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BENZOFURAN AND OTHER CONSTITUENTS OF THE ESSENTIAL OIL OF *AGERATUM CONYZOIDES*

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Key Word Index—*Ageratum conyzoides*; Compositae; essential oil; chromenes; chromones; isodihydroeuparin derivative; nymphal mortality; *Schistocerca gregaria*.

Abstract—An investigation of the essential oil of *Ageratum conyzoides* afforded, in addition to known precocenes I and II, four new compounds, an isodihydroeuparin derivative, a chromene, chromone, and a chromanone. Their structures were established by spectroscopic methods as 2-(2′-methylethyl)-5,6-dimethoxybenzofuran, 2-(1′-oxo-2′-methylpropyl)-2-methyl-6,7-dimethoxy-chromene, 3-(2′-methylpropyl)-2-methyl-6,8-dimethoxy-chrom-4-one and 2-(2′-methylprop-2′-enyl)-2-methyl-6,7-dimethoxy-chroman-4-one respectively. The topical application of the oil to the nymphs of *Schistocerca gregaria* showed high nymphal mortality (91%).

INTRODUCTION

The genus *Ageratum* (Compositae), is well-known for its chromenes [1–6] and flavonoids [7–9]. The species *Ageratum conyzoides* found in India and elsewhere, has been used in folk medicine for the treatment of several diseases [10]. It has also been used as an insecticide [6]. In recent years, the concept of controlling insects with juvenoids has attracted much attention. Precocenes I (**1**) and II (**2**) are the first anti-allatotropins isolated from *Ageratum* species [5]. In our studies, *A. conyzoides* oil showed greater activity than precocenes against *Schistocerca gregaria* when compared with earlier reported data [14], and this prompted us to search for active constituents other than precocenes. We report the isolation of four new compounds, an isodihydroeuparin derivative (**3**), a chromene (**4**), chromone (**5**) and a chromanone (**6**) in addition to the known **1** and **2**.

RESULTS AND DISCUSSION

The essential oil was column chromatographed on silica gel to give different fractions. The fractions were then, subjected to multiple prep. TLC developed in petrol:diethyl ether:acetic acid (90:10:1) to give compounds **1**–**6**. The compound **1**, $C_{12}H_{14}O_2$ (EIMS ; 190 M^+) and **2**, $C_{13}H_{16}O_3$ (EIMS m/z; 220 M^+) were unambiguously identified as precocene I and precocene II respectively by comparing their IR, ^{1}H NMR and mass spectral fragmentation data which are in excellent agreement with those reported in the literature [4].

The molecular formula of **3**, a red substance and an isomer of **2**, was confirmed as $C_{13}H_{16}O_3$ by EI-mass spectrum [$(M)^+$ 220] and the elemental analysis together with ^{13}C NMR data. In addition to the presence of aromatic ring and an isopropyl group (1568, 1517, 1330 and 1336 cm^{-1}), the IR spectrum revealed the absorption of aromatic methoxyl groups (1138 and 1113 cm^{-1}) which were confirmed by the ^{13}C signal at δ_C 56.87 and 56.61. Besides an isopropyl group (1330, 1336 cm^{-1}, δ_H; 1.72, 1.72, both *d*, $J = 7$ Hz, H-11/12), the presence of a vinylic proton (δ_H 6.50, *s*, H-3), was confirmed by the IR and ^{1}H NMR spectroscopic data. The spectra revealed also the presence of two aromatic protons (δ_H 7.64 and 6.67, both singlets, H-4 and H-7). The location of an isopropyl group at C-2 was further supported by the peak due to the ion at m/z 43 in the mass spectrum.

The EI mass spectrum exhibited characteristic peaks at 205 and 175, besides the molecular ion. As in alkyl furans [11], cleavage β- to the furan ring was the dominant fragmentation process, resulting in the stable ring-expanded aromatic chromenyl ion (m/z 205) as the base peak. On the basis of above-mentioned spectroscopic evidence and comparing the data with reported isodihydroeuparin derivatives [12], structure **3** is assigned to this new dimethoxy benzo-

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furan and it is named as 2-(2′-methylethyl)-5,6-dimethoxybenzofuran.

Compound **4**, a pale yellow substance with an oily consistency, was assigned the molecular formula $C_{16}H_{20}O_4$ on the basis of the $(M)^+$ peak in the EI-MS (m/z 276) and the elemental analysis data. In addition to an isopropyl group (1370, 1364 cm^{-1}), the IR spectrum showed the absorption of a carbonyl group (1719 cm^{-1}). The ^1H NMR spectrum of **4** was remarkably simple and displayed the signals characteristic of a monomethyl chromene. Two singlet signals at δ_H 3.89 and 3.81 were attributed to aromatic methoxyl protons at C-6 and C-7. The olefinic protons H-3 and H-4 displayed an AB pattern of doublets at δ_H 5.42 and 6.40 (1H each, $J = 10$ Hz), of which the signal at δ_H 5.42 was assigned to H-3, as the β-proton of *p*-methoxystyrene system is more shielded than the α-proton [4]. Also, the spectrum had signals for two aromatic protons, of which the signal at δ_H 7.68 was assigned to H-5 which is deshielded by the styrene double bond [4], and the other at δ_H 6.52 was assigned for H-8. These two aromatic protons and their observed multiplicity indicated a *para* relationship. A doublet at δ_H 0.99 (6H, $J = 6$ Hz, H-13/14) and a multiplet at δ_H 1.97–2.20 (1H, H-12) corresponded to an isopropyl system. The mass spectral fragmentation supports the structure assigned. The most facile cleavage giving rise to the peak at m/z 205 could be visualised due to the loss of butanoyl group of the chromene. Thus **4** is established as 2-(1′-oxo-2′-methylpropyl)-2-methyl-6,7-dimethoxychromene.

Compound **5**, also obtained as a pale yellow amorphous substance, showed an IR spectrum indicating a carbonyl (1720 cm^{-1}) and an isopropyl group (1364, 1336 cm^{-1}). The EI-MS showed $(M)^+$ at 276, corresponding to the molecular composition $C_{16}H_{20}O_4$. The carbon content was corroborated by ^{13}C NMR showing resonances that are readily assigned to a carbonyl group (δ_C 168.00) and an isopropyl side chain (δ_C 27.82 and 19.23). The two singlets arising from the meta-coupled H-5 and H-7 protons stand out clearly. The UV spectrum displayed a band at 270 nm ($\varepsilon = 489$ in MeOH) due to the enone system. ^{13}C NMR data, compared to ^{13}C chemical shifts of numerous chromones, matched with the basic skeleton of 4-chromone for ring B. The side chain shows the characteristic isopropyl end group (^1H-NMR: 0.98, *d*, $J = 7$ Hz, H-13/14; 1.96–2.20, 1H, *m*, H-12) as well as the C-2 methyl (δ_C 29.77, δ_H 1.25, *s*). The attachment of this side chain at C-3 position was demonstrated by ^1H NMR data and the absence of NMR peaks associated with C-3 and C-4 position (ca δ 5.40 and 6.20) in the spectra of 3,4-chromenes [16]. This view is confirmed by the cleavage of butyl moiety at C-3 in mass spectrum to give the base peak at m/z 57 which arises through fission of the bond β- to the chromene ring, with the associated formation [11] of another prominent ion at m/z 219. Based on all these data, it is apparent that **5** is 3-(2′-methylpropyl)-2-methyl-6,8-dimethoxychrom-4-one.

Compound **6** was isolated as a red amorphous solid. The EI-MS showed the $(M)^+$ ion peak at m/z 276, which was in agreement with the molecular formula $C_{16}H_{20}O_4$. The ^1H and ^{13}C NMR spectroscopic data indicated another chromanone type structure and were partly reminiscent of those of **5**. In addition to the ^1H and ^{13}C NMR signals observed in **5**, **6** showed the ^1H signals due to β-methylallyl group (δ_H 1.72, 6H, *s*, H-13/14 and 6.64, 1H, *s*, H-11) and methylene protons (δ_H 2.10, *d*, $J = 7$ Hz, H-3). The IR spectrum showed the absorption of a carbonyl group (1720 cm^{-1}), which was supported by the ^{13}C signal at δ_C 168.00. The ^{13}C NMR signals of two aromatic methoxyl carbons (δ_C 56.92 and 56.59) are in agreement with the two aromatic ring carbons (δ_C 145.10 and 150.14). Their location at C-6 and C-7 were established by comparing the NMR data of **6** with those of **3** and **1**. The attachment of β-methylallyl group at C-2 position was determined by a close examination of NMR spectrum of the compound, as there was only one methyl group (δ_H 1.30, *d*) at C-2, at which normally chromenes will have gem-dimethyl groups. The structure of **6** was further supported by mass spectral fragmentation in EIMS to give the most prominent ion peaks at m/z 217 and 149. Thus, **6** is 2-(2′-methylprop-2′-enyl)-2-methyl-6,7-dimethoxychroman-4-one.

Compounds **3** and **4** are thought to arise from the alkylation of precocene II (**2**) by DMAPP, to the chromene and benzofuran system. These compounds have also come to be known as aromatic hemiterpenes [12]. *Ageratum* appears to be the only source of these precocene derived metabolites which a methyl group of gem-dimethyl group has undergone further chemical modification. It is the first report on the nature of a 5,6-dimethoxybenzofuran (**3**) in which 2-isopropenyl substituent of euparin has undergone reduction.

Biogenesis of chromone (**5**) and chromanone (**6**) involves a side chain of eight carbon atoms, of which some have become a part of the heterocyclic ring. It has been suggested that the successive loss of one terminal carbon atom a monoterpene moiety may explain the existence of such compounds. Also, 2,3-disubstituted chromone (**5**) could be derived biogenetically from an anthraquinone [16]. It has been suggested that their unusual skeleton could arise via a poly ketide chain, with C-2 being inserted at a latter stage or via a degradative pathway from an oxygenated isoflavone [17].

Regarding the biological activity of the *A. conyzoides* oil, it was observed that topical application of the oil (250 μg) to 96–120 h old 4th instar nymphs of *Schistocerca gregaria* caused 91% total nymphal mortality and 9% deformed adults. This data when compared with the earlier reports [14] on the activity of precocenes against the same insects, the oil has greater activity. Obviously compounds other than precocenes in the oil may be responsible for this toxicity. The biological activity of the isolated and identified

compounds (i.e. **3**–**6**) against the same insects is in progress.

EXPERIMENTAL

General

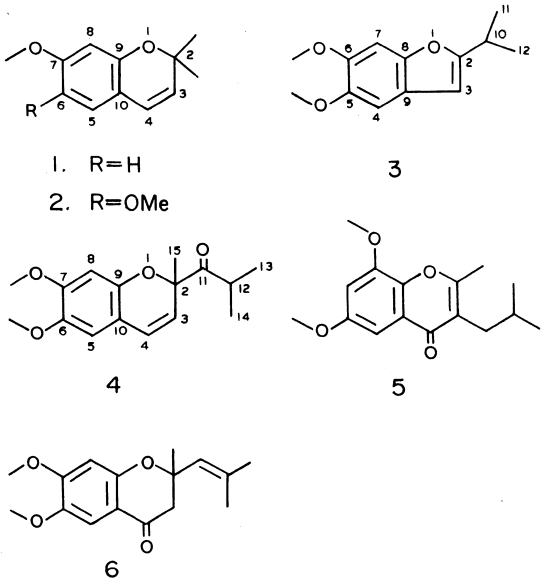
IR were measured in CCl_4. NMR were recorded in 100 MHz for ^1H and 200 MHz for ^{13}C, in $CDCl_3$ using TMS as int. standard. UV were measured in MeOH. MS were recorded using a direct inlet system at 70 eV. TLC and prep. TLC (0.75 mm thick) were carried out on silica gel G, using petrol–Et_2O–HOAc (90:10:1) CC was carried out on silica gel (60–120).

Plant Material

Ageratum conyzoides was collected at the peak of the flowering stage (March 1995) in the Institute Campus where it blossoms during periods of March–April. The plant was duly identified by Dr P. R. Bhagwat, Raw Materials Herbarium & Museum Delhi (RHMD) at National Institute of Science Communication (CSIR), New Delhi, where a voucher specimen (Field No. 1793) has been deposited.

Essential oil of A. conyzoides

Freshly collected whole plant (9.4 kg) was hydrodistilled and the distillate was repeatedly extracted with CH_2Cl_2. The organic part was separated and the solvent was evaporated under reduced pressure to give a red essential oil (12.04 g, yield 0.13% of fresh wt). d_4^{28} 0.984, η_D^{27} 1.5231.



Chromatography of the oil

Chromatography of the oil (8.5 g) was carried out using silica gel (60–120 mesh, 300 g) column (100 × 4 cm). Elution with hexane:benzene gave frs 1–4; benzene gave frs 5–8; benzene:chloroform, 9–15, chloroform 16–17; and chloroform:methanol gave frs 18–26. Fractions of 200 ml each were collected.

Fractions 1, 2 (A_0), and 3, 4 (A_1) yielded precocene I, and frs 5–8 (A_2, 0.5 g) and 9–10 (A_3, 2.3 g) afforded precocene II as the major compound. TLC of frs 11–15, 16–17, 18–20 and 21–26 showed each of them to be a complex mixture. According to TLC conditions, these frs were pooled and referred to as A_4 (1.5 g, frs 11–15), A_5 (0.3 g, frs 16–17), A_6 (0.53 g, frs 18–20) and A_7 (0.53 g, frs 21–26) respectively.

Isolation of compounds **3**, **4**, **5** *and* **6**

Pre. TLC of frs. Using petrol–Et_2O–HOAc (90:10:1) [5] as the solvent system in TLC, fr. A_4 was resolved into 4 spots. By cutting the band of R_f 0.4, by repeated prep. TLC **3** (12 mg) could be obtained as a red solid. By cutting the bands corresponding to R_f 0.6 and 0.8 by repeated preparative TLC of fr. A_5, a chromene **4** (10 mg) and a chromone **5** (8 mg) were isolated. Similarly **6** (15 mg) was obtained (R_f 0.25) as a red solid from fr. A_6. Acetone was used as an eluting solvent in the isolation of each compound.

2-(2′-*Methylethyl*)5,6-*dimethoxybenzofuran* (**3**). Red amorphous solid, $UV\lambda_{max}$ MeOH nm (log ε): 410 (973), 320 (714) and 270 (1369). $IR\nu_{max}$ (CCl_4) cm^{-1}. 2940 (CH), 1614, 1568, 1517 (aromatic C=C), 1466, 1330 (gem-dimethyl), 1274, 1206 (C—O), 1138 and 1013 (OMe). ^1H NMR (δ from TMS). 6.56 (1H, *s*, H-3), 7.24 (1H, *s*, H-4), 6.67 (1H*s*, H-7), 3.89 (3H, *s*, 5-OMe), 3.84 (3H, *s*, 6-OMe), 1.25 (1H, *m*, $J = 7$ Hz, H-10), 1.72 (6H, *d*, $J = 7$ Hz, 11- and 12-Me). ^{13}C NMR (Table 1): EIMS (pos. ion): *m*/*z* (rel. int.); $(M)^+$ 220 (15), [Found: C, 70.90; H, 8.03. Calcd for $C_{13}H_{16}O_3$: C, 70.92; H, 8.20%], 205 $(M^+ - Me)^+$ (90), 175 $(M^+ - Me - 30)$ (15), 128 (10), 97 (10) and 43 $((H_3C)_2CH)^+$ (15).

2-(1′-*Oxo*-2′-*methylpropyl*)-2-*methyl*-6,7-*dimethoxychromene* (**4**). Pale yellow amorphous solid. $UV\lambda_{max}$ MeOH nm (log ε): 319 (766), 273 (584) and 217 (980). $IR\nu_{max}$ (CCl_4) cm^{-1}: 2964 (CH), 1719 (C=O), 1612, 1550, 1535 (aromatic C=C), 1467, 1443 (C=C), 1370, 1364 (gem-dimethyl), 1260, 1216 (C—O), 1136, 1072 (OMe), 1007 and 981 (Ph-H out of plane bend). ^1H NMR (δ from TMS). 5.42 (1H, *d*, $J = 10$ Hz, H-3), 6.40 (1H, *d*, $J = 10$ Hz, H-4), 7.68 (1H, *s*, H-5), 6.52 (1H, *s*, H-8), 1.97–2.30 (1H, *m*, H-12), 0.99 (6H, *d*, $J = 7$ Hz, 13- and 14-Me), 1.41 (3H, *s*, 15-Me), 3.89 (3H, *s*, 6-OMe) and 3.81 (3H, *s*, 7-OMe). EIMS (pos. ion): *m*/*z* (rel. int.); $(M)^+$ 276 (10), [Found: C, 69.61; H, 7.34. Calcd for $C_{16}H_{20}O_4$: C, 69.56; H, 7.24%], 205 $(M^+ - 71)^+$ (25), 167 $(M^+ - 71 - H_2C \cdot CCH)^+$ (30), 149 $(M^+ - 109 - H_2O)^+$ (100), 104 (70), 76 (60), 57 (60) and 56 (65).

3-(2′-*Methylpropyl*)-2-*methyl*-6,8-*dimethoxychrom*-4-*one* (**5**). Pale yellow amorphous solid. UV^{λ}_{max} MeOH nm (log ε): 315 (256), 270 (489), 216 (1167). IR^{ν}_{max} (CCl_4) cm^{-1}. 2964 (CH), 1720 (C═O), 1577, 1535 (aromatic), 1522, 1458, 1364, 1216, 1198, 1163, 1135 (COC), 1072 (OMe), and 981. ^1H NMR (δ from TMS). 7.65 (1H, *s*, H-5), 7.53 (1H, *s*, H-7), 4.11 (3H, *s*, 6-OMe), 4.04 (3H, *s*, 8-OMe), 1.25 (3H, *s*, 15-Me), 1.60 (2H, *t*, $J = 10$ Hz, H-11), 1.98–2.20 (1H, *m*, $J = 6$ Hz, H-12), 0.98 (6H, *d*, $J = 7$ Hz, 13- and 14-Me). ^{13}C NMR (Table 1). EIMS (pos. ion): (rel. int.); 276 $(M)^+$ (5), [Found: C, 69.72; H, 7.30. Calcd for $C_{16}H_{20}O_4$: C, 69.57; H, 7.24%], 219 $(M^+ - (CH_3)_2CH \cdot CH2)^+$ (15), 205 (5), 167 (10), 149 $(M^+ - 71 - CH_2 \cdot CH{=}C{=}O)^+$ (100), 123 (20), 89 (40), 69 (38) and 57 (65).

2-(2′-*Methylprop*-2′-*enyl*)-2-*methyl*-6,7-*dimethoxy*-*chroman*-4-*one* (**6**). Red amorphous solid. UV^{λ}_{max} MeOH nm (log ε): 430 (282), 320 (260) and 270 (480). IR^{ν}_{max} (CCl_4) cm^{-1}: 2923 (CH), 1727 (C═O), 1577 (s, aromatic), 1257, 1200 (C═C), 1138, 1007. ^1H NMR (δ from TMS). 2.10 (2H, *d*, $J = 12$ Hz, H-3), 7.64 (1H, *s*, H-5), 6.60 (1H, *s*, H-8), 3.89 (3H, *s*, 6-OMe), 3.85 (3H, *s*, 7-OMe), 1.30 (3H, *d*, $J = 10$. Hz, 15-Me), 6.44 (1H, *s*, H-11), 1.72 (6H, *s*, 13- and 14-Me). ^{13}C NMR (Table 1). EIMs (pos. ion): (rel. int.); 276 $(M)^+$ (10), [Found: C, 69.68; H, 7.36. Calcd for $C_{16}H_{20}O_4$: C, 69.56; H, 7.24%], 250 (85), 217 $(M^+ - 30 - CO)^+$ (100), 205 (78), 149 $(M^+ - 58 - CO)^+$ (85), 83 (20) and 57 (35).

Table 1. ^{13}C NMR (200 MHz, $CDCl_3$) spectral data (δ, ppm) of the compounds **3**, **5** and **6**

	Compound		
C-atom	**3**	**5**	**6**
2	145.34	71.86	79.33
3	79.34	130.00	39.18
4	110.51	168.00	168.00
5	154.70	128.50	110.53
6	150.12	129.62	145.10
7	101.25	134.02	150.14
8	129.87	129.62	101.29
9	111.41	132.00	131.25
10	30.12	111.20	111.00
11	26.05	26.10	129.20
12	26.05	27.82	129.78
13	—	19.23	26.07
14	—	19.23	14.48
15	—	29.77	29.48
5-OMe	56.87	—	—
6-OMe	56.61	52.40	56.92
7-OMe	—	—	56.59
8-OMe	—	52.42	—

Bioassay studies

Nymphs of 96–120 h old *Schistocerca gregaria* maintained according to Mehrotra and Rao [15] were topically treated with *A. conyzoides* oil using a Hamilton syringe. The mortality count and the developmental abnormalities were recorded until adult emergence.

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