nucleus proton singlets at 6.59, 7.15 and 7.42, which suggested that agecorynin D should have the same substitution pattern as agecorynin C (**3a**). Methylation of **3b** produced the fully methoxylated derivative which was identical in all respects with agecorynin C (**3a**), thus establishing the substitution pattern. Since the ^{1}H NMR spectrum of agecorynin D indicated the presence of four methoxy groups (δ 3.82, 3.84, 3.94, 4.03), the remaining substituents should be three hydroxyl groups, one of them already having been placed at C-5. Acetylation of agecorynin D afforded the triacetate **3c**, confirming the presence of three hydroxyl groups, one of them at the C-5 position (δ 2.46) [12].

The MS of **3b** showed a molecular ion peak at *m/e* 390 and a base peak at *m/e* 375 (M – Me), indicating the presence of a C-6 methoxy group [9], and peaks at *m/e* 211 ($C_9H_7O_6$), 183 ($C_9H_7O_6$ – CO), which suggested that the A-ring was fully substituted with three methoxy groups and a hydroxy group. Therefore two hydroxy groups and one methoxy group should be placed at the B-ring (MS peak at *m/e* 164 ($C_9H_8O_3$, 6.6%)). The relative position of these substituents was determined by the shifts observed in the UV absorption bands produced with diagnostic reagents (NaOMe, $AlCl_3$/HCl and $NaOAc/H_3BO_3$) and which indicated the presence of a C-4′ hydroxy group but the absence of *ortho*-dihydroxyl groups [6].

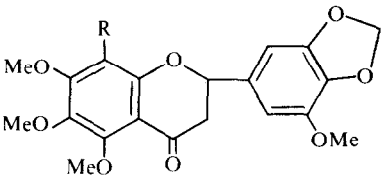
Based on the above data, we propose **3b** as the more likely structure for agecorynin D.

EXPERIMENTAL

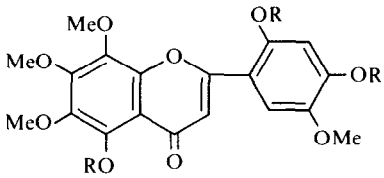
Ageratum corymbosum Zucc. was collected in Mexico City at U.N.A.M. in October 1978. A voucher, Calderon 24, has been



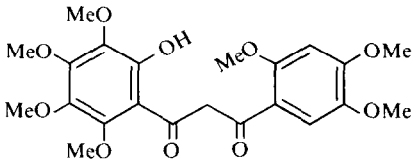
1a R.R$_1$ = O – CH$_2$ – O, R$_2$ = Me
1b R = R$_1$ = OMe, R$_2$ = Me
1c R = R$_1$ = OMe, R$_2$ = H



2a R = H
2b R = OMe



3a R = Me
3b R = H
3c R = Ac



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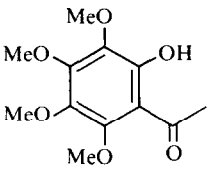

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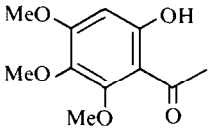

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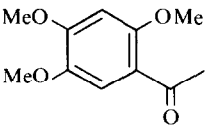

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deposited at the Herbarium of the Instituto de Biologia (UNAM), México.

Dried leaves and flowers (1.5 kg) were extracted first with petrol (twice), and then with $CHCl_3$. The petrol extract, after elimination of the solvent, was dissolved in 1 l. MeOH, cooled, filtered, and concd *in vacuo*, yielding 23 g of crude syrup. The $CHCl_3$ extract prepared as described before yielded 29 g of crude syrup. Both extracts were chromatographed separately on Si gel (1 kg), using petrol–$CHCl_3$ and $CHCl_3$–EtOAc as eluants.

Eupalestin (**1a**). Compound **1a** was obtained by crystallization from the crude syrup of the petrol extract (500 mg), mp 187–188° (lit. [5] 185°). (Found: C, 60.27; H, 4.82; O, 34.40. $C_{21}H_{20}O_9$ requires: C, 60.57; H, 4.84; O, 34.58%). The UV, IR, ^{1}H NMR and MS were identical with the reported data [5]. Compound **1a** was also isolated by CC (500 mg) of the petrol extract.

Alkaline degradation of **1a**. Compound **1a** (200 mg) was refluxed with 50% KOH (30 ml) in MeOH (7 ml) under N_2 for 9 hr. The reaction mixture was cooled, acidified with HCl and extracted with EtOAc. The EtOAc extract was washed with 10% $NaHCO_3$, then H_2O, dried (Na_2SO_4) and distilled *in vacuo* to afford a bright yellow oil identified as 2-hydroxy-3,4,5,6-tetramethoxyacetophenone (**5**) (164 mg) [5, 13]. The aq. phase was acidified with HCl, extracted with EtOAc (3 ×), washed with H_2O, dried (Na_2SO_4) and concd *in vacuo* to give 3-methoxy-4,5-methylenedioxybenzoic acid (20 mg), mp 212–214° (lit. [14] 210°).

Gardenin A methyl ether (**1b**). Further TLC (pentane–Et_2O–EtOAc, 2:2:1, 3 ×) of latter chromatography fractions eluted with $CHCl_3$, and earlier fractions eluted with $CHCl_3$–EtOAc (95:5) afforded 280 mg of gardenin A methyl ether (**1b**), mp 95–97° (lit. [3] 102°). The UV, IR, ^{1}H NMR and MS were completely in agreement with the reported data [5].

Alkaline degradation of **1b**. The alkaline degradation of 100 mg **1b**, under the same conditions as described above, afforded the same acetophenone (**5**), and 3,4,5-trimethoxybenzoic acid identical with an authentic sample.

Agecorynin A (**2a**). Chromatography fractions eluted with petrol–$CHCl_3$ (2:8) afforded **2a** (110 mg), mp 174–176° from Me_2CO–petrol, $[\alpha]_D$ – 11.2° ($CHCl_3$). IR ν_{max} cm^{-1}: 1680, 1605, 1500. UV λ_{max}^{EtOH} nm (ε): 276 (20 300), 323 (5456). MS *m/e* (rel. int.): 388 (M^+, 45.2), 210 ($C_{10}H_{10}O_5$, 100.0), 195 ($C_{10}H_{20}O_5$ – Me, 92.3), 178 ($C_{10}H_{10}O_3$, 51.9), 167 (195 – CO, 63.4).

Alkaline degradation of **2a**. Alkaline degradation of **2a**, under the same conditions as mentioned before, yielded two neutral products, which were separated by TLC (pentane–Me_2CO, 4:1, twice). The less polar compound was an oil identified as 3-methoxy-4,5-methylenedioxybenzaldehyde, mp 128–130° (lit. [7] 131–132°), IR ν_{max} cm^{-1}: 2920, 2825, 1688, 1620. ^{1}H NMR ($CDCl_3$): δ 3.94 (*s*, 3 H), 6.06 (*s*, 2 H), 7.02 (*d*, *J* = 2 Hz, 1 H), 7.1 (*d*, *J* = 2 Hz, 1 H), 9.74 (*s*, 1 H). MS *m/e* (rel. int.): 180 (M^+, 100.0), 179 (M^+ – H, 89.0), 165 (M^+ – Me, 9.7), 149 (M^+ – OMe, 36.5). The more polar degradation product was the oily 2-hydroxy-4,5,6-trimethoxyacetophenone (**6**). The UV, IR, ^{1}H NMR and MS were in agreement with the reported data [8].

Agecorynin B (**2b**). Compound **2b** (15 mg) was isolated from the mother liquor of **2a** by TLC (pentane–Me_2CO, 3:1), mp 135–137° from Me_2CO–petrol, $[\alpha]_D$ 0° ($CHCl_3$): IR ν_{max} cm^{-1}: 1680, 1608, 1540. UV λ_{max}^{EtOH} nm (ε): 277 (18 548), 332 (5094). MS *m/e* (rel. int.): 418 (M^+, 36.0), 388 (M – 30, 13.0), 240 ($C_{11}H_{12}O_6$, 100.0), 225 ($C_{11}H_{12}O_6$ – Me, 76.0), 178 ($C_{10}H_{10}O_3$, 19.0). The ^{1}H NMR spectrum (Table 1) indicated that agecorynin B (**2b**) still contained a small amount of **1b**, which could not be separated satisfactorily.

Agecorynin C (**3a**). Chromatography fractions eluted with $CHCl_3$–EtOAc (95:5) and (90:10), after purification by TLC (pentane Et_2O–EtOAc, 5:3:2), afforded 200 mg **3a**, mp 158–160° from Me_2CO–pentane. IR ν_{max} cm^{-1}: 1640, 1570, 1520. UV λ_{max}^{EtOH} nm (ε): 256 (19 320), 267 (18 514), 361 (18 514). MS *m/e* (rel. int.): 432 (M^+, 20.0), 417 (M − 15, 100.0), 225 ($C_{10}H_9O_6$, 10.4), 192 ($C_{11}H_{12}O_3$, 8.6).

Alkaline degradation of **3a**. A 55 mg sample of agecorynin C (**3a**), treated under the same degradative conditions as described before, afforded the same acetophenone **5** obtained from **1a** and **1b**, and 2,4,5-trimethoxybenzoic acid, mp 141–142° (lit. [10] 143–144°). In addition, two neutral products were isolated by TLC. The less polar product was identified as 2,4,5-trimethoxyacetophenone (**7**). IR ν_{max} cm^{-1}: 1650, 1602, 1530, 1520. ^1H NMR ($CDCl_3$) δ: 2.58 (*s*, ArCOMe), 3.86, 3.90, 3.93 (*s*, 3 OMe), 6.48 (*s*, H-3), 7.40 (*s*, H-6). MS *m/e* (rel. int.): 210 (M^+, 37.9), 195 (M − 15, 100.0), 167 (M − 43, 11.2), 43 (MeCO, 98.0). The third neutral product was the keto–enol **4**, mp 129–131° from MeOH. IR ν_{max} cm^{-1}: 3100–3600 broad (OH), 1615 (chelated β-diketone), 1570, 1525 (aromatics). ^1H NMR ($CDCl_3$): δ 3.84 (3 H), 3.87 (3 H), 3.90 (6 H), 3.96 (6 H), 4.05 (3 H) (*s*, 7 OMe); 6.54 (*s*, 1 H), 7.54 (*s*, 1 H), 7.78 (*s*, 1 H), 12.48 (*s*, 1 H). MS *m/e* (rel. int.): 450 (M^+, 18.8), 419 (M − 31, 46), 240 ($C_{11}H_{12}O_6$, 16.8), 225 ($C_{11}H_{12}O_6$ − 15, 12.8), 195 ($C_{10}H_{11}O_4$, 100.0).

Agecorynin D (**3b**). Chromatography fractions of the $CHCl_3$ extract, eluted with $CHCl_3$–EtOAc (1:1) afforded 160 mg agecorynin D (**3b**), mp 258–260° from Me_2CO–$CHCl_3$. [Found: C, 58.62; H, 4.87; O, 36.3. $C_{19}H_{18}O_9$ requires: C, 58.46; H, 4.65; O, 36.89%]. IR ν_{max} cm^{-1}: 2500–3600 broad, 1650, 1600. UV λ_{max}^{MeOH} nm (ε): 273 (13 985), 380 (16 150); λ_{max} (MeOH + NaOAc): 272, 430; λ_{max} (MeOH + NaOAc + H_3BO_3): 274, 376; λ_{max} (MeOH + $AlCl_3$): 284, 302, 418; λ_{max} (MeOH + $AlCl_3$ + HCl): 287, 302, 410. MS *m/e* (rel. int.): 390 (M^+, 39.3), 375 (M − 15, 100.0), 211 ($C_9H_7O_6$, 48.4), 183 ($C_9H_7O_6$–CO, 18.6).

Agecorynin D triacetate (**3c**). A 66 mg sample of **3b** acetylated with Ac_2O–Py as usual, gave the triacetate **3c**, mp 154°. IR ν_{max} cm^{-1}: 1770, 1650, 1600, 1510. λ_{max}^{EtOH} nm (ε): 243 (24 187), 265 (22 575), 314 (15 950). MS *m/e* (rel. int.): 516 (M^+, 5.4), 474 (M − CH_2CO, 100.0), 432 (M − $2CH_2CO$, 68.1), 417 (M − $2CH_2CO$ − Me, 43.9), 390 (M − $3CH_2CO$, 30.1), 375 (M − $3CH_2CO$ − Me, 99.0), 211 ($C_9H_7O_6$, 34.4), 183 ($C_9H_7O_6$ − CO, 28.4).

Agecorynin D trimethyl ether (**3a**). 17 mg of **3b** in dry Me_2CO (50 ml), Me_2SO_4 (1 ml) and 500 mg dry K_2CO_3 were refluxed for 10 hr and worked up as usual. Purification by TLC of the reaction residue yielded a crystalline compound which was identical to agecorynin C (**3a**) in all respects.

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