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POLYOXYGENATED FLAVONES FROM *AGERATUM CONYZOIDES*

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Key Word Index—*Ageratum conyzoides*; Asteraceae; 5,6,7-trimethoxy-3',4'-methylenedioxyflavone; 5,6,7,3'-tetramethoxy-4'-hydroxyflavone; 5,6,7,3',5'-pentamethoxy-4'-hydroxyflavone; polyoxygenated flavones.

Abstract—Twelve polyoxygenated flavones have been isolated from *Ageratum conyzoides*, three of which are new natural flavones, namely ageconyflavones A (5,6,7-trimethoxy-3',4'-methylenedioxyflavone), B (5,6,7,3'-tetramethoxy-4'-hydroxyflavone) and C (5,6,7,3',5'-pentamethoxy-4'-hydroxyflavone). The other nine compounds were identified as linderoflavone B, eupalestin, nobiletin, 5'-methoxynobiletin, 5,6,7,5'-tetramethoxy-3',4'-methylenedioxyflavone, sinensetin, 5,6,7,3',4',5'-hexamethoxyflavone, 5,6,7,8,3'-pentamethoxy-4'-hydroxyflavone and 5,6,7,8,3',5'-hexamethoxy-4'-hydroxyflavone.

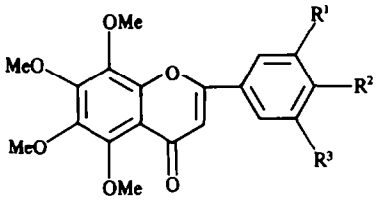
INTRODUCTION

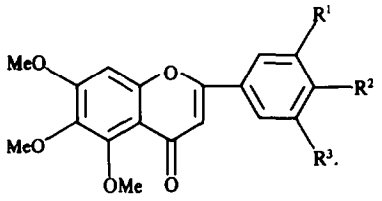
Plants of the genus *Ageratum* (Asteraceae) are known to contain chromenes [1, 2], benzofurans [3], terpenoids [4, 5] and flavonoids. Many polyoxygenated flavonoids have been isolated from *A. houstonianum* [6, 7], *A. conyzoides* [8], *A. strictum* [9] and *A. corymbosum* [10]. During the course of our previous reinvestigation [11] of a novel chromone, *viz.* conyzorigun, which was found to be a flavone, we isolated several other flavones from *A. conyzoides*. The present communication describes three new flavones, ageconyflavones A (**5**), B (**11**) and C (**12**), along with nine other compounds (**1–4**, **6–10**) which have been isolated earlier from *Ageratum* and *Eupatorium* species. This is the first report of flavones **6** and **8** from the Asteraceae, but they have been recorded recently from *Bauhinia championii* (Leguminosae) [12] as new natural products. Also, flavones (**1–3** and **5–10**) are reported for the first time from *A. conyzoides*.

RESULTS AND DISCUSSION

The concentrated ethanolic extract of *A. conyzoides* (whole plants) was treated with 1 N NaOH overnight and then extracted with diethyl ether. Repeated column chromatography of the ether extract followed by multiple TLC of the less polar fractions eluted with benzene–ethyl acetate resulted in the isolation of all the non-phenolic flavones (**1–8**). The known compounds 5,6,7,8-tetramethoxy-3',4'-methylenedioxyflavone (linderoflavone B, **1**), 5,6,7,8,5'-pentamethoxy-3',4'-methylenedioxyflavone (eupalestin, **2**), 5,6,7,8,3',4'-hexamethoxyflavone (nobiletin, **3**), 5,6,7,8,3',4',5'-heptamethoxyflavone (5'-methoxynobiletin, **4**), 5,6,7,5'-tetramethoxy-3',4'-methylenedioxyflavone (**6**), 5,6,7,3',4'-pentamethoxyflavone (sinensetin, **7**) and 5,6,7,3',4',5'-hexamethoxyflavone (**8**) were identified by their mp, UV, IR, ^{1}H NMR, and MS fragmentation data, which are in good agreement with those reported in the literature [12–15]. The structural determination of the three new ageconyflavones A, B and C is described below.

Ageconyflavone A (**5**), $C_{19}H_{16}O_7$, mp 189–190° (EtOAc–Et_2O), displayed UV (330, 267 nm) and IR (1635, 1600, 1505 cm^{-1}) absorptions typical of a non-phenolic flavone [16]. The presence of 5,6,7,3',4' penta-substitution in compound **5** was evident from its ^{1}H NMR spectrum showing three methoxyls (δ3.96, 4.02 and 4.03), a methylenedioxy group (δ6.10) and five ring protons, the





1 $R^1 + R^2 = OCH_2O$, $R^3 = H$
2 $R^1 + R^2 = OCH_2O$, $R^3 = OMe$
3 $R^1 = R^2 = OMe$, $R^3 = H$
4 $R^1 = R^2 = R^3 = OMe$
9 $R^1 = OMe$, $R^2 = OH$, $R^3 = H$
10 $R^1 = R^3 = OMe$, $R^2 = OH$

5 $R^1 + R^2 = OCH_2O$, $R^3 = H$
6 $R^1 + R^2 = OCH_2O$, $R^3 = OMe$
7 $R^1 = R^2 = OMe$, $R^3 = H$
8 $R^1 = R^2 = R^3 = OMe$
11 $R^1 = OMe$, $R^2 = OH$, $R^3 = H$
12 $R^1 = R^3 = OMe$, $R^2 = OH$

pattern being similar to that observed in the case of 5,6,7,3',4'-pentamethoxyflavone (7). The three B-ring protons were observed at δ6.94 (1H, *d*, J = 8 Hz, H-5'), 7.48 (1H, *dd*, J = 2, 8 Hz, H-6') and 7.35 (1H, *d*, J = 2 Hz, H-2'). One of the two singlets at δ6.82 was assigned to the A-ring aromatic proton H-8 (as observed for the flavones **6**, **7** and **8** at δ6.81, 6.81 and 6.84, respectively) while the other at δ6.57 corresponded to the pyrone ring proton H-3 (as observed for the flavones **1**–**4** and **6**–**8** at δ6.57, 6.58, 6.64, 6.67, 6.56, 6.60 and 6.64, respectively). The substitution pattern of methoxyls in the A-ring could be determined by studying C_6D_6 induced shifts ($\Delta = \delta\ CDCl_3 - \delta\ C_6D_6$) [17]. One of the methoxyls exhibited a large up-field shift (0.68 ppm) while the others shifted to lesser extents (0.21 and −0.09) indicating either 5,6,7 or 6,7,8 substitution. However, the absence of a down-field aromatic signal (*ca* δ8.80) [17] confirmed 5,6,7-trimethoxyl substitution in the A-ring. On the basis of these observations along with the characteristic MS fragmentation [18] (Fig. 1), ageconyflavone A was assigned the structure 5,6,7-trimethoxy-3',4'-methylenedioxyflavone (**5**). It may be mentioned here that an isoflavone, *viz.* odoratine, with the same substitution pattern has been reported [9]. However, in general, the presence of a methylenedioxy group is comparatively rare in the case of flavones [20].

The phenolic flavones (**9**–**12**) were isolated by CC of the ethanolic extract on silica from the more polar fractions eluted with ethyl acetate–methanol (7:3). The fractions contained only low amounts of these flavones along with much resinous material. The compounds were purified by reversed phase TLC using paraffin impregnated silica gel plates, followed by normal TLC. Although these phenolic flavones were isolated in low yields, their structures could be determined by their UV spectra with classical reagents [17], by MS of their acetates and by their conversion to known polymethoxyflavones which have been isolated in larger amounts from the same plant material.

The UV spectra of all these flavones (**9**–**12**), on addition of sodium methoxide and sodium acetate, exhibited a large Band-I bathochromic shift (*ca* 45–85 nm) with both reagents, suggesting the presence of a C-4' hydroxyl group [17]. Since Band-II, in all cases, did not undergo any shift, no hydroxyl could be assigned to the A-ring. The presence of additional hydroxy groups at C-5, C-3, C-3' and C-5' was ruled out as neither $AlCl_3$ nor $CH_3COONa + H_3BO_3$ induced any bathochromic shift. The presence of a hydroxyl group at C-2' or C-6' could also be eliminated since these flavones (**9**–**12**), upon treatment with CH_2N_2, yielded the known flavones (**3**), (**4**), (**7**) and (**8**), respectively. The identity of the methyl derivatives was confirmed by multiple co-TLC with authentic markers in different solvent systems, HPLC and the superimposibility of the MS fragmentation pattern. The fact that all these flavones contained only one hydroxyl, at C-4', was also evident since only mono-acetate derivatives were formed (MS). Although the mass spectra of **9** and **10** have been reported earlier [15], we failed to obtain satisfactory spectra for these flavones, probably because of low volatility. On the basis of these observations, the flavones were assigned the structures 5,6,7,8,3'-pentamethoxy-4'-hydroxyflavone (**9**), 5,6,7,8,3',5'-hexamethoxy-4'-hydroxyflavone (**10**), 5,6,7,3'-tetramethoxy-4'-hydroxyflavone (**11**) (ageconyflavone B) and 5,6,7,3',5'-pentamethoxy-4'-hydroxyflavone (**12**) (ageconyflavone C).

EXPERIMENTAL

Plant material. Whole plants along with the roots were collected from our campus (identified by Dr. V. Abraham, Nuclear Agriculture Division, Bhabha Atomic Research Centre) and a voucher specimen (No. 1510-NBM/BOD) has been deposited in the Herbarium of Landscape and Cosmetic Maintenance Section, BARC.

Compounds were detected on TLC under UV light (254, 365 nm), by exposing the plates to I_2 vapour and by heating at 110° after spraying with 10% H_2SO_4. Acetate derivatives of **9**–**12** were prepared by the usual method ($Ac_2O + C_5H_5N$, overnight). Methylation was carried out using CH_2N_2 in Et_2O prepared by the reaction of *N*-nitroso-*N*-methyl-*p*-toluenesulphonamide with aq. ethanolic KOH. The methyl derivatives of **9**–**12** were

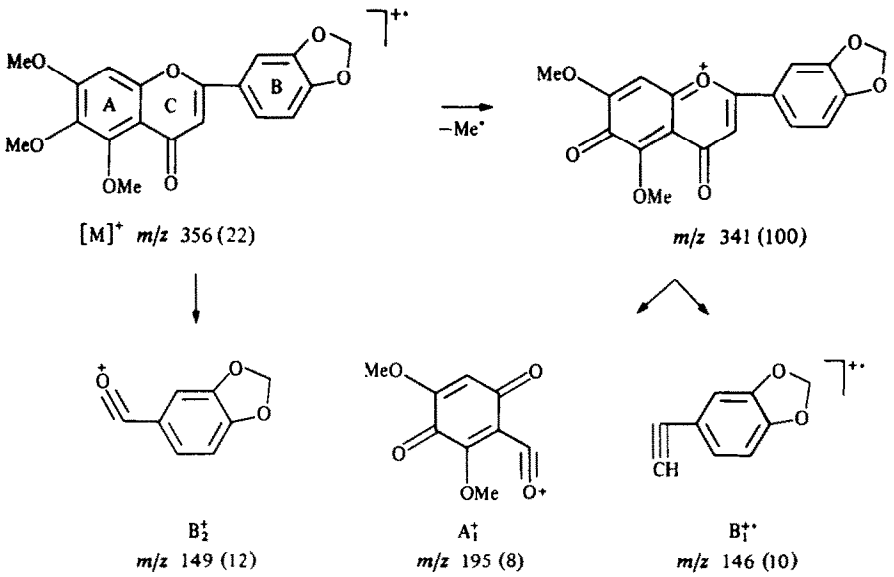


Fig. 1. Mass spectral fragmentation of **5**.

compared with reference samples of the corresponding known flavones **3**, **4**, **7** and **8**, respectively, by multiple co-TLC on silica gel G and alumina G (C_6H_6–EtOAc, 13:7 and $CHCl_3$–MeOH, 19:1) as well as by HPLC on a μ-Bondapak-C_{18} column (MeOH–H_2O).

^{1}H NMR (100.1 MHz): $CDCl_3$, C_6D_6. MS (70 eV) direct insertion. UV: MeOH. IR: KBr.

Isolation of non-phenolic flavones (**1–8**). The air dried plant material was finely ground (1.3 kg) and extracted with EtOH (4 × 4 l.). The *in vacuo* concd extract was stirred with 1 N aq. NaOH for 3 hr, allowed to stand overnight and extracted with Et_2O (7 × 0.5 l.). The Et_2O extract, washed with H_2O and dried over Na_2SO_4, was concd to a yellowish brown viscous residue which was subjected to CC on silica using petrol (60–80°), C_6H_6, EtOAc and MeOH. The fractions eluted with C_6H_6–EtOAc (EtOAc 25–70%) contained different mixtures of flavones (**1–8**) which were purified by repeated multiple TLC on silica gel G (C_6H_6–EtOAc, 13:7). The flavones **1**, 8 mg; **2**, 41 mg; **5**, 5 mg; **6**, 18 mg; **4**, 72 mg; **3**, 44 mg; **8**, 23 mg; and **7**, 29 mg were isolated in increasing order of polarity. The UV, IR, ^{1}H NMR, MS and mp of **1–4** and **6–8** were in agreement with the published data [12–15].

Ageconyflavone A (5,6,7-*trimethoxy*-3′,4′-*methylenedioxyflavone*) (**5**). Needles from EtOAc–Et_2O, mp 189–190°, UV λ_{max} nm (log ε): 330 (4.25), 267 (4.17). IR ν_{max}^{KBr} cm^{-1}: 1635, 1610, 1600, 1505, 1495, 1455, 1440, 1418, 1329, 1243, 1200, 1117, 1038, 988, 930, 830, 800. ^{1}H NMR (100.1 MHz, $CDCl_3$): δ3.96 (3H, *s*, Ar–OMe), 4.02 (3H, *s*, Ar–OMe), 4.03 (3H, *s*, Ar–OMe), 6.10 (2H, *s*, –OCH_2O–), 6.57 (1H, *s*, H-3), 6.82 (1H, *s*, H-8), 6.94 (1H, *d*, J = 8 Hz, H-5′), 7.35 (1H, *d*, J = 2 Hz, H-2′), 7.48 (1H, *dd*, J = 2, 8 Hz, H-6′); C_6D_6: δ3.28 (3H, *s*, Ar–OMe), 3.81 (3H, *s*, Ar–OMe), 4.12 (3H, *s*, Ar–OMe), 5.31 (2H, *s*, –OCH_2O–), 6.59 (1H, *s*, H-3), 6.32 (1H, *s*, H-8), 6.58 (1H, *d*, J = 8 Hz, H-5′), 7.04 (1 H, *br s*, H-2′) (H-6′ signal, being merged with C_6D_6 peak, could not be detected). MS *m/z* (rel. int.) 356 [M]$^+$ (22), 341 (100), 313 (4), 297 (9), 267 (5), 195 (8), 152 (7), 149 (12), 146 (10), 137 (4). (Found: C, 64.18; H, 4.40. $C_{19}H_{15}O_7$ requires: C, 64.04; H, 4.49.)

Isolation of phenolic flavones (**9–12**). The concd EtOH extract of another batch of plant material (0.9 kg) was subjected directly to CC. Examination of polar fractions eluted with EtOAc–MeOH on TLC showed some bright UV fluorescent spots in low concentration. Preliminary separation of these compounds was carried out using reversed phase prep. TLC. Activated silica gel plates were impregnated with paraffin by developing in petrol–paraffin (19:1) and the plates reactivated for 10 min before chromatography of the concd mixture in MeOH–H_2O (3:2). The required bands were eluted with MeOH, the eluates dried over Na_2SO_4 and concd *in vacuo*. Flavones (**9–12**) were isolated from the residue by normal prep. TLC ($CHCl_3$–EtOAc–MeOH, 25:24:1). Yields of these minor constituents were as follows: **9**, 0.8 mg; **10**, 1.2 mg; **11**, 2 mg and **12**, 2 mg.

5,6,7,8,3′-*Pentamethoxy*-4′-*hydroxyflavone* (**9**). Colourless solid. UV λ_{max}^{MeOH} nm (log ε): 337 (4.21), 270 (4.20); $\lambda_{max}^{MeOH+NaOMe}$ 418, 266; $\lambda_{max}^{MeOH+NaOAc}$ 415, 268; $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ 337, 270; $\lambda_{max}^{MeOH+AlCl_3}$ 338, 273. MS of acetate *m/z* (rel. int.): 430 [M]$^+$ (25), 415 (27), 372 [415 – MeCO]$^+$ (100). MS of methyl ether (**3**) *m/z* (rel. int.): 402 [M]$^+$ (32), 387 (100).

5,6,7,8,3′,5′-*Hexamethoxy*-4′-*hydroxyflavone* (**10**). Colourless solid. UV λ_{max}^{MeOH} nm (log ε): 310 (4.24), 272 (4.26); $\lambda_{max}^{MeOH+NaOMe}$ 355, 268; $\lambda_{max}^{MeOH+NaOAc}$ 370, 268; $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ 308, 268; $\lambda_{max}^{MeOH+AlCl_3}$ 310, 268. MS of acetate *m/z* (rel. int.): 460 [M]$^+$ (34), 445 (45), 402 [445 – MeCO]$^+$ (100). MS of methyl ether (**4**) *m/z* (rel. int.): 432 [M]$^+$ (32), 417 (100).

Ageconyflavone B (5,6,7,3′-*tetramethoxy*-4′-*hydroxyflavone*) (**11**). Yellow solid. UV λ_{max}^{MeOH} nm (log ε): 332 (4.32), 267 (4.23); $\lambda_{max}^{MeOH+NaOMe}$ 418, 267; $\lambda_{max}^{MeOH+NaOAc}$ 415, 267; $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ 335, 270; $\lambda_{max}^{MeOH+AlCl_3}$ 332, 267. MS of acetate *m/z* (rel. int.): 400 [M]$^+$ (28), 385 (48), 342 [385 – MeCO]$^+$ (100). MS of methyl ether (**7**) *m/z* (rel. int.): 372 [M]$^+$ (23), 357 (100).

Ageconyflavone C (5,6,7,3′,5′-*pentamethoxy*-4′-*hydroxyflavone*) (**12**). Yellow solid. UV λ_{max}^{MeOH} nm (log ε): 323 (4.30), 265 (4.27); $\lambda_{max}^{MeOH+NaOMe}$ 394, 265, $\lambda_{max}^{MeOH+NaOAc}$ 388, 264; $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ 330, 268; $\lambda_{max}^{MeOH+AlCl_3}$ 327, 265. MS of acetate *m/z* (rel. int.): 430 [M]$^+$ (20), 415 (24), 387 [430 – MeCO]$^+$ (9), 372 [415 – MeCO]$^+$ (100). MS of methyl ether (**8**) *m/z* (rel. int.): 402 [M]$^+$ (26), 387 (100).

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