The Synthesis of the Epoxide and some other possible Metabolites of Precocene I

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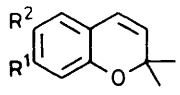
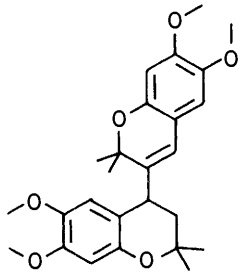
The synthesis of precocene I epoxide, 3,4-epoxy-7-methoxy-2,2-dimethylchroman (**8**), is described. This compound is the highly reactive metabolite responsible for the biological effects of the precocenes. The syntheses of some other oxygenated derivatives of precocene I, which are themselves possible metabolites, are also described.

The chromene (**1**)† was isolated first from *Ageratum houstonianum* (Mill) and from *A. conyzoides* (L.)[2] and was given the name ageratochromene. The desmethoxy analogue (**2**) and a dimeric form of ageratochromene (**6**) were isolated subsequently from *A. conyzoides*.[3] During a search for naturally occurring compounds with anti-juvenile hormone activity, both (**1**) and (**2**) were found in *A. houstonianum* and these compounds were found to induce precocious metamorphosis when administered to the larval stages of certain sensitive species of insects.[4] As a result of their biological properties, the compounds were renamed precocene I(**2**) and II(**1**) and it is by these names that they have been known during the extensive biological and chemical investigations that have been carried out following the discovery of their unique properties.





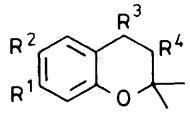
(**6**)

(**1**) $R^1 = R^2 = OMe$
(**2**) $R^1 = OMe, R^2 = H$
(**3**) $R^1, R^2 = OCH_2O$
(**4**) $R^1 = OH, R^2 = H$
(**5**) R^1, R^2 = either OH or OMe

The potentially important role of these compounds in the search for new, specific insecticides with novel modes of action[4,5] prompted immediate investigations into their mode of action. The reports that the diols (**9**) were metabolites of (**1**) in some insect tissues[6] coupled with the fact that compounds such as bromobenzene and the carcinogenic polycyclic aromatic hydrocarbons are known to be converted into their biologically active forms by epoxidation, led to the speculation[7] that the epoxides (**7**) and (**8**) could be the biologically active species in this case. The methylenedioxy analogue (**3**) was synthesised and was found to protect the insects against the effects of the precocenes.[8] Aromatic methylenedioxy compounds are well known inhibitors of mixed-function oxidases of the cytochrome P_{450} type and this seemed to confirm the initial speculation.

† The compounds in this paper are named as derivatives of chroman and chromen as this system of nomenclature seems to be the simplest and is in common use. Reference 1 contains a full discussion of this and of alternative systems of nomenclature.

It was shown by experiments with sensitive species of insect[9] that the precocenes cause the specific destruction of the *corpora allata*, the glands where the juvenile hormones are biosynthesised, and that the precocious moulting results from the lack of juvenile hormone in the treated insect. Since the *corpora allata* are the target organs for the precocenes, the metabolism of [4-^{3}H]precocene I *in vitro* by isolated glands from adult female *Locusta migratoria* was investigated.[10] It was found that not only were the *cis*- and *trans*-diols (**10a, b**) the sole low molecular weight metabolites produced but that extensive incorporation of tritium into macromolecular components of the cells which make up the *corpora allata* also occurred.



(**7**) $R^1 = R^2 = OMe, R^3R^4 = O$
(**8**) $R^1 = OMe, R^2 = H, R^3R^4 = O$
(**9**) $R^1 = R^2 = OMe, R^3 = R^4 = OH$, (a) *cis*, (b) *trans*
(**10**) $R^1 = OMe, R^2 = H, R^3 = R^4 = OH$, (a) *cis*, (b) *trans*
(**11**) $R^1 = OMe, R^2 = H, R^3 = m\text{-}ClC_6H_4CO_2, R^4 = OH$, (a) *cis*, (b) *trans*
(**12**) $R^1 = OMe, R^2 = H, R^3 = Bu^tO_2, R^4 = OH$
(**13**) $R^1 = OMe, R^2 = H, R^3 = OH, R^4 = Cl$
(**14**) $R^1 = OMe, R^2 = H, R^3 = OH, R^4 = Br$
(**15**) $R^1 = OMe, R^2 = H, R^3 = NCOCH_2CH_2CO, R^4 = Br$
(**16**) $R^1 = R^3 = OMe, R^2 = H, R^4 = OH$
(**17**) $R^1 = OMe, R^2 = R^4 = H, R^3 = OH$
(**18**) $R^1 = OMe, R^2 = R^3 = H, R^4 = OH$
(**19**) $R^1 = R^2 = OMe, R^3 = H, R^4 = OH$
(**20**) $R^1 = R^3 = OH, R^2 = R^4 = H$

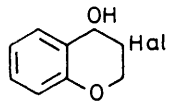
These findings reinforce the hypothesis that the precocenes require activation by oxidation to the epoxide. The synthesis of (**8**), described previously in preliminary form[11] and the study of its reactions[12] showed that it is an extremely reactive molecule which possesses all the properties appropriate for a potent cytotoxin. It appears, therefore, that the precocene I is converted into this highly reactive species within the insect's *corpora allata* and that it either reacts with water by an S_N1 process to form the *cis*- and *trans*-diols[12] (**10a, b**) or with other cell nucleophiles. The latter reaction leads to cell death, cessation of juvenile hormone production, and the consequent biological effects upon the insect. Only glands that are actively synthesising juvenile hormones are affected, and since the terminal step of juvenile hormone biosynthesis involves an epoxidation by a P_{450} linked epoxidase,[13] it appears that this could be the enzyme also responsible for the epoxidation of the precocenes.

The diols that are produced by the metabolism of precocene I are formed stereospecifically[14] and their configurations have

been assigned.[15] This provides more evidence for the intermediacy of the epoxide and confirms that it is produced enzymatically but hydrated by a non-enzymatic process. The metabolism of the precocenes by other insect tissues has been investigated[16,17] in both sensitive and insensitive species but specific cytotoxic effects have only been described for the *corpora allata*. The metabolism of precocene I and II by rat liver has been studied[15,18] and precocene II has been found to cause liver and kidney necrosis in rats.[18] A similar biological mechanism has been invoked to explain these findings since again the diols (**9**) are found as metabolites and the labelling of macromolecules is observed.

The investigation of the metabolism of precocene I in insects necessitated the synthesis of some potential metabolites and the methods used and some associated reactions are the subject of this paper. In the initial study of the metabolism of precocene II, it was claimed[6] that the epoxide (**7**) could be isolated not only from the reaction between precocene II and *m*-chloroperoxybenzoic acid but also as a metabolite from *in vitro* preparations, along with the 3,4-diols (**9**), of unspecified stereochemistry, and the corresponding chroman-3-ol (**19**) which was itself synthesised by reducing the epoxide with sodium borohydride. The synthesis of the epoxide by this method was queried[17] and a careful reassessment and successful synthesis by an alternative route[19] showed that the original work was in error. The reaction of (**2**) with the same peracid, even under buffered conditions, failed to give the epoxide and produced a 1:1 mixture of the *cis*- and *trans*-3,4-diols (**10**) esterified at the 4-position with *m*-chlorobenzoic acid. These esters (**11**) were separated by preparative high-performance liquid chromatography and were then hydrolysed with alcoholic potassium hydroxide to the corresponding diols (**10**).

The configurations of the half-esters and of the diols were assigned from their ^{1}H n.m.r. spectra. In these compounds, and in all related structures, $J_{3,4\text{-}trans}$ was greater than 7 Hz and $J_{3,4\text{-}cis}$ was less than 5 Hz.[20] These assignments were substantiated by the unambiguous synthesis of the *cis*-diol (**10a**) by reaction of precocene I with osmium tetraoxide and by the fact that the epoxide (**8**), of fixed *cis*-configuration, has $J_{3,4}$ 4 Hz. The size of the coupling constants suggests a diequatorial arrangement for the 3 and 4 substituents in the *trans*-compounds[20] and a 4-equatorial-3-axial arrangement in the *cis*-series. By contrast, in the related halohydrins (**21**) $J_{3,4\text{-}trans}$



(**21**)

is 2.5 Hz[21] and the 3 and 4 substituents are thought to adopt a diaxial arrangement to minimise dipole–dipole repulsions and to alleviate the interaction between the 4-equatorial substituent and the 5-hydrogen on the aromatic ring. In the present series, such an arrangement would lead to an unfavourable interaction between the 4-axial substituent and the axial C-2 methyl group and thus the pyran ring adopts the configurations described to minimise this[22] (see Figure).

The reaction of olefins with t-butyl hydroperoxide in the presence of certain transition-metal catalysts has proved to be a highly effective way of preparing epoxides.[23] In this case, however, no reaction was observed between precocene I and the peroxide in the presence of vanadyl acetylacetonate but in the presence of hexacarbonylmolybdenum, a more polar product was formed which was assigned the hydroxy-peroxide structure (**12**). A similar product was tentatively identified when (**1**) was subjected to these reaction conditions.[17]

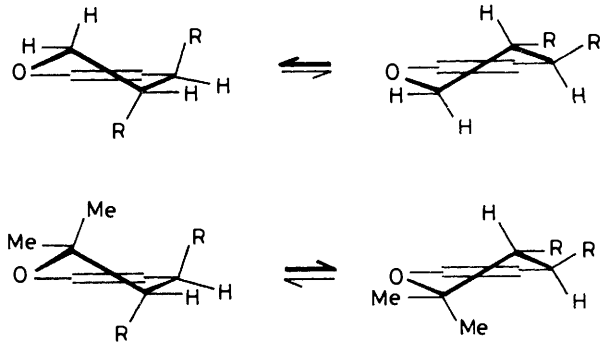
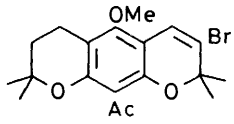


Figure.

An attempt was made previously to synthesise the epoxide (**8**) by treating the chlorohydrin (**13**) with sodium hydroxide in ether.[24] The expected epoxide was not formed and the product obtained was assigned the 3,4-diol structure (**10**) but of unspecified stereochemistry. The melting point of this substance (115 °C) does not correspond to the melting point of either of the pure isomers and suggests that a mixture of the two may have been formed. Since the epoxide could not be obtained by direct methods, reinvestigation of the ring-closure of a suitable halohydrin was undertaken. The chlorohydrin (**13**) was prepared previously by the addition of chlorine to the 3,4-double bond of (**2**) followed by displacement of the 4-chlorine with acetate and then hydrolysis of this group. The bromohydrin could not be prepared in a similar way since the initial bromine adduct was found to be highly unstable.[24] Reaction of (**2**) with *N*-bromosuccinimide in aqueous tetrahydrofuran yielded the desired product (**14**), however, as well as the product (**15**) resulting from the direct addition of *N*-bromosuccinimide to the 3,4-double bond. This compound could be obtained as the sole product from the reaction of (**2**) with *N*-bromosuccinimide in anhydrous dichloromethane and is a highly crystalline, stable material which survives chromatography. Similar compounds have been postulated[25] as intermediates in the formation of the vinyl bromides (**22**) and (**23**) obtained by treating the appropriate chromans with the same reagent but in these cases ready elimination of succinimide occurred and the vinyl bromides shown were obtained.

(**22**)

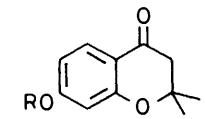


(**23**)

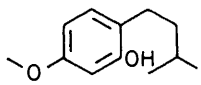
The bromohydrin (**14**) was treated with anhydrous potassium carbonate in methanol but instead of the epoxide, a good yield of the monomethyl ether of the *trans*-diol (**16**) was obtained. All these approaches suggested that the epoxide was being formed but was reacting with any nucleophiles present in the system to give the observed products. Reaction of the bromohydrin with a non-nucleophilic base was, therefore, attempted and it was found that the desired epoxide (**8**) could be obtained in high yield by treating the bromohydrin with sodium hydride in anhydrous conditions. The epoxide was obtained as an analytically pure, crystalline substance which can only be purified by crystallisation and is indeed highly reactive.[12] The role that this compound plays in the biological activity of these

compounds has been discussed[10] but other potential metabolites were required for a full investigation of the metabolic transformations of precocene I.

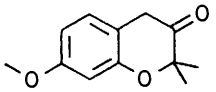
Oxidative demethylation of methoxy groups is a common metabolic pathway and a synthesis of the phenol (**4**) was required. Conjugates of the phenols (**5**) have been identified as metabolites of (**1**) in several species of insect[17] and the free phenols as metabolites in a fat body homogenate from the cabbage looper *Trichoplusia ni*.[19] The phenol (**4**) is a useful intermediate for the synthesis of analogues of the precocenes[26] and has been mentioned briefly in a paper[27] describing the n.m.r. of this and of related compounds but was prepared by an extremely low-yielding route. The hydroxychromanone (**25**)



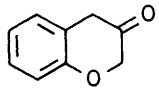
(**24**) R = Me
(**25**) R = H



(**26**)



(**27**)



(**28**)

was prepared as described[28] and was reduced to the chromanol (**20**) with lithium aluminium hydride. This somewhat unstable substance was characterised as the corresponding dibenzoate, and was readily dehydrated to the desired phenol by exposure to aqueous hydrochloric acid.[4] Alternative conditions which are often very effective for the dehydration of chroman-4-ols, namely a trace of toluene-*p*-sulphonic acid in refluxing benzene or toluene,[29] and which have been claimed to render all other methods obsolete,[30] in this case yielded only polymeric products.

The chroman-3- and -4-ones (**27**), and (**24**) and the chroman-3- and -4-ols (**17**), (**18**) are all, in principle, possible metabolites. The 4-oxygenated derivatives are available as intermediates *en route* to the precocenes themselves[4] but whereas the chemistry of such compounds is well known, that of the 3-oxygenated derivatives has been much less investigated.[1]

The chroman-3-ol (**19**) was identified as a metabolite in the initial study of the metabolism of (**1**)[4] but it was claimed that it had been synthesised by the reduction of the epoxide (**7**) with sodium borohydride. The identity of the metabolite has been confirmed rigorously in a subsequent study[19] although it was synthesised by a different method, as this study also showed that the identity of the epoxide prepared in the intitial work was in doubt.

Hydroboration of the precocenes, followed by oxidation, would be expected to introduce a hydroxy group at the 3 position[31] and the reaction of diborane, generated *in situ*, with (**2**) has been described previously.[32] In this case, however, an oxidative work-up gave an unstable mixture of alcohols which was oxidised with chromic acid to a mixture of ketones from which only the chroman-4-one (**24**) was isolated, in 22% yield. This reaction was repeated using a commercial solution of borane in tetrahydrofuran and, again, after treatment of the hydroboration reaction mixture with alkaline hydrogen peroxide, a mixture of alcohols was obtained. This mixture was separable by chromatography and the chroman-3-ol (**18**) was isolated in 31% yield. The ring-opened product (**26**) was also isolated in 38% yield and both of these products result from the addition of boron to the 3-position, followed presumably, in the latter case, by elimination of the phenolate anion and re-hydroboration of the newly introduced double bond.[31] Only a small amount (3%) of the chroman-4-ol (**17**) was obtained by this route. By contrast similar treatment of (**1**) is reported[19] to give mainly (**19**) (41%) and the chroman-4-ol (24%).

Attempts to oxidise the chroman-3-ol to the chroman-3-one (**27**) with chromium-based reagents gave complex mixtures of products which probably reflects the instability of the product to further oxidation[1] and so an alternative route to this compound was sought. The unsubstituted chroman-3-one (**28**) has been synthesised by dehydration of the corresponding 3,4-diol with anhydrous copper sulphate in benzene at 200 °C in a sealed tube.[33] Treatment of a mixture of the *cis*- and *trans*-3,4-diols (**10**) with anhydrous copper sulphate in refluxing toluene gave a moderate yield of the desired ketone (**27**). This was obtained as an unstable oil which was characterised by the formation of crystalline derivatives and which, on treatment with lithium aluminium hydride, gave the same chroman-3-ol as that prepared by the route described above.

The mass spectra of the chroman-3-one (**27**) and the epoxide (**8**) are very similar. Both show ions formed by the loss of carbon monoxide, methyl and carbon monoxide, and C_4H_5O, a retro-Diels–Alder fragment.[34] Thermal or acid-catalysed rearrangement of the 3,4-epoxides to the corresponding chroman-3-ones should be easy, owing to the relative stability of the C_4 carbonium ion,[12] and this may occur within the mass spectrometer. The chroman-3-ones can also be formed by dehydration of the 3,4-diols and these observations may, therefore, help to explain the apparent identification by g.l.c.–m.s. of the epoxide (**7**) in a supposedly pure sample of the *cis*-diol (**9a**).[19]

Reduction both of the 3,4-epoxides and of the chroman-3-ones with hydride-type reducing agents gives the chroman-3-ols. The chroman-3-ol (**19**) was apparently synthesised from (**7**) by this method and was used to confirm the identity of (**19**) formed as a metabolite.[6] The subsequent study[19] confirmed the identity of the metabolite but showed that it could not have been synthesised by the route described. If, however, the chroman-3-one had been obtained from the reaction of (**1**) with *m*-chloroperoxybenzoic acid by rearrangement of the initially formed unstable epoxide (**7**) then the above observations could be explained.

Experimental

M.p.s are uncorrected. I.r. spectra of solids (paraffin mulls) and liquids (neat) were recorded on a Perkin-Elmer 257 spectrophotometer. N.m.r. spectra were obtained on a Jeol FX90Q machine for solutions in deuteriochloroform with tetramethylsilane as the internal standard. Mass spectra were obtained on Varian CH5 or Ribermag R10-10C mass spectrometers. Preparative thin layer chromatography (p.l.c.) was carried out on plates coated with Merck Kieselgel GF_{254} (0.5 mm). Preparative high pressure liquid chromatography (h.p.l.c.) was carried out on a Waters LC500 machine and preparative radial centrifugal chromatography (p.r.c.c.) on a Chromatotron model 7924 using a plate coated with Merck Kieselgel 60 PF_{254} (2 mm) and the solvent indicated at a flow rate of 5 ml min^{-1}.

Solutions were dried over anhydrous sodium sulphate and ether refers to diethyl ether throughout.

Reaction of Precocene I (**2**) *with* m-*Chloroperoxybenzoic Acid.*—*m*-Chloroperoxybenzoic acid (14.2 g, 85%) was added in portions to a stirred solution of precocene I[4] (12 g) in dichloromethane (250 cm^3). The reaction was stirred for 3 h and

then washed successively with aqueous sodium sulphite, aqueous sodium hydrogen carbonate, and brine. The organic phase was dried and evaporated to leave a sticky brown foam which was dissolved in 30% ethyl acetate–hexane and filtered through a short column of alumina. The eluant was evaporated to give a cream foam (18 g) from which two compounds were isolated by preparative h.p.l.c. (2 PREPAK cartridges, 30% ether–pentane, 200 cm^3 min^{-1}). The faster eluting component, trans-4-(3-*chlorobenzoyloxy*)-3-*hydroxy*-7-*methoxy*-2,2-*dimethylchroman* (**11b**) (4.7 g), a viscous oil, had $\nu_{max.}$ 3 465, 1 720, 1 625, 1 590, 1 255, 1 130, and 750 cm^{-1}; δ 1.38, 1.52 (6 H, 2 s, CMe_2), 2.15br (1 H, d, *J* 7 Hz, 3-OH), 3.76 (3 H, s, OMe), 3.92 (1 H, m, 3-H), 6.04 (1 H, d, *J* 7 Hz, 4-H), 6.38 (1 H, d, *J* 2.5 Hz, 8-H), 6.50 (1 H, dd, *J* 2.5, 8 Hz, 6-H), 7.10 (1 H, d, *J* 8 Hz, 5-H), 7.20—8.00 (4 H, m, ArH); addition of D_2O caused the signal at 2.15 to vanish and the multiplet at 3.92 to collapse to a doublet, *J* 7 Hz (Found: M^+, 364.0886, 362.0918. $C_{19}H_{19}ClO_5$ requires M^+, 364.0876, 362.0906). The second component, cis-4-(3-*chlorobenzoyloxy*)-3-*hydroxy*-7-*methoxy*-2,2-*dimethylchroman* (**11a**) (6.1 g) crystallised with time and was recrystallised from ether–pentane; it had m.p. 105—106 °C, $\nu_{max.}$ 3 460, 1 720, 1 625, 1 590, 1 250, 1 105, and 750 cm^{-1}; δ 1.43, 1.52 (6 H, 2 s, CMe_2), 2.9br (1 H, d, *J* 5 Hz, 3-OH), 3.76 (3 H, s, OMe), 4.05 (1 H, d, *J* 4.5 Hz, 3-H), 6.22 (1 H, d, *J* 4.5 Hz, 4-H), 6.40 (1 H, d, *J* 2.5 Hz, 8-H), 6.49 (1 H, dd, *J* 2.5, 8 Hz, 6-H), 7.10 (1 H, d, *J* 8 Hz, 5-H), and 7.20—8.00 (4 H, m, ArH) (Found: C, 62.8; H, 5.25. $C_{19}H_{19}ClO_5$ requires C, 62.9; H, 5.3%).

cis- *and* trans-3,4-*Dihydroxy*-7-*methoxy*-2,2-*dimethylchroman* (**10**).—The *trans*-half-ester (**11b**) (310 mg) in ethanol (2.5 cm^3) and aqueous sodium hydroxide (2M; 2.5 cm^3) was stirred at room temperature for 30 min. The bulk of the solvent was removed under reduced pressure and the residue was partitioned between brine and ether. The organic layer was washed with water, dried, and evaporated to give the *trans*-diol (**10b**) (175 mg) which had m.p. 124.5—125.5 °C (ethyl acetate–hexane), $\nu_{max.}$ 3 370, 3 250, 1 062, and 1 025 cm^{-1}; δ 1.24, 1.50 (6 H, 2 s, CMe_2), 2.10br (1 H, d, *J* 8 Hz, 4-OH), 2.58br (1 H, s, 3-OH), 3.56br (1 H, d, *J* 8 Hz, 3-H), 3.74 (3 H, s, OMe), 4.50br (1 H, t, *J ca.* 8 Hz, 4-H), 6.30 (1 H, d, *J* 2.5 Hz, 8-H), 6.50 (1 H, dd, *J* 2.5, 8 Hz, 6-H), 7.28 (1 H, d, *J* 8.5 Hz, 5-H); addition of D_2O caused the signals at 2.10 and 2.58 to disappear, the signal at 3.56 to sharpen, and the signal at 4.50 to collapse to a doublet, *J* 8 Hz (Found: C, 64.3; H, 7.2%; M^+, 224.1055. $C_{12}H_{16}O_4$ requires C, 64.25; H, 7.2%; M^+, 224.1044).

The *cis* half-ester (**11a**) (230 mg) was treated analogously to give the *cis*-diol (132 mg) (**10a**) which had m.p. 101—102 °C (ethyl acetate–hexane), $\nu_{max.}$ 3 430, 3 340, 1 145, 1 035, and 990 cm^{-1}; δ 1.28, 1.48 (6 H, 2 s, CMe_2), 2.05 (1 H, d, *J* 8 Hz, OH), 2.48 (1 H, d, *J* 8 Hz, OH), 3.60—3.78 (4 H, m, OMe, 3-H), 4.74 (1 H, dd, *J* 5 Hz, 8 Hz, 4-H), 6.32 (1 H, d, *J* 2.5 Hz, 8-H), 6.55 (1 H, dd, *J* 2.5, 8.5 Hz, 6-H), 7.39 (1 H, d, *J* 8.5 Hz, 5-H); addition of D_2O caused the signals at 2.05 and 2.48 to disappear and the signals at *ca.* 3.6 and *ca.* 4.7 to collapse to doublets at 3.64 and 4.7 *J* 5 Hz (Found: C, 64.2; H, 7.15%; M^+, 224.1035. $C_{12}H_{16}O_4$ requires C, 64.25; H, 7.2%; M^+, 224.1044).

Hydroxylation of Precocene I *with Osmium Tetraoxide.*—Osmium tetraoxide (142 mg) was added to a solution of precocene I (103 mg) in pyridine (3 cm^3) and the black reaction mixture which resulted was stirred for 3 h. Aqueous sodium metabisulphite solution was added and the mixture was stirred for a further 30 min and then partitioned between dichloromethane and water. The organic layer was washed well with water and aqueous copper sulphate and then dried. Evaporation of the solvent left the *cis*-diol (**10a**) (110 mg) identical in all respects with the material prepared as described above.

Reaction of Precocene I *with t-Butyl Hydroperoxide.*—Hexacarbonylmolybdenum (10 mg) and t-butyl hydroperoxide (300 μl, 70%) were added to a stirred solution of precocene I (190 mg) in benzene (10 cm^3). The reaction mixture was brought to reflux and after 4 h the same amounts of reagents were again added. After the mixture had been heated for a further 4 h under reflux, the reaction appeared to be complete (t.l.c.); the mixture was then cooled and partitioned between ether and aqueous sodium sulphite. The organic layer was washed with brine, dried, and evaporated to leave an oil (206 mg) which was purified by p.l.c. (20% ethyl acetate–hexane). Starting material (10 mg, R_F 0.75) was recovered and trans-3-*hydroxy*-7-*methoxy*-2,2-*dimethyl*-4-*t-butylperoxychroman* (**12**) (103 mg, R_F 0.6) was obtained as an oil which had $\nu_{max.}$ 3 535, 1 625, 1 590, 1 505, 1 365, 1 200, and 990 cm^{-1}; δ 1.28, 1.48 (6 H, 2 s, CMe_2), 1.36 (9 H, s, Bu^t), 3.14 (1 H, d, *J* 4 Hz, OH), 3.72 (3 H, s, OMe), 3.95 (1 H, dd, *J* 4 Hz, 7 Hz, 3-H), 4.90 (1 H, d, *J* 7 Hz, 4-H), 6.24 (1 H, d, *J* 2.5 Hz, 8 Hz), 6.44 (1 H, dd, *J* 2.5, 8 Hz, 6-H), 7.20 (1 H, d, *J* 8 Hz, 5-H); addition of D_2O caused the signal at δ 3.14 to disappear and the signal at δ 3.95 to collapse to a doublet *J* 7 Hz (Found: M^+, 296.1616. $C_{16}H_{27}O_5$ requires M^+, 296.1617).

Reaction of Precocene I *with* N-*Bromosuccinimide.*—(i) *In aqueous tetrahydrofuran.* Freshly recrystallised *N*-bromosuccinimide (184 mg) was added in small portions to a stirred solution of precocene I (190 mg) in 50% aqueous tetrahydrofuran (10 cm^3) at 0 °C. The reaction was allowed to warm to ambient temperature and then poured into brine. The products were extracted into ether and the extract was washed with aqueous sodium hydrogen carbonate, water and brine, dried, and evaporated to leave an oil (334 mg). Three components were separated by p.l.c. (30% ethyl acetate–hexane). The least polar (41 mg, R_F 0.45) appeared from its n.m.r. and mass spectra to be a mixture of ring brominated materials and was not investigated further. The middle band gave trans-3-*bromo*-4-*hydroxy*-7-*methoxy*-2,2-*dimethylchroman* (**14**) (231 mg, R_F 0.4) which had m.p. 77.0—78.5° (ethyl acetate–hexane), $\nu_{max.}$ 3 360, 1 625, 1 590, 1 505, 1 165, and 1 110 cm^{-1}; δ 1.40, 1.60 (6 H, 2 s, CMe_2), 2.46 (1 H, d, *J* 5 Hz, OH), 3.76 (3 H, s, OMe), 4.08 (1 H, d, *J* 9 Hz, 3-H), 4.86 (1 H, dd, *J* 5 Hz, 9 Hz, 4-H), 6.25 (1 H, d, *J* 2.5 Hz, 8-H), 6.52 (1 H, dd, *J* 2.5, 8.5 Hz, 6-H), and 7.30 (1 H, d, *J* 8.5 Hz, 5-H) (Found: C, 50.35; H, 5.3%; M^+ 286.0195, 288.0178. $C_{12}H_{15}BrO_3$ requires C, 50.2; H, 5.3%; M^+, 286.0171, 288.0181). The most polar material (49 mg, R_F 0.15) was identified as trans-3-*bromo*-7-*methoxy*-2,2-*dimethyl*-4-*succinimidochroman* (**15**) and had m.p. 162.5—164 °C (dichloromethane–pentane), $\nu_{max.}$ 1 705, 1 620, 1 590, 1 200, 1 165, and 1 110 cm^{-1}; δ 1.44, 1.64 (6 H, 2 s, CMe_2), 2.77br (4 H, s, CH_2CH_2), 3.72 (3 H, s, OMe), 4.98 (1 H, d, *J* 11 Hz, 3-H), 5.46 (1 H, d, *J* 11 Hz, 4-H), 6.28—6.42 (2 H, m, 6-,8-H), and 6.60 (1 H, d, *J* 8 Hz, 5-H) (Found: C. 52.15; H, 5.0; N, 3.65. $C_{16}H_{18}BrNO_4$ requires C, 52.2; H, 4.95; N, 3.8%).

(ii) *In dichloromethane.* Freshly recrystallised *N*-bromosuccinimide (180 mg) was added to a stirred solution of precocene I (190 mg) in dry dichloromethane (5 cm^3) at 0 °C. After 45 min the solvent was evaporated and the residue purified by p.r.c.c. (50% ether–hexane) to give the adduct (**15**) (303 mg) which was identical in all respects with that formed in (i).

Reaction of the Bromohydrin (**14**) *with Bases.*—(i) *Potassium carbonate.* Powdered, anhydrous potassium carbonate (200 mg) was added to a stirred solution of the bromohydrin (160 mg) in dry methanol (5 cm^3). The reaction mixture was stirred for 20 h, diluted with ether, filtered, and evaporated. The residue was taken up in ethyl acetate which was then washed with brine, dried, and evaporated to leave an oil which was purified by p.l.c. (30% ethyl acetate–hexane) to give trans-3-*hydroxy*-4,7-*dimeth*-

oxy-2,2-*dimethylchroman* (**16**) (106 mg), m.p. 139.5—140.5 °C (ethyl acetate–hexane), $\nu_{max.}$ 3 420, 1 620, 1 590, 1 270, 1 210, and 1 120 cm^{-1}; δ 1.24, 1.44 (6 H, 2 s, CMe_2), 2.20 (1 H, d, *J* 5 Hz, 3-OH), 3.44 (3 H, s, 4-OMe), 3.72—3.84 (4 H, s, and m, 7-OMe, 3-H), 4.14 (1 H, dd, *J* 7 Hz, 4-H), 6.24 (1 H, d, *J* 3 Hz, 8-H), 6.48 (1 H, dd, *J* 3 Hz, 8.5 Hz, 6-H), and 7.15 (1 H, d, *J* 8.5 Hz, 5-H) (Found: C, 65.25; H, 7.9%; M^+, 238.1228. $C_{13}H_{18}O_4$ requires C, 65.55; H, 7.6; M^+, 238.1200).

(ii) *Sodium hydride.* Sodium hydride (167 mg, 60% in oil) was washed free of oil and then suspended with stirring in dry tetrahydrofuran (15 cm^3) with protection from atmospheric moisture. The bromohydrin (287 mg) was added and after the initial effervescence had subsided, the reaction was stirred for 1 h. The reaction mixture was filtered and the filtrate evaporated to leave an oil. Trituration with dry hexane gave 3,4-*epoxy*-7-*methoxy*-2,2-*dimethylchroman* (189 mg) (**8**), m.p. 45—46 °C (hexane), $\nu_{max.}$ 1 620, 1 580, 1 160, 1 135, 1 035, and 870 cm^{-1}; δ 1.25, 1.57 (6 H, 2 s, CMe_2), 3.42 (1 H, d, *J* 4.5 Hz, 3-H), 3.74 (3 H, s, OMe), 3.85 (1 H, d, *J* 4.5 Hz, 4-H), 6.25—6.50 (2 H, m, 6-, 8-H), and 7.15 (1 H, d, *J* 8 Hz, 5-H); *m/z* 206 (M^+, 100%), 178 (42, $M - CO$), 177 (17), 163 (71, $M - CH_3$, CO), 150 (27), 149 (11), 137 (72, $M - C_4H_5O$), 135 (17), 120 (28), 105 (15), 91 (13), 89 (12), 77 (27), 69 (15), 65 (14), 57 (23), 56 (10), 55 (12), and 51 (37) (Found: C, 69.85; H, 6.8%; M^+, 206.0958. $C_{12}H_{14}O_3$ requires C, 69.9; H, 6.8%; M^+, 206.0939).

4,7-*Dihydroxy*-2,2-*dimethylchroman* (**20**).—7-Hydroxy-2,2-dimethyl-4-chromanone[28] (**25**) (195 mg) was dissolved in dry tetrahydrofuran (15 cm^3) and treated with an excess of lithium aluminium hydride (70 mg). The reaction mixture was stirred for 2 h, quenched with a few drops of saturated aqueous sodium sulphate, and partitioned between brine and ethyl acetate. The organic extract was washed with water, dried, and evaporated to give an oil which was purified by p.r.c.c. (ether) to give the title compound as a powdery solid (183 mg) which was crystallised with difficulty; it had m.p. 113—118 °C (ether–hexane), $\nu_{max.}$ 3 320, 3 090, 1 620, 1 605, 1 165, 1 120, and 835 cm^{-1}; δ 1.31, 1.42 (6 H, 2 s, CMe_2), 1.85—2.27 (2 H, m, 3-H), 4.80 (2 H, m, 4-H, OH), 6.26 (1 H, d, *J* 3 Hz, 8-H), 6.42 (1 H, dd, *J* 3 Hz, 8 Hz, 6-H), and 7.28 (1 H, d, *J* 8 Hz, 5-H).

The compound formed a *dibenzoate*, m.p. 95.5—98 °C (ether–hexane), δ 1.45, 1.51 (6 H, s, CMe_2), 2.30 (2 H, m, 3-H), 6.27 (1 H, m, 4-H), 6.80 (2 H, m, 6-, 8-H), 7.30 (1 H, d, *J* 7.6 Hz, 5-H), and 7.3—8.30 (10 H, m, ArH) (Found: C, 74.85; H, 5.5. $C_{25}H_{22}O_5$ requires C, 74.6; H, 5.5%).

7-*Hydroxy*-2,2-*dimethylchromene* (**4**).—The chromanol (**20**) (64 mg) in tetrahydrofuran (1 cm^3) and hydrochloric acid (4M; 1 cm^3) was stirred under an atmosphere of nitrogen for 1 h. The reaction mixture was partitioned between ether and brine and the organic extract was washed with aqueous sodium hydrogen carbonate and brine, dried, and evaporated. The residual oil was purified by p.r.c.c. (30% ether–hexane) to give the *title phenol* (**4**) as an oil (31 mg), $\nu_{max.}$ 3 350, 1 620, 1 505, 1 160, 1 120, and 990 cm^{-1}; δ 2.05 (6 H, s, CMe_2), 5.23br (1 H, s, OH), 5.45 (1 H, d, *J* 10.7 Hz, 3-H), 6.30 (3 H, m, 4-, 6-, 8-H), and 6.82 (1 H, d, *J* 9.7 Hz, 5-H) (Found: M^+, 176.0836. $C_{11}H_{12}O_2$ requires M^+, 176.0834).

Hydroboration of Precocene I (**2**).—A solution of borane in tetrahydrofuran (1M; 2 cm^3) was added to precocene I (500 mg) in dry tetrahydrofuran (10 cm^3). The reaction mixture was left at room temperature for 20 h and then quenched cautiously with a few drops of water. Aqueous sodium hydroxide (3M; 3 cm^3) and aqueous hydrogen peroxide (30%; 3 cm^3) were added with stirring and cooling and the reaction mixture was then warmed to 50 °C for 1 h.

The reaction mixture was cooled, poured into brine, and the products extracted into ether. The organic extract was washed with aqueous sodium metabisulphite and brine, dried, and then filtered through a short column of silica gel. Evaporation of the solvent left an oil which was purified by p.r.c.c. (50% ether–hexane, 4 mm plate, 6 cm^3 min^{-1}). The first compound eluted was 1-(2-*hydroxy*-4-*methoxyphenyl*)-3-*methylbutan*-2-*ol* (**26**) (170 mg), m.p. 89.5—91 °C (ethyl acetate–hexane). $\nu_{max.}$ 3 340, 2 730, 1 630, 1 585, 1 515, 1 290, 1 165, and 1 000 cm^{-1}; δ 0.98 (6 H, d, *J* 7.5 Hz, CMe_2), 1.70 (1 H, m, 3-H), 2.75 (3 H, m, 1-H and 2-OH), 3.62 (1 H, m, 2-H), 3.76 (3 H, s, OMe), 6.40 (2 H, m, 3-, 5-ArH), 6.90 (1 H, d, *J* 8.6 Hz, 6-ArH), and 8.46br (1 H, ArOH); addition of D_2O caused the signals at *ca.* 2.6 Hz and 8.46 to disappear and the signals at 2.75 and 3.62 to sharpen (Found: C, 68.8; H, 8.65. $C_{12}H_{18}O_3$ requires C, 68.5; H, 8.65%). The next compound eluted was 3-*hydroxy*-7-*methoxy*-2,2-*dimethylchroman* (**18**) (137 mg), m.p. 64.5—66 °C (hexane and a trace of ethyl acetate), $\nu_{max.}$ 3 420, 1 630, 1 590, 1 505, 1 200, 1 140, 1 035, and 990 cm^{-1}; δ 1.31, 1.36 (6 H, 2 s, CMe_2), 1.76 (1 H, d, *J* 8.6 Hz, OH), 2.85 (2 H, m, 4-H), 3.75 (3 H, s, OMe), 3.80 (1 H, m, 3-H), 6.41 (2 H, m, 6-, 8-H), and 6.95 (1 H, d, *J* 9 Hz, 5-H) (Found: C, 69.0; H, 7.75. $C_{12}H_{16}O_3$ requires C, 69.2; H, 7.75%). The final compound, an oil (16.4 mg), was identified as the chroman-4-ol (**17**) from its spectral data.

7-*Methoxy*-2,2-*dimethylchroman*-3-*one* (**27**).—The diol (**10**), a mixture of the two isomers (80% *trans*) (150 mg) in toluene (15 cm^3) containing anhydrous copper sulphate (35 mg) was refluxed with stirring for 15 min. The reaction mixture was cooled and decanted from the residual copper salts which were washed well with ether. The combined organic extracts were evaporated to leave an oil which was purified by p.r.c.c. (30% ether–hexane) to give the *title compound* as an oil (81 mg), $\nu_{max.}$ 1 730, 1 625, 1 590, 1 505, and 1 160 cm^{-1}; δ 1.41 (6 H, s, CMe_2), 3.53 (2 H, s, 4-CH_2), 3.79 (3 H, s, OMe), 6.55—6.65 (2 H, m, 6-, 8-H), and 6.98 (1 H, d, *J* 10 Hz, 5-H); *m/z* 206 (M^+, 100%), 191 (11), 178 (51, $M-CO$), 177 (16), 164 (11), 163 (81, $M - CH_3$, CO), 151 (17), 137 (70, $M - C_4H_5O$), 135 (18), 134 (22), 124 (11), 121 (13), 120 (30), 106 (11), 105 (13), 91 (16), 89 (10), 77 (24), 69 (16), 65 (15), 63 (16), 57 (21), 55 (16), and 51 (39).

The ketone formed a 2,4-*dinitrophenylhydrazone*, m.p. 203—206 °C (decomp.) (ethyl acetate–hexane) (Found: C, 55.9; H, 4.45; N, 14.5. $C_{18}H_{18}N_4O_6$ requires C, 55.95; H, 4.7; N, 14.5%) and a *semicarbazone*, m.p. 187—195 °C (decomp.) (ethyl acetate) (Found: C, 59.2; H, 6.5; N, 16. $C_{13}H_{17}N_3O_3$ requires C, 59.5; H, 6.5; N, 16%).

Reduction of 7-*Methoxy*-2,2-*dimethylchroman*-3-*one* (**27**).—The chroman-3-one (**27**) (100 mg) in dry ether (10 cm^3) was treated with lithium aluminium hydride (20 mg) at 0 °C. The reaction was allowed to warm to room temperature over 30 min and was then quenched with a few drops of saturated aqueous sodium sulphate and filtered through a small column of anhydrous sodium sulphate. The solvent was evaporated and the residue was purified by p.r.c.c. (50% ether–hexane) to give the chroman-3-ol (**18**) (78 mg), identical with the material synthesised by the route described previously.

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