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BIOSYNTHESIS OF PRECOCENES-I AND -II, ANTI-JUVENILE HORMONES

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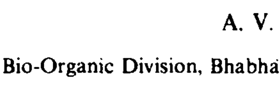
Key Word Index—*Ageratum conyzoides*; Compositae; precocenes-I and -II; isolation and biosynthesis; 2,2-dimethylchromenes.

Abstract—Administration of [2-^{14}C]-sodium acetate and [2-^{14}C]-mevalonic acid to *Ageratum conyzoides* plants has shown that the aromatic moiety of the precocenes is derived from acetate and the other five carbon atoms are of terpenoid origin.

2,2-Dimethylchromenes [precocenes-I and -II (**1** and **2**)] isolated from plants of the genus *Ageratum* have been found to exhibit antijuvenile insect hormone activity [1]. The most active component of the plant extract, precocene-II (**2**), led to precocious metamorphosis of insect larvae to give juvenile or moribund miniature adults.



Precocene-I, **1** R = H
Precocene-II, **2** R = OMe

It was thought worthwhile to study the biosynthesis of these compounds. It was earlier suggested that the biogenesis of 2,2-dimethylchromenes started from a *C*-dimethylallyl phenol precursor. The reaction is initiated by oxidation of the phenol ion followed by the loss of benzylic proton to give an *O*-methylene intermediate which cyclized to furnish the chromene [2]. However, this does not explain the substitution in the aromatic ring.

We suggest that the aromatic ring arises from the polyketide route and condenses with dimethylallyl pyrophosphate arising from mevalonic acid. Subsequent hydroxylation followed by methylation of the aromatic ring would yield the precocenes. To prove this hypothesis, [2-^{14}C]-sodium acetate and [2-^{14}C]-mevalonic acid were administered to *Ageratum conyzoides* plants by the wick technique and the plants harvested after 9 days. The radiolabelled precocenes-I and -II were isolated and purified to constant activity. Precocene-II from [2-^{14}C]-mevalonic acid was degraded to locate the activity of the label [3] as was precocene-I obtained from [2-^{14}C]-sodium acetate [4]. It was found that a major part of the activity of precocene-II obtained from [2-^{14}C]-mevalonic acid is located at one of the *gem*-dimethyl groups (**2**). Similarly the major part of the activity of precocene-I obtained from [2-^{14}C]-sodium acetate is located in the aromatic nucleus. It thus seems probable that precocenes are synthesized by a pathway involving three units of acetate and one of mevalonic acid.

EXPERIMENTAL

IR spectra were taken as either liquid films or KBr pellets. NMR spectra were run in CCl_4 with TMS as int. standard. MS analysis

Table 1. Specific activities of undiluted precocenes obtained from [2-^{14}C]-mevalonic acid and [2-^{14}C]-sodium acetate and their degradation products

	[2-^{14}C]-Mevalonic acid (0.3 mCi, 4.23 mg, 9.23 mCi/mM)			[2-^{14}C]-Sodium acetate (0.74 mCi, 4.14 mg, 19.8 mCi/mM)		
	Yield (%)	Activity (10^7 dpm/mM)	Incorporation (%)	Yield (%)	Activity (10^7 dpm/mM)	Incorporation (%)
Precocene-I	0.03	0.475	0.08	0.02	0.973	0.03
Precocene-II	0.03	0.589	0.10	0.03	1.32	0.05
Acetone–semicarbazone	—	0.541	—	—	—	—
Resorcinol	—	—	—	—	0.768	—

was carried out at the National Chemical Laboratory, Poona. TLC was performed on Si gel G, spots being detected by observing the plates under UV lamp, I_2 staining or spraying the plates with H_2SO_4 (10%) and heating at 100°.

Islation of precocenes. Fresh plants were homogenized with Me_2CO (*ca* 2 ml/g) and the macerate was then extracted with 5 × 500 ml Et_2O–Me_2CO (1:1). The extracts were pooled and concd under red. pres. to a dark green crude mass (*ca* 1.9 %). This was subjected to steam distillation for 8 hr and the distillate was extracted with 5 × 200 ml $CHCl_3$, the $CHCl_3$ extract dried and concd to give a liquid (*ca*. 0.25 %) which was chromatographed on Si gel G TLC plates, using C_6H_6–EtOAc (99:1) as solvent. Precocenes-I and -II having R_fs 0.75 and 0.38, respectively were obtained and further purified. The identity of the two precocenes was established by comparing reported spectral data [5].

Degradation of [^{14}C]*-precocene-*II *obtained from* [2-^{14}C]*-mevalonic acid.* [^{14}C]-Precocene-II and carrier precocene-II in MeOH (free of Me_2CO) were heated with aq. KOH (30 %) for 4 hr at 140–145°. The mixture was distilled into semicarbazide hydrochloride reagent and the Me_2CO-semicarbazone was purified by $CHCl_3$ extraction and prep. TLC (Si gel G, $CHCl_3$–MeOH (95:5), R_f 0.55). [^{14}C]-Me_2CO-semicarbazone was further purified by crystallization from 95 % EtOH (0.541 × 10^7 dpm/mM).

Degradation of [^{14}C]*-precocene-*I *obtained from* [2-^{14}C]*-sodium acetate.* [^{14}C]-Precocene-I and carrier material in Me_2CO was refluxed with powdered $KMnO_4$ for 6 hr. The reaction mixture was refluxed for an additional 2 hr and Me_2CO was removed from the reaction mixture by evapn. Distilled H_2O was added and the mixture decolorized by SO_2 gas and then extracted with EtOAc. The EtOAc soln was concd under red. pres. to obtain the acid product which was further purified by crystallization from EtOAc–petrol, bp 60–80°. It was characterized by comparing with cold diacid obtained in a similar way from cold precocene-I. Diacid had mp 146°, UV λ_{max}^{MeOH} nm (ε): 283 (3161), 253 (11 990). IR ν_{max}^{KBr} cm^{-1}: 3195 (OH), 3225–2500 (COOH), 1761 (C=O, aliphatic COOH), 1681 (C=O, aromatic COOH), 1613, 1567 (C=C aromatic), 1389, 1372 (*gem*-dimethyl), 756.4 (aromatic). ^{1}H NMR ($CDCl_3$): δ 1.6 (*s*, 6H, *gem*-dimethyl), 3.71 (*s*, 3H, OMe), 6.61 (*m*, 2H, aromatic), 8.1 (*m*, 3H). MS, M^+ 254. The acid was refluxed with HBr (45 %) for 2.5 hr at 120–125° and the product extracted with EtOAc (20 × 5 ml). After drying (Na_2SO_4), it was concd under red. pres. and chromatographed by prep. TLC (Si gel G, C_6H_6–EtOAc–HOAc, 79:14:7) to obtain resorcinol (3.8 mg, 7.68 × 10^6 dpm/mM) which was further purified by prep. TLC.

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