Structure Reinvestigation of Conyzorigun, a New Chromone from *Ageratum conyzoides*

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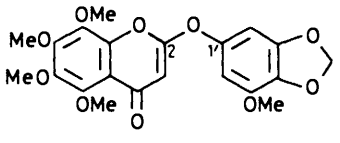
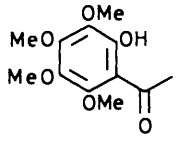
A highly oxygenated chromone, conyzorigun (**1**), from *Ageratum conyzoides* plants was earlier reported to contain a novel stable ketene acetal system by virtue of an oxygen linkage between the benzopyrone and phenyl moieties. A reinvestigation of this structure in the light of biosynthetic experiments followed by chemical degradation is now described. The results obtained indicate that conyzorigun does not contain the above-described oxygen linkage, being a flavone of a known structure, *viz.* eupalestin (**4**).

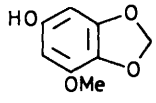
Adesogan and Okunade[1] have reported the isolation of a new chromone, conyzorigun (**1**), $C_{21}H_{20}O_{10}$, from *Ageratum conyzoides* plants as a minor constituent. This structure was assigned by them on the basis of u.v., i.r., ^1H and ^{13}C n.m.r. data together with evidence from alkali degradation of the compound which yielded the products (**2**) and (**3**). In view of the presence of such an unusual oxygen linkage between the benzopyrone and phenyl moieties, constituting a ketene acetal system, we were interested in the biosynthesis of this compound.



(**1**)



(**2**)

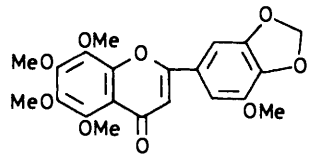


(**3**)

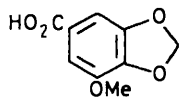
Adesogan and Okunade have mentioned that, biogenetically, conyzorigun is possibly related to chromenes which are quite abundant in this plant. However, this does not seem to be likely. In our earlier work on the biosynthesis of precocenes,[2] the 2,2-dimethylchromenes present in *Ageratum conyzoides*, it was shown that these chromenes arise from 3 acetate units and an isoprenoid moiety. Moreover, naturally occurring chromones are synthesised *via* acetogenesis[3] and involve 2-alkyl substitution. Thus, biogenesis of conyzorigun with an oxygen function at C-2 is difficult to explain.

Results and Discussion

We isolated conyzorigun by repeated column chromatography of an ethanol extract of the plant, followed by repeated multiple t.l.c. of the required fraction. The crystallised sample (from methanol, m.p. 192 °C (lit.,[1] 192—193 °C) was homogeneous with the reference sample (m.p., co-t.l.c., co-h.p.l.c.) kindly supplied by the authors. Its u.v., i.r., and ^1H n.m.r. spectral data were in agreement with those reported for conyzorigun. However, these values also resemble very closely those of eupalestin (**4**), a flavone isolated from *Ageratum*[4] (m.p. 187—188 °C), *Eupatorium*[5] (m.p. 185 °C), and *Conoclinium*[6] (m.p. of natural sample 190—190.5 °C; m.p. of synthesised sample 191—192 °C) species (Experimental section). Eupalestin has all the structural features of conyzorigun, except for the oxygen



(**4**)



(**5**)

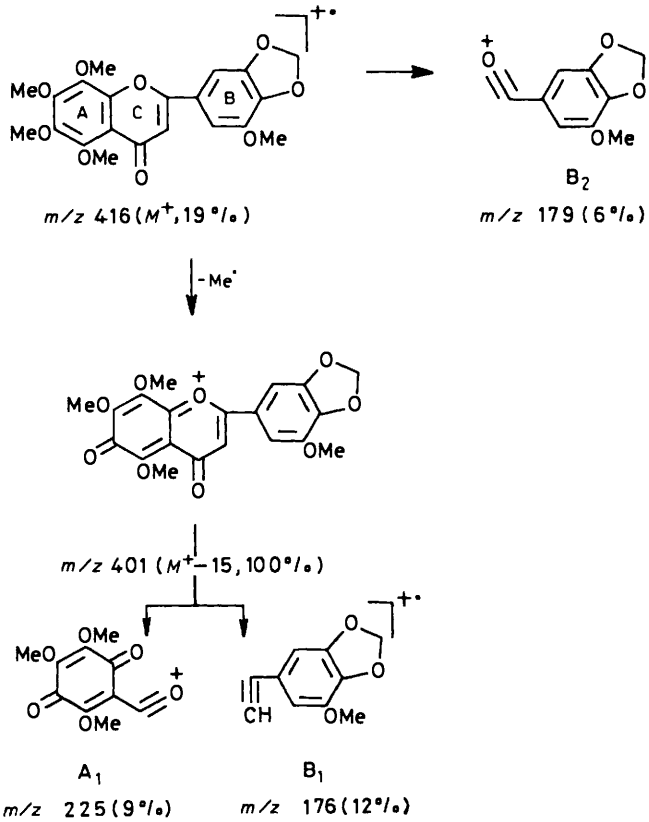


Figure. Mass spectral fragmentation of conyzorigun, *i.e.* eupalestin (**4**)

linkage between benzopyrone and phenyl rings. Comparison of the chemical shifts of the vinyl and aromatic protons as observed for eupalestin and conyzorigun, indicates that in spite of the oxygen linkage involved in the latter, there is hardly any change in the chemical shifts. Moreover, the ^{13}C n.m.r. results reported for the compound give evidence for 5 aromatic methoxy groups and a carbonyl function, whereas no mention

of the chemical shifts for carbon atoms C-2 and C-1′, which form the C–O–C linkage between the benzopyrone and phenyl moieties, has been made. In the case of eupalestin these have been reported at δ 160.63 and 125.90 respectively.[6] Adesogan and Okunade have not reported the mass spectral fragmentation of conyzorigun. In our investigation (see Figure), M^+ was observed at *m/z* 416 (19%) rather than 432 as would be expected from structure (**1**). The mass spectrum also showed a base peak at *m/z* 401 ($M^+ - 15$, 100) followed by peaks at *m/z* 225 (9), 176 (12), and 179 (6) corresponding to fragment ions (A_1^+), ($B_1^{+\bullet}$), and (B_2^+) in confirmation with the established fragmentation pattern of flavones.[7] Furthermore, this was in agreement with the reported fragmentation of eupalestin isolated from *Conoclinium coelestinum*[6] [416 (M^+, 47.1%), 401 (100), 225 (9.4), 176 (8.2), and 179 (5.8)].

During biosynthetic investigations, *in vivo* incorporation of sodium [2-^{14}C]acetate and L-[U-^{14}C]phenylalanine into *Ageratum conyzoides* plants was examined. In view of structure (**1**), a C_6–C_3 precursor like phenylalanine will not be expected to incorporate into the B and C rings of conyzorigun. However, the results obtained did indicate efficient incorporation of L-[U-^{14}C]phenylalanine (1.134×10^6 d.p.m. mmol^{-1}, incorporation 0.02%), in addition to that of sodium [2-^{14}C]acetate (1.177×10^6 d.p.m. mmol^{-1}, incorporation 0.002%).

This incorporation of precursors observed in the case of conyzorigun is in close agreement with that of nobiletin, 5,6,7,8,3′,4′-hexamethoxyflavone, isolated during these experiments (1.943×10^6 d.p.m. mmol^{-1}, incorporation 0.02%; 1.566×10^6 d.p.m. mmol^{-1}, incorporation 0.002% respectively for the same precursors). The incorporation of a C_6–C_3 precursor is suggestive of a flavonoid skeleton for conyzorigun.

Adesogan and Okunade reported, that conyzorigun formed 2-hydroxy-3,4,5,6-tetramethoxyactophenone (**2**) and 3,4-methylenedioxy-5-methoxyphenol (**3**) with alkali (4M-sodium hydroxide in n-propanol, 6 h). In contrast, however, we observed that the compound yielded [(i) 4M sodium hydroxide in n-propanol, 6 h; (ii) 50% potassium hydroxide in aqueous methanol, 9 h] the same acetophenone (**2**) but not the phenol (**3**). Instead, the other product was found to be an aromatic acid, obtained by sodium hydrogen carbonate treatment of an ethyl acetate extract of the acidified reaction mixture. This was characterised as 3,4-methylenedioxy-5-methoxybenzoic acid (**5**) [myristicic acid; m.p. 209 °C (from methanol) (lit.,[5] 208 °C; lit.,[8] 209—210 °C)]. Its i.r. spectrum showed characteristic absorptions for an aromatic acid at 3 200—2 350 (br, CO_2H), 1 690 (PhCO), 1 045, and 926 cm^{-1} (OCH_2O). Myristicic acid (**5**) could be easily converted into its methyl ester, by treatment with diazomethane, which showed i.r. absorption at 1 715 cm^{-1} (PhCOO). The mass spectrum of the ester indicated M^+ at *m/z* 210 (88%), followed by peaks at *m/z* 179 ($M^+ - OCH_3$, 100), and 151 ($M^+ - CO_2CH_3$, 14). It is interesting to note that the methyl ester of myristicic acid gave the same M^+ at *m/z* 210, as reported for the acetate of the phenol (**3**), by Adesogan and Okunade. Formation of an acetophenone and a benzoic acid by the alkaline degradation is well known in the case of flavones, *e.g.* eupalestin.[4,5] The above degradation thus confirms that conyzorigun is a flavone.

To ascertain the incorporation of L-[U-^{14}C]phenylalanine into the B and C rings of conyzorigun, the compound obtained was subjected to alkali degradation in a similar manner. It was observed that the major part of the activity of conyzorigun (1.027×10^5 d.p.m. mmol^{-1}) could be located in myristicic acid (**5**), the B-ring degradation product (0.700×10^5 d.p.m. mmol^{-1}; expected activity as per ^{14}C ratio 7:9 would be 0.799×10^5 d.p.m. mmol^{-1}).

On the basis of these observations it is concluded that conyzorigun (**1**) is not a chromone with the oxygen linkage between the benzopyrone and phenyl moieties but it is a flavone with 5,6,7,8,5′-pentamethoxy-3′,4′-methylenedioxy substitution, *i.e.* eupalestin (**4**).

Experimental

M.p.s were determined on a Fisher-Johns melting point apparatus and are uncorrected. U.v. spectra of the compounds were taken in methanol on a Carl-Zeiss RPQ 20A spectrophotometer. I.r. spectra were recorded either in KBr or as thin film on a Perkin-Elmer Infracord 137-B spectrophotometer. ^{1}H N.m.r. spectra were obtained by using a Varian A-60A spectrometer with $CDCl_3$ as solvent and $SiMe_4$ as the internal standard. Mass spectral studies were carried out on a VG Micromass 7070 F instrument. H.p.l.c. was performed on Waters Associates 440 Liquid Chromatograph. The radiochemicals used in biosynthetic experiments were obtained from the Isotope Division, Bhabha Atomic Research Centre, Bombay-400085, India. The radioactivity of the compounds was assayed on a Beckman LS 100 liquid scintillation counter using BBOT in toluene as the medium.

Isolation of Conyzorigun (**1**).—Dried *Ageratum conyzoides* plants were finely powdered (1.5 kg) and extracted with ethanol (16 l) in a percolator. The extract was concentrated under reduced pressure and the resultant mass was treated overnight with aqueous sodium hydroxide (1M; 800 ml). This was then extracted with diethyl ether (4 l). The ether extract, washed thoroughly with water and dried over anhydrous sodium sulphate, was concentrated to give a viscous yellow liquid (34.3 g) which was subjected to a preliminary chromatographic separation [neutral alumina, (ratio 1:25); elution with 0—50% ethyl acetate in benzene]. A fraction obtained by elution with 20—25% ethyl acetate in benzene contained conyzorigun [comparison with the reference sample by multiple t.l.c. (× 3) on silica gel, benzene–ethyl acetate 7:3 v/v] along with three other aromatic compounds identified later as flavones.

This fraction (3.1 g) was further chromatographed on a long column [silica gel, (ratio 1:120), 0—100% ethyl acetate in benzene as the eluant system]. The fraction containing conyzorigun as the major component (0.24 g) was purified by multiple (× 3) preparative t.l.c. The compound obtained (0.16 g; m.p. 186 °C) still showed the presence of 5,6,7,8-tetramethoxy-3′,4′-methylenedioxyflavone, as an impurity. It was further purified by repeated crystallisations from hot methanol (0.076 g; m.p. 192 °C) (lit.,[1] m.p. 192—193 °C). Conyzorigun isolated thus was found to be homogeneous with the reference sample with respect to mixed m.p., multiple co-t.l.c. and co-h.p.l.c. (carried out on a μ-Bondapak-C_{18} column with methanol–water eluant system) [Found: C, 60.65; H, 4.85. Calc. for $C_{21}H_{20}O_{10}$ (**1**): C, 58.33; H, 4.63%. Calc. for $C_{21}H_{20}O_9$ (**4**): C, 60.58; H, 4.81%; $\lambda_{max.}$(MeOH) 274 and 336 nm (ε 18 650 and 21 600 dm^3 mol^{-1} cm^{-1}]; $\nu_{max.}$ 1 639 (cross conjugated C=O), 1 585, 1 563, 1 511(Ph), 1 370, 1 048, 934 (OCH_2O) cm^{-1}; δ_H 3.95 (6 H, s, Ar-OMe), 3.97 (3 H, s, Ar-OMe), 4.02 (3 H, s, Ar-OMe), 4.10 (3 H, s, Ar-OMe), 6.08 (2 H, s, OCH_2O), 6.55 (1 H, s, vinyl-H), 7.09 (1 H, d, *J* 1 Hz, ArH), 7.15 (1 H, d, *J* 1 Hz, ArH); *m/z* 416 (M^+, 19%), 401 (100), 387 (6), 373 (8), 371 (5), 358 (16), 340 (15), 225 (9), 197 (21), 182 (19), 179 (6), and 176 (12) [lit.,[1] $\lambda_{max.}$ 272 and 338 nm (ε 19 000 and 22 000 dm^3 mol^{-1} cm^{-1}); $\nu_{max.}$ 1 638 cm^{-1}; δ_H 3.99 (6 H, s, Ar-OMe), 4.00 (3 H, s, Ar-OMe), 4.03 (3 H, s, Ar-OMe), 4.13 (3 H, s, Ar-OMe), 6.02 (2 H, s, OCH_2O), 6.50 (1 H, s, vinyl-H), 6.95 (1 H, d, *J* 1 Hz, ArH), 7.08 (1 H, d, *J* 1 Hz, ArH); literature[5] values for eupalestin: $\lambda_{max.}$ 272 and 333 nm; $\nu_{max.}$ 1 640 (cross conjugated C=O), 1 590, and 1 515 cm^{-1} (Ph); δ_H 3.94 (6 H, s, Ar-OMe), 3.97 (3 H, s, Ar-OMe), 4.00 (3 H, s, Ar-OMe), 4.08 (3 H, s, Ar-OMe), 6.06 (2 H, s, OCH_2O), 6.55 (1 H, s, vinyl-H), 7.09 (1 H, d, *J* 1 Hz, ArH), 7.15 (1 H, d, *J* 1 Hz, ArH); *m/z* 416.111 (M^+, 27.7%), 401 (100), 387 (25.7), 371 (17), 340 (7.1), and 358 (14.6)].

Incorporation of Sodium [2-^{14}C]*Acetate and* L-[U-^{14}C]-*Phenylalanine into Conyzorigun* (**1**).—Sodium [2-^{14}C]acetate (1.694×10^9 d.p.m.) was diluted with carrier sodium acetate (0.014 g) and dissolved in distilled water. L-[U-^{14}C]Phenylalanine (0.099×10^9 d.p.m., available as a solution in 2 ml 0.05M-hydrochloric acid) was diluted with carrier L-phenylalanine (0.012 g) dissolved in aqueous hydrochloric acid (0.05M; 8 ml). These solutions were then administered into two sets of flowering *Ageratum conyzoides* plants by the 'wick method'. The plants were harvested after 7 days (fresh weight 264 and 152 g respectively) and worked up to isolate [^{14}C]conyzorigun (0.013 g, 1.177×10^6 d.p.m. mmol^{-1} and 0.0072 g, 1.134×10^6 d.p.m. mmol^{-1} respectively) in the same manner as described above.

Alkali Degradation of Conyzorigun (**1**).—Conyzorigun (**1**) (0.025 g, 0.06 mmol) was suspended in methanol (2 ml) and boiled until most of it was dissolved. Aqueous potassium hydroxide (50%, 5 ml) was then added and the reaction mixture refluxed under nitrogen for 9 h (oil-bath, 130 °C). The reaction mixture was cooled, diluted with water, and extracted once with diethyl ether to remove any unchanged conyzorigun. It was then acidified with aqueous hydrochloric acid (20%) and extracted thoroughly with ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate (10%) followed by water, dried (Na_2SO_4), and concentrated under reduced pressure to obtain a viscous bright yellow liquid (0.0105 g). This was purified by preparative t.l.c. (silica gel) to give 2-hydroxy-3,4,5,6-tetramethoxyacetophenone (**2**) (0.008 g, 52%) which was identified by comparison (co-t.l.c., u.v., i.r., mass spec.) with a reference sample obtained by a similar alkali degradation of 5,6,7,8,3′,4′,5′-heptamethoxyflavone, isolated earlier from the same plant.

The sodium hydrogen carbonate extract was acidified with dilute aqueous hydrochloric acid and extracted with ethyl acetate. This was washed with water, dried (Na_2SO_4) and concentrated under reduced pressure to give a pale brownish solid (0.0073 g). It was purified by crystallisation from methanol to give colourless needles (0.0049 g, 42%) and was identified as 3,4-methylenedioxy-5-methoxybenzoic acid (**5**) (myristicic acid), m.p. 209 °C (lit.,[5] 208 °C; lit.,[8] 209—210 °C); $\nu_{max.}$ 3 200—2 350, 1 690, 1 613, 1 471, 1 433, 1 370, 1 333, 1 277, 1 045, 943, and 926 cm^{-1}. The acid (**5**) was readily converted into its methyl ester by the addition of ethereal diazomethane, $\nu_{max.}$ 1 715, 1 615, 1 471, and 1 042 cm^{-1}; *m/z* 210 (M^+, 88%), 179 (100), and 151 (14).

Degradation of [^{14}C]*Conyzorigun.*—[^{14}C]Conyzorigun (**1**) (0.001 g, 2.84×10^3 d.p.m.) obtained by incorporation of L-[U-^{14}C]phenylalanine was diluted with the carrier compound (0.0105 g) (resultant specific activity of conyzorigun being 1.027×10^5 d.p.m. mmol^{-1}), and was degraded in the same way as described earlier to give 2-hydroxy-3,4,5,6-tetramethoxyacetophenone (**2**) and 3,4-methylenedioxy-5-methoxybenzoic acid (**5**). The acetophenone was purified by preparative t.l.c. and was assayed for radioactivity (0.0023 g, 0.067×10^5 d.p.m. mmol^{-1}). The acid was crystallised twice from methanol and assayed (0.0014 g, 0.700×10^5 d.p.m. mmol^{-1}).

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