Inhibition of Rat Liver Microsomal Lipid Peroxidation Elicited by Simple 2,2-Dimethylchromenes and Chromans Structurally Related to Precocenes

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The effects on rat liver microsomal lipid peroxidation elicited by 2,2-dimethylchromenes and chromans structurally related to precocenes was investigated in NADPH-dependent incubations by using the thiobarbituric acid reactive substances (TBARS) and oxygen uptake rate tests as evaluation methods. Precocene II (1) exhibited an unexpected inhibitory activity on lipid peroxidation (IC_{50} = 11.4 μM, TBARS production test). Among the compounds tested, those which had a hydroxyl group on the aromatic ring, particularly at the C-6 position (i.e., 6-hydroxy-7-methoxy-2,2-dimethyl-1-2*H*-benzopyran (2) and its 3,4-dihydro derivative 5), appeared to be the best inhibitors. In this context, the inhibitory effect elicited by the hydroxychroman 5 (IC_{50} = 0.3 μM, TBARS production test) was higher than that shown by 2,6-di-*tert*-butyl-4-methylphenol (BHT, 11). These results suggested that the lipid peroxidation inhibitory effect exhibited by precocene II and related derivatives could be due to a vitamin E-like free radical scavenger based mechanism. The identification of phenolic metabolites in the incubations performed with precocene II or its benzopyranyl analogues lacking aromatic hydroxyl substituents supported the above postulated mechanism.

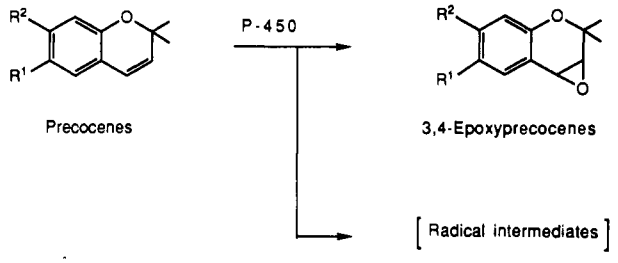
INTRODUCTION

Precocenes (see Scheme I) are naturally occurring 2,2-dimethylchromenes which inhibit insect juvenile hormone (JH) biosynthesis as a result of a specific cytotoxic action on the corpora allata, the JH secretory glands [Bowers et al., 1976; for a review see Staal (1986)]. However, the narrow range of biological activity showed by these toxins caused a gradual decrease in their interest as potential insecticides. Another fact that advised against the potential application of precocenes was their reported hepato- and nephrotoxicity in mammals (Duddy and Hsia, 1989, and references cited therein). Nevertheless, the peculiarities exhibited by precocenes, particularly in relation to their bioactivation mechanisms, have been interesting enough for considering these xenobiotics as useful models for toxicological studies.

In this respect, there is strong evidence supporting the assumption that precocenes undergo a cytochrome P-450 mediated bioactivation to give a highly reactive 3,4-epoxy intermediate, which would be responsible for the cytotoxicity exhibited by the parent compounds (Pratt et al., 1980; Soderlund et al., 1980). However, an unequivocal confirmation of this hypothesis either from in vivo or in vitro assays is still missing, which has been attributed to the extreme chemical reactivity of the above epoxides (Hamnett et al., 1981; Conchillo et al., 1990).

On the other hand, data obtained on chemically generated radical species derived from precocene II (P II, 1) (unpublished results) suggested that radical intermediates could be also contemplated for explaining the cytotoxicity of this compound. Therefore, we were interested in obtaining some evidence about the putative formation of radical intermediates in biological matrices incubated with P II, by looking at some effect linked to free radical mediated mechanisms such as lipid peroxidation. Unexpectedly, preliminary results obtained from the incubation with rat liver microsomes in the presence of NADPH showed that P II exerted an inhibitory effect on lipid peroxidation. With the aim of finding a plausible mechanism which would rationalize the observed effect, further assays were contemplated. Thus, the inhibitory effect on rat liver microsomal lipid peroxidation elicited by a series of 2,2-dimethylchromans and chromenes structurally related to precocenes was evaluated. In addition, identification of primary metabolites formed during the incubations performed with selected substrates was also carried out. The results obtained on this study are herein reported.

Scheme I. Bioactivation Pathways of Precocenes



EXPERIMENTAL PROCEDURES

Synthesis of Compounds. *General.* The IR spectra were recorded with a Perkin-Elmer 399 B spectrometer. The ^1H (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded with a Varian Unity 300 spectrometer. All NMR spectra were performed in neutralized $CDCl_3$ solutions, and chemical shifts are given in parts per million downfield from tetramethylsilane. The gas chromatographic and mass spectrometric (GC–MS, electron impact) analyses were carried out with a Hewlett-Packard 5980 gas chromatograph (12-m OV-1 bonded-phase capillary column) coupled to a Hewlett-Packard 5970 mass spectrometer.

Test Compounds and Metabolites (Chart I and Scheme II). Preparation of precocene II (1) and chroman-4-ol (17) has been previously reported (Teixidor et al., 1988). Synthesis of chromenes 2, and 14 and of diols 13 (cis/trans isomeric mixture) was described by Soderlund et al. (1980). Compounds 3 and 7-hydroxy-2,2-dimethyl-1(2*H*)-benzopyran (standard not shown in Scheme II)

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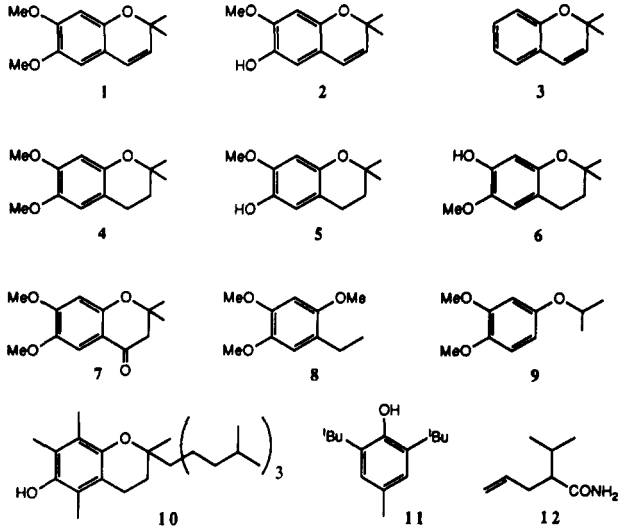
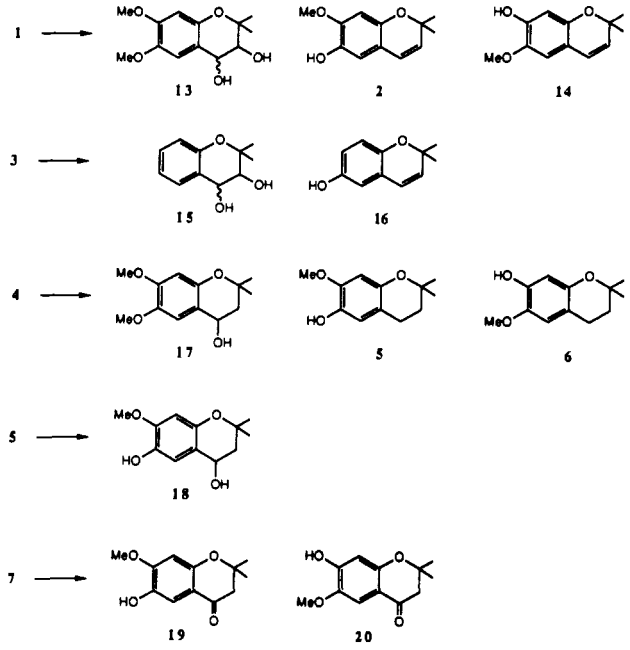
Chart I. Prococene Analogues and Reference Compounds Tested as Lipid Peroxidation Inhibitors



Scheme II. Metabolites Generated in the Incubation of Prococene Analogues with Rat Liver Microsomes



(Schweizer and Meeder-Nycz, 1977) were prepared according to the procedure reported by Teixidor et al. (1988). Chroman-4-ones **7**, **19**, and **20** were prepared according to the method of Camps et al. (1980). Standard metabolite **18** was prepared following the procedure of Anastasis and Brown (1982). α-Tocopherol (**10**) and 2,6-di-*tert*-butyl-4-methylphenol (BHT, **11**) were commercially available. 2-Isopropylpent-4-enamide (AIA, **12**) was a generous gift from Hoffman-La Roche. A sample of 6-hydroxychromene (**16**) was kindly supplied by Dr. J. L. Bernier (Inserm, Lille, France).

(*a*) *Preparation of Chromans 4–6. General Procedure.* A mixture of Zn pellets (0.6 g), $HgCl_2$ (0.06 g), H_2O (1 mL), and concentrated HCl (0.03 mL) was vigorously stirred for 5 min at 25 °C. After the supernatant was discarded, 8 N HCl (1.5 mL) and a solution of the corresponding 4-chromanone (1 mmol) in toluene (2–3 mL) were sequentially added to the residue and the mixture was stirred at 100 °C until reaction was completed (GC monitoring). Then the crude reaction mixture was allowed to cool, the organic layer was separated, and the aqueous phase was extracted with methyl *tert*-butyl ether (2 × 25 mL). The collected organic fractions were washed with $NaHCO_3$ saturated solution (1 × 25 mL) and brine (1 × 25 mL) and dried over $MgSO_4$. The residue obtained after solvent removal was purified by flash chromatography eluting with hexane/ethyl acetate mixtures to give the expected chromans as pure compounds, which were identified by comparison with authentic samples or by comparison of their spectral data (^1H and ^{13}C NMR, MS) with those previously reported. 3,4-Dihydro-6,7-dimethoxy-2,2-dimethyl-1(2*H*)-benzopyran (**4**) was isolated as a solid in 80% yield (Camps et al., 1979). 3,4-Dihydro-6-hydroxy-7-methoxy-2,2-dimethyl-1(2*H*)-benzopyran (**5**) was isolated as a solid in 76% yield (Anastasis and Brown, 1982). 3,4-Dihydro-7-hydroxy-6-methoxy-2,2-dimethyl-1(2*H*)-benzopyran (**6**) was isolated as a solid in 72% yield (Al-Khayat et al., 1987).

(*b*) *1-Ethyl-2,4,5-trimethoxybenzene* (**8**). This compound was synthesized from 2,4,5-trimethoxyacetophenone by using the above reduction procedure and isolated as a pale yellow oil in 85% yield (Högberg et al., 1990).

(*c*) *1-Isopropoxy-3,4-dimethoxybenzene* (**9**). A solution of isopropyl iodide (0.51 g, 3 mmol) in *N*,*N*-dimethylformamide (3 mL) was added to a suspension of 3,4-dimethoxyphenol (0.30 g, 2 mmol) and K_2CO_3 (0.63 g, 5 mmol) in the same solvent (15 mL), and the mixture was stirred for 1 h at 25 °C. The crude reaction mixture was poured into 2 N HCl (100 mL) and extracted with hexane (2 × 30 mL). The collected organic fractions were washed with 1 N NaOH (2 × 30 mL), H_2O (30 mL), and brine (30 mL) and dried over $MgSO_4$. Evaporation of solvents yielded 0.15 g of pure compound **9** in 40% yield. This poor conversion yield was due to the concomitant formation of 2-isopropyl-4,5-dimethoxyphenol under the reaction conditions used. **9**: IR (CCl_4) 2990, 2820, 1610, 1510 cm^{-1}; ^1H NMR δ 6.76 (d, 1 H, J = 8 Hz), 6.51 (d, 1 H, J = 2.5 Hz), 6.40 (dd, 1 H, J_1 = 8 Hz, J_2 = 2.5 Hz), 4.45 (h, 1 H, J = 6 Hz), 3.85 (s, 3 H, CH_3O), 3.83 (s, 3 H, CH_3O), 1.32 (d, 6 H, J = 6 Hz); ^{13}C NMR δ 152.3, 149.8, 143.4, 111.8, 105.8, 102.4, 70.6, 56.4, 55.7, 22.1; MS, m/z (%) 196 (M^+, 44), 154 (85), 139 (100).

(*d*) *3,4-Dihydro-3,4-dihydroxy-2,2-dimethyl-1(2H)-benzopyran* (**15**). A mixture of chromene **3** (0.080 g, 0.5 mmol) and *m*-chloroperoxybenzoic acid (0.10 g) in dichloromethane (5 mL) was allowed to react for 2 h at 25 °C. Then the solvent was removed under vacuum, and the residue was redissolved in dimethoxyethane (5 mL) and treated with 1 N NaOH (2 mL). The crude reaction mixture was stirred for 3 h at 25 °C, concentrated under vacuum, and extracted with ethyl acetate (3 × 10 mL). The combined organic fractions were washed with brine (1 × 10 mL) and dried over $MgSO_4$. The residue obtained after solvent removal was purified by preparative thin-layer chromatography (hexane/ethyl acetate 1:2) to give pure compound **15** as a cis:trans 1:3 isomeric mixture (0.085 g, 86% yield): IR ($CHCl_3$) 3600, 3400, 1580, 1480, 1400 cm^{-1}; ^1H NMR δ 6.6–7.5 (8 H, Ar H), 4.71 (d, 1 H, J = 4.5 Hz, H-4, cis isomer), 4.46 (d, 1 H, J = 8.5 Hz, H-4, trans isomer), 3.92 (br, s, 4 H, OH), 3.57 (d, 1 H, J = 4.5 Hz, H-3, cis isomer), 3.51 (d, 1 H, J = 8.5 Hz, H-3, trans isomer), 1.40 (s, 6 H), 1.20 (s, 3 H, cis isomer), 1.13 (s, 3 H, trans isomer); ^{13}C NMR δ 153.0 (Ar CO), 130.1 (Ar CH), 129.4 (Ar CH), 128.2 (Ar CH), 124.1 (Ar CH), 122.8 (Ar C), 121.8 (Ar C), 121.4 (Ar CH), 79.4 (Me_2CO), 79.2 (CHOH, trans isomer), 78.5 (Me_2CO), 72.2 (CHOH, cis isomer), 70.4 (CHOH, trans isomer), 66.0 (CHOH, cis isomer), 27.4 (CH_3, trans isomer), 25.6 (CH_3, cis isomer), 24.2 (CH_3, cis isomer) 19.5 (CH_3, trans isomer). Treatment of **15** with an excess of acetic anhydride in pyridine led to the formation of the corresponding 3,4-diacetoxy derivatives: MS, m/z (%) 278 (M^+, 4), 218 (7), 203 (19), 161 (100).

Log *P* and Dipolar Moment Values. Log *P* and dipolar moment values for test compounds **1–9** and for BHT (**11**) were calculated according to the method described by Bodor et al. (1989), by using fully optimized structures based on AM1 calculations.

Evaluation of Lipid Peroxidation. *Incubations.* Suspensions of male Sprague-Dawley rat liver microsomes in Krebs–Ringer phosphate buffer, pH 7.4 (KRP), were prepared according to the method of Fraga et al. (1988). Microsomal protein (2 mg) in 1 mL of KRP buffer was incubated during 1 h at 37 °C in the presence of 0.1 mM NADPH and 2 μL of dimethyl sulfoxide (DMSO) containing the test compound at the appropriate concentration. Incubations were stopped by addition of 4% BHT (0.2 mL).

Incubations in the Presence of 2-Isopropylpent-4-enamide (AIA, 12). To show the importance of oxidative metabolism for

Table I. Effect of Compounds Structurally Related to Precocenes on Rat Liver Microsomal Lipid Peroxidation, Expressed as IC_{50} Values for the Inhibition of TBARS Production and as Oxygen Uptake Percentage of Inhibition[a]

compd	IC_{50} TBARS production, μM	% inhibition of oxygen uptake[b]
1	11.4	8.5
2	1.4	45.2
3	3.8	16.0
4	15.0	28.7
5	0.3	39.8
6	3.4	25.6
7	31.4	24.8
8	14.0	ND[c]
9	17.0	ND
10	204	4.6
11	0.8	43.8
12	NI[d]	4.6

[a] For details, see Experimental Procedures. [b] Determinations were carried out at 10 μM inhibitor concentration. [c] Not determined. [d] No inhibitory effect was observed up to 1 mM concentration.

eliciting the observed lipid peroxidation inhibitory effects, incubations with selected substrates were carried out in the presence of AIA, a well-known inhibitor of cytochrome P-450 (Ortiz de Montellano and Mico, 1981). Accordingly, rat liver microsomes were incubated for 15 min as described above in the presence of 0.5 mM AIA (**12**) and 0.1 mM NADPH. Then, compound **4** or **5** was added to the medium at two different final concentrations (1 and 25 μM for **4**; 0.1 and 1 μM for **5**), which corresponded approximately to those causing a 10% and 50% inhibitions of TBARS production, respectively. Then, incubations were pursued during 1 h. Finally, lipid peroxidation was determined as described below. Values of percentage of inhibition were calculated considering as zero the TBARS production value at minute 15. Incubations carried out with 0.5 mM AIA and 0.1 mM NADPH were taken as controls.

TBARS Test. After incubations were completed, lipid peroxidation was evaluated by the production of thiobarbituric acid reactive substances (TBARS) as described by Fraga et al. (1988). Briefly, 0.5 mL of 3% sodium dodecyl sulfate was added to 0.5-mL aliquots of microsomal incubations. After mixing, 2 mL of 0.1 N HCl, 0.3 mL of 10% phosphotungstic acid, and 1 mL of 0.7% 2-thiobarbituric acid were added. The mixture was heated for 30 min at 100 °C, and TBARS were extracted with 3 mL of 1-butanol. After centrifugation, the fluorescence of the butanol layer was measured at 515-nm excitation and 555-nm emission. The values obtained were expressed as nanomoles of TBARS (malondialdehyde equivalents) per gram of liver. Malondialdehyde standards were prepared from 1,1,3,3-tetramethoxypropane. Assays were performed in triplicate. The IC_{50} values were determined by plotting percent inhibition vs log [*I*], using at least five different concentrations. Percentage inhibition values are referred to a measurement carried out in the presence of DMSO (2 μL). No chemical interference in the TBARS test was observed when P II (**1**) or the hydroxy derivative **5** was added to standard amounts of malondialdehyde.

Oxygen Uptake. Microsomal oxygen consumption was measured polarographically by utilizing a Clark-type electrode (Model 5300 biological oxygen monitor, Yellow Springs Instrument Co.). Assays were performed at 37 °C during 10 min in the presence of NADPH (0.1 mM) and the corresponding inhibitor at 10 μM final concentration. Percentage inhibition values depicted in Table I are referred to a measurement carried out in the presence of DMSO (2 μL).

Metabolism Studies. Microsomal protein (2 mg) in 1 mL of KRP buffer was incubated during 1 h at 37 °C in the presence of 0.1 mM NADPH and 2 μL of dimethyl sulfoxide (DMSO) containing the test compound at 50 μM final concentration. Incubations were stopped by extraction of unaltered substrate and generated metabolites with methyl *tert*-butyl ether (2 × 2 mL). Organic extracts were evaporated under nitrogen, redissolved in methanol, and stored at −20 °C until used. Identification of metabolites was carried out by comparison of their relative retention times in HPLC with those of authentic samples from synthetic origin. The HPLC/UV analyses were carried out with an Applied Biosystems set constituted by a Model 400 solvent delivery system, a Model 491 injector, and 1000S diode array detector. Samples were injected onto a Lichrospher 100 RP-18 column (125 × 4 mm, 5 μm, Merck), using mixtures of methanol/formic acid/triethylamine (50 mM) buffer, pH 3.5, as mobile phase. Identification of metabolites was confirmed by GC–MS analysis, using the above extracts as crude samples or after trimethylsilyl derivatization.

RESULTS AND DISCUSSION

Although there is strong evidence supporting the assumption that the deleterious effects elicited by precocenes in insects and mammals are linked to a cytochrome P-450 mediated bioactivation of the C-3,C-4 double bond of the molecule, which would generate a 3,4-epoxy derivative as key cytotoxic intermediate (Pratt et al., 1980; Hsia et al., 1981), this hypothesis has not been unequivocally demonstrated. On the other hand, it is accepted that monooxygenase-mediated pathways may involve the formation of substrate-derived radical species (White, 1991). If this were the case for precocenes, these species could participate in the etiology of the observed cellular damage. Therefore, we were interested in obtaining some evidence on the potential generation of P II derived radical intermediates in NADPH-dependent incubations with biological matrices by looking at some effect linked to free radical mediated mechanisms such as lipid peroxidation.

Somehow, contrary to our expectations, P II (**1**) showed an inhibitory activity of rat liver microsomal lipid peroxidation (IC_{50} = 11.4 μM, TBARS production test). Therefore, to elucidate the structural features of **1** that could be responsible for the observed effect, the lipid peroxidation inhibitory activity of different precocene analogues, including chromenes, chromans, and nonheterocyclic structures (cf. compounds **2–9**, Chart I) was investigated. The TBARS production test and the oxygen consumption rate were the methods used for this evaluation, and results obtained are shown in Table I. Values for α-tocopherol (**10**) and BHT (**11**) were also determined for comparison purposes.

Some interesting observations could be derived from the results concerning the TBARS production test. First, chromenes **2** and **3** exhibited higher inhibitory activities (IC_{50} = 1.4 and 3.8 μM, respectively) than that elicited by P II. On the other hand, results of incubations with chromans **4–6** indicated that the presence of the C-3,C-4 double bond was not a structural requisite for obtaining inhibitory activity. Thus, the 3,4-dihydro analogue of precocene II (**4**) showed an activity (IC_{50} = 15.0 μM) within the same range as that exhibited by compound **1**, whereas chromans **5** and **6** elicited higher inhibitory effects (IC_{50} = 0.3 and 3.4 μM, respectively). Remarkably, hydrochroman **5** was the most potent antioxidant within the compounds tested, and its activity was even higher than that obtained with a well-known antioxidant such as BHT (**11**) (IC_{50} = 0.8 μM). Finally, chromanone **7**, a molecule containing a deactivating group at C-4, showed a moderate antioxidant effect. As it has been described elsewhere (Battioni et al., 1991), and probably due to its high lipophilicity, α-tocopherol (**10**) elicited a poor antioxidant activity under the in vitro conditions used.

A study was performed to derive possible relationships between the antioxidant activities of compounds **1–9** and physicochemical parameters such as their partition coefficient (log *P*) or dipolar moment (μ). Values for these parameters were determined by using the method reported by Bodor et al. (1989) and are shown in Table II. In this

Table II. Calculated Log *P* and Dipolar Moment Values for Compounds Structurally Related to Precocenes[a]

compd	log *P*[b]	dipolar moment (μ), D
1	2.97 (2.77)	1.42
2	2.65	1.63
3	3.16	1.07
4	2.94 (2.74)	2.00
5	2.67	1.84
6	2.62	2.51
7	2.48	2.32
8	2.70	1.12
9	2.68	1.72
11	4.00	1.38

[a] For details, see Experimental Procedures. [b] Values in parentheses are referred to experimental log *P* values determined as described by Camps et al. (1986).

respect, calculated log *P* values for derivatives 1 and 4 agreed satisfactorily with those previously determined by HPLC techniques (Camps et al., 1986). As shown, log *P* values for compounds 1–9 were within a relatively narrow range (2.48 and 3.16). However, neither these values nor those derived from the calculated dipolar moments could be correlated with TBARS production data for compounds 1–9. These results suggest that under our incubation conditions, and provided that lipophilicity of all substrates appeared to be within the range required for eliciting the observed inhibitory activity, the basic mechanism of this effect had to be related to other structural features, probably more connected with the aromatic ring substitution pattern.

An important structural feature common to compounds eliciting the highest inhibitory activities, i.e., 4 and 5, was the presence of a hydroxyl group at C-6. From this point of view, these derivatives could be considered α-tocopherol analogues (cf. with 10). As it has been demonstrated by the group of Ingold (Burton and Ingold, 1986), the spatial arrangement between the hydroxyl group and the oxygen atom of the 2,2-dialkylpyranyl moiety present in vitamin E permits an optimal stabilization of phenoxyl radicals, which accounts for the high efficiency of this compound as free radical scavenger. A similar mechanism would explain that hydroxychroman 5, the more closely related analogue of α-tocopherol, would be the best inhibitor among the compounds tested. In this sense, the presence of a more reactive moiety toward metabolic degradation in hydroxychromene 2 could justify its lower inhibitory activity in comparison with that of hydroxychroman 5.

Assuming the hypothesis of a vitamin E-like based mechanism to account for the observed inhibitory effect, the question was how to explain the activities of precocene II and related analogues lacking hydroxyl substituents in the aromatic ring. An obvious possibility was that the required phenol groups are generated under the incubation conditions. In this context, an in vitro study on the metabolism of P II in fat body homogenates of *Trichoplusia ni* showed the major formation of the corresponding *cis/trans*-3,4-dihydrodiol derivatives 13 and the presence of minor amounts of compounds 2 and 14 (cf. Scheme II) (Soderlund et al., 1980). In addition, an in vivo study performed on different insect species revealed that O-demethylation followed by glucosidation was the major metabolic route for P II (Bergot et al., 1980). Therefore, to confirm our hypothesis, the metabolism of compounds 1, 3–5, and 7 under the incubation conditions was investigated, and results obtained are shown in Scheme II.

Thus, for the case of precocene II, HPLC/UV and GC–MS analysis of the organic extract revealed, besides the

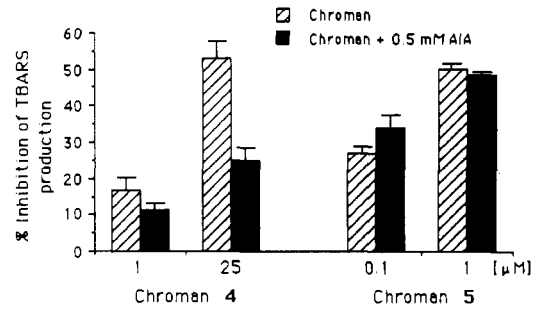


Figure 1. Effect of AIA (12) on the inhibition of lipid peroxidation elicited by chromans 4 and 5. Incubations were carried out as described under Experimental Procedures. TBARS production was not affected by AIA at the concentration of 0.5 mM. Percentage inhibition values are referred to a measurement carried out in the presence of DMSO (2 μL). Error bars indicate standard deviations of triplicates.

formation of the corresponding cis/trans mixture of 3,4-dihydrodiol derivatives 13 as major metabolites, the presence of the hydroxychromenes 2 and 14. Although a quantitative study was not performed, compound 2 was clearly the predominant phenolic compound. Likewise, metabolites demethylated at C-6 or C-7 were identified in the incubation extracts of chroman 4 (cf. compounds 5 and 6) and 4-chromanone (7) (cf. compounds 19 and 20). In the first case, the presence of the 4-hydroxy derivative 17 was detected as major metabolite. Similarly, a compound resulting from a benzylic hydroxylation (i.e., 18) was the only metabolite identified in the case of incubations performed with hydroxychroman 5, a substrate already containing a phenolic substituent. Finally, incubations carried out with chromene 3 led to the identification of three metabolites: the 3,4-dihydrodiols 15 (as a cis:trans isomer mixture) as major components and 6-hydroxychromene 16. In this case, the corresponding phenolic isomer at C-7, available as standard, was not detected under our analytical conditions.

Results derived from these metabolic studies showed that the major metabolic pathway for all derivatives, with the exception of ketone 7, involved a C-4 or C-3,C-4 oxidation. In addition, demethylation leading to phenolic derivatives at C-6 or C-7 was a common metabolic pathway for all dimethoxylated precocene analogues assayed, and a phenolic metabolite was also formed in the case of the only demethoxy precocene analogue tested (i.e., 3). Therefore, the fact that all substrates lacking hydroxyl aromatic substituents were capable of generating phenolic metabolites made possible the presence of species with a potential vitamin E-like lipid peroxidation inhibitory activity.

In this context, the importance of oxidative metabolism for the lipid peroxidation effects elicited by the above derivatives was also confirmed by the measure of the TBARS production in incubations carried out with chroman 4 or its hydroxy derivative 5 in the presence of AIA (12), a well-known inhibitor of cytochrome P-450 (Ortiz de Montellano and Mico, 1981). As shown in Figure 1, incubations performed in the presence of AIA decreased the inhibitory activity of compound 4. Thus, at 25 μM 4 inhibition was 52%, whereas coincubation with 0.5 mM AIA reduced this to 25%. Therefore, inhibition of cytochrome P-450 mediated metabolism resulted in a clear decrease of the antioxidant effect exhibited by chroman 4. Conversely, coincubations of hydroxychroman 5 with AIA did not alter or only caused a slight increase of the inhibitory activity. These results suggest that inhibition of lipid peroxidation found for hydroxychroman 5 seemed to be related to the compound itself, whereas that exhibited

by the dimethoxychroman 4 appeared to be due to its cytochrome P-450 generated metabolite(s).

With all of these considerations, the data obtained on the inhibitory activity measured by the oxygen rate consumption test deserve some comments. As shown in Table I, values of inhibition percentages of oxygen uptake for compounds 1–7 did not follow the pattern obtained with the TBARS production test. Thus, incubations carried out in the presence of hydroxy derivatives 2 and 5 showed the highest oxygen uptake inhibition percentages (45.2 and 39.8%, respectively). These values were comparable to those found in incubations with BHT (43.8%). However, oxygen uptake was less inhibited in the presence of P II or chromene 3 (8 and 16%, respectively). As it has been shown, under the incubation conditions used, these substrates underwent metabolic degradation involving oxygen consumption. Although the incubation time of this assay (10 min) was considerably lower than that used for the TBARS production tests, the results obtained suggest that the inhibition percentages found for phenols 2, 5, and 11 might reflect more properly their antioxidant capability. Conversely, in the cases of P II and chromene 3, the oxygen consumption due to an activated oxidative metabolism may counterbalance the inhibition caused by the phenolic metabolites generated in the incubation medium. These considerations advise that data derived from oxygen consumption rate tests should be taken with caution as an evaluation method for lipid peroxidation, particularly when substrates undergo a concomitant oxidative metabolism during the incubation.

In summary, our results show that precocenes and related compounds inhibit rat liver microsomal lipid peroxidation and that this effect appears to be due to a free radical scavenger mechanism operated by phenolic species already present or generated in the incubation. These results would question the exclusive consideration of precocenes as harmful compounds, since their antioxidant effects could play a protective role under specific circumstances [cf. Howard et al. (1979)]. Thus, the high doses needed for eliciting antigonadotropic activity after topical application on *Blattella germanica* adult females (100–200 μg/insect; Bellés et al., 1985) or for inducing irreversible cytotoxic effects in in vitro incubations of the corpora allata of this cockroach (Bellés et al., 1988) could be the result of a complex combination of protective and deleterious factors, particularly in the case of the in vivo treatments. Finally, we believe that a design of precocene analogues in which metabolic deactivation at C-7 and at the double bond could be minimized would give rise to molecules with a highly potent antioxidant activity that could find application as peroxyl radical scavengers. Work along this line is in progress in our laboratory.

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Registry No. **1**, 644-06-4; **2**, 74094-51-2; **3**, 2513-25-9; **4**, 69888-42-2; **4** (chromanone), 65383-61-1; **5**, 83923-51-7; **5** (chromanone), 74094-44-3; **6**, 115546-37-7; **6** (chromanone), 74094-45-4; **7**, 65383-61-1; **8**, 125198-30-3; **9**, 77106-50-4; **10**, 10191-41-0; **11**, 128-37-0; **12**, 299-78-5; **13**, 62471-04-9; **14**, 74094-52-3; **15**, 91970-66-0; **16**, 19012-99-8; **17**, 62470-72-8; **18**, 74094-59-0; **19**, 74094-44-3; **20**, 74094-45-4; 2,4,5-trimethoxyacetophenone, 1818-28-6; isopropyl iodide, 75-30-9; 3,4-dimethoxyphenol, 2033-89-8; 2-isopropyl-4,5-dimethoxyphenol, 139585-02-7.