# Reactions of Precocene II Epoxide with Model Nucleophiles

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The reactivity of precocene II epoxide (3,4-epoxy-6,7-dimethoxy-2,2-dimethyl-2*H*-benzo[*b*]pyran, I) toward several nucleophiles in tetrahydrofuran solution decreased in the following order: L-cysteine methyl ester > thiophenol > morpholine > methanol = water > adenine. Adducts (4-substituted-3,4-di-hydro-3-hydroxy-6,7-dimethoxy-2,2-dimethyl-2*H*-benzo[*b*]pyrans) of all six nucleophiles with I were isolated and characterized. Water, methanol, and adenine gave both cis- and trans-substituted adducts, whereas morpholine, thiophenol, and L-cysteine methyl ester gave trans-substituted adducts only. Three new degradation products of I formed in aqueous media were also isolated and characterized. These findings suggest that nucleophilic attack on I may proceed by either $S_n1$ or $S_n2$ mechanisms. Also, the high reactivity of thiol nucleophiles toward I implicates tissue thiols as possible nucleophiles in the cytotoxic reactions of I with cellular macromolecules.

## INTRODUCTION

Precocene II (6,7-dimethoxy-2,2-dimethyl-2*H*-benzo-[*b*]pyran) is the more potent of two naturally occuring chromenes having antijuvenile hormone activity in several insect species (Bowers, 1976; Bowers et al., 1976). Bioassays have established that oxidative bioactivation of the precocenes is essential to their biological activity (Brooks et al., 1979). Previous studies in this laboratory and elsewhere have suggested the 3,4-epoxide as a critical transient intermediate in the metabolism of precocene II in insects in vivo and in subcellular preparations of insect tissues in vitro (Ohta et al., 1977; Soderlund et al., 1980, 1981; Bergot et al., 1980; Pratt et al., 1980). The synthesis of precocene II epoxide confirmed the extreme lability of this compound and further implicated it as the bioactivated reactive intermediate involved in the antihormonal action of precocene II (Soderlund et al., 1980).

Precocenes selectively destroy the insect corpus allatum (CA), the gland that synthesizes and secretes juvenile hormone (Bowers and Martinez-Pardo, 1977; Unnithan et al., 1977; Pener et al., 1978; Schooneveld, 1979). These tissue-specific cytotoxic effects are hypothesized to result from the alkylation by the reactive epoxide of critical endogenous nucleophiles in the CA (Bowers, 1981; Bowers et al., 1982). Macromolecular labeling by products of precocene oxidation is observed in intact CA (Pratt et al., 1980; Hamnett and Pratt, 1983) and in CA homogenates (Soderlund et al., 1981).

Nucleophilic attack on epoxides by water and other nucleophiles may proceed by either an $S_n1$ or $S_n2$ mechanism, depending on the pH of the reaction medium and the nature of the attacking nucleophile (Long and Pritchard, 1956; Pritchard and Long, 1956; Parker and Isaacs, 1959; Buchanan and Sable, 1972). Studies of the hydration of precocene I epoxide (7-methoxy-2,2-dimethyl-2*H*-benzo[*b*]pyran 3,4-epoxide) implicate an $S_n1$ mechanism in which a trigonal carbonium ion at C-4 acts as the ultimate electrophile in the reaction of this compound with water (Hamnett et al., 1981). Further information of the reactivity of precocene epoxides with a greater variety of nucleophiles is critical to an under-

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standing of their fate and action in living systems. We now report the rates of degradation of precocene II epoxide by several model nucleophiles, the structures of the adducts formed, and the structures of additional degradation products of precocene II epoxide formed in aqueous media.

## MATERIALS AND METHODS

**Chemicals.** Adenine, morpholine, and thiophenol were purchased from Sigma Chemical Co., St. Louis, MO. L-Cysteine methyl ester, which is unstable, was prepared from the hydrochloride (Sigma) by treatment with saturated $NaHCO_3$ prior to each experiment. Precocene II epoxide (I) was synthesized from precocene II (Aldrich Chemical Co., Milwaukee, WI) by a published procedure (Soderlund et al., 1980). All solvents were reagent grade and were glass-distilled before use.

**Chromatography.** Precoated 20 × 20 cm chromatoplates (silica gel 60 $F_{254}$, EM Laboratories, Elmsford, NY) having a gel thickness of 0.25 mm (analytical separations) or 0.50 mm (preparative separations) were used for thin-layer chromatography (TLC). Eleven TLC solvent systems were used: (A) chloroform–methanol (19:1); (B) hexane–ethyl acetate (1:1); (C) ethyl acetate–methanol (5:1); (D) ethyl acetate–methanol (3:1); (E) benzene–ethyl acetate (5:1); (F) benzene–ethyl acetate (1:1); (G) benzene–tetrahydrofuran (1:1); (H) benzene–ethyl acetate (3:1); (I) chloroform–methanol (50:1); (J) chloroform–methanol (9:1); (K) ethyl acetate. The use of these solvent systems is designated by letters throughout the text. Gas–liquid chromatography (GLC) was performed by using a Packard Model 7400 chromatograph equipped with a flame ionization detector and glass column (2 mm × 1.8 m packed with 3% OV-101 on Gas-Chrom W-HP, 100–120 mesh). Gas flow rates were 400, 40, and 30 mL/min for air, $H_2$, and $N_2$ (carrier), respectively. TLC $R_f$ values and GLC retention times and operating temperatures for precocene II epoxide and the adducts and their derivatives described below are summarized in Table I.

**Spectroscopy.** Routine electron impact mass spectra (MS) were obtained with a Hewlett Packard Model 5985 mass spectrometer with data system operated in the direct inlet mode at 20 eV. High resolution mass spectra were obtained with a Hitachi Model M80 spectrometer operated in the direct inlet mode at 70 eV. Infrared spectra (IR, reported in cm$^{-1}$ for major absorption frequencies) were recorded with a Perkin Elmer Model 257 grating spectrophotometer for samples as liquid films, KBr pellets, or chloroform solutions. Nuclear magnetic resonance (NMR) spectra ($^1$H) were determined for dilute solutions in chloroform-*d*, with tetramethylsilane as the internal

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**Table I. Chromatographic Properties of I, Its Adducts and Degradation Products, and Their Derivations**

| compd | TLC $R_f$ (solvent system)[a] | GLC $t_R$, min (condn)[b] |
|---|---|---|
| I | | 13.8 (1), 5.2 (2) |
| precocene II[c] | | 9.5 (1), 4.0 (2) |
| II | 0.11 (A), 0.07 (H), 0.02 (I) | 5.6 (3) |
| II-Ac₂ | 0.35 (E) | 3.9 (4) |
| III | 0.21 (A), 0.09 (H), 0.04 (I) | 5.4 (3) |
| III-Ac₂ | 0.32 (E) | 4.2 (4) |
| IV | 0.65 (A), 0.43 (B), 0.27 (H), 0.12 (I) | 9.6 (3) |
| IV-Ac | 0.34 (E) | 12.8 (3), 4.0 (4) |
| V | 0.82 (A), 0.43 (B), 0.32 (H), 0.11 (I) | 10.2 (3) |
| V-Ac | 0.25 (E) | 12.4 (3), 3.9 (4) |
| VI | 0.64 (D) | |
| VI-Ac₂ | 0.58 (J) | |
| VII | 0.51 (D) | |
| VII-Ac₂ | 0.49 (J) | |
| VIII | 0.30 (F), 0.17 (H), 0.06 (I) | 5.5 (5) |
| VIII-Ac | 0.75 (F) | 6.5 (5) |
| IX | 0.38 (E), 0.78 (H), 0.53 (I) | 8.5 (5) |
| IX-Ac | 0.88 (F) | 10.3 (5) |
| X | 0.50 (C), 0.35 (G) | |
| X-Ac₂ | 0.25 (F), 0.63 (K) | |
| XI | 0.70 (E), 0.60 (H), 0.31 (I) | |
| XI-Ac | 0.59 (E) | |
| XII | 0.40 (E), 0.47 (H), 0.20 (I) | |
| XIII | 0.20 (E) | |
| XIII-Ac | 0.33 (E) | |

[a] See Materials and Methods for descriptions of solvent systems. [b] GLC operating conditions: (1) column 140 °C, injector and detector 150 °C; (2) column 205 °C, injector and detector 230 °C; (3) column 210 °C, injector and detector 250 °C; (4) column 250 °C, injector and detector 280 °C; (5) column 270 °C; injector and detector 280 °C. [c] Internal standard for reaction rate determinations.

standard (δ 0.00), with a Perkin Elmer Model R600 spectrometer at 60 MHz. MS, IR, and NMR data for I and its reaction products and derivatives described below are summarized in Table II.

*trans*-3,4-Dihydro-3,4-dihydroxy-6,7-dimethoxy-2,2-dimethyl-2*H*-benzo[*b*]pyran (II) and *cis*-3,4-Dihydro-3,4-dihydroxy-6,7-dimethoxy-2,2-dimethyl-2*H*-benzo[*b*]pyran (III) (Figure 1). I (120 mg) in tetrahydrofuran (20 mL) was reacted with water (3 mL) with stirring at room temperature overnight, then concentrated in vacuo, and extracted with ethyl acetate. The extract was dried over Na₂SO₄ and concentrated to give a yellow oil (152 mg). TLC fractionation (A) gave II (14.1 mg) and III (10.4 mg).

*trans*-3,4-Dihydro-3-hydroxy-4,6,7-trimethoxy-2,2-dimethyl-2*H*-benzo[*b*]pyran (IV) and *cis*-3,4-Dihydro-3-hydroxy-4,6,7-trimethoxy-2,2-dimethyl-2*H*-benzo[*b*]pyran (V) (Figure 1). I (88 mg) was reacted with dry methanol (3 mL) overnight at room temperature. Methanol was removed in vacuo, and the residue was purified by preparative TLC (B) to give a mixture of IV and V (44 mg). The isomers were separated by preparative TLC (A) to give IV (20 mg, pale yellow oil) and V (18 mg, colorless crystals).

*trans*-3,4-Dihydro-3-hydroxy-6,7-dimethoxy-2,2-dimethyl-4-(9*H*-purin-6-amino)-2*H*-benzo[*b*]pyran (VI) and *cis*-3,4-Dihydro-3-hydroxy-6,7-dimethoxy-2,2-dimethyl-4-(9*H*-purin-6-amino)-2*H*-benzo[*b*]pyran (VII) (Figure 1). Adenine (68 mg) suspended in 5 mL of dry tetrahydrofuran was added to a solution of I (120 mg) in 20 mL of the same solvent. The mixture was refluxed for 2 h and then concentrated in vacuo to give a brown solid, which was dissolved in ethyl acetate–methanol (5:1) and filtered to remove insoluble material. The filtrate was concentrated and fractionated by preparative TLC (D)
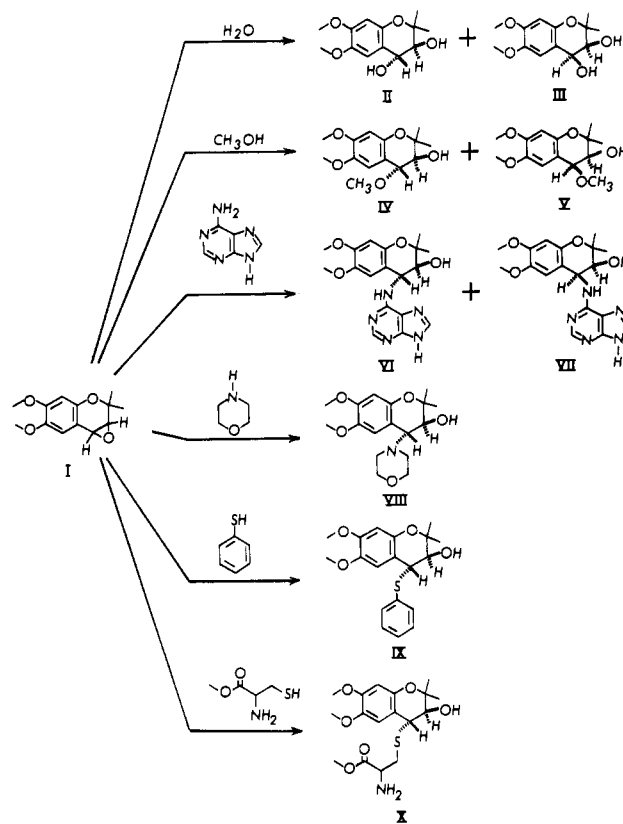


**Figure 1.** Structures of the adducts formed in the reaction of I with six nucleophiles.

to give submilligram amounts of **VI** and **VII**.

*trans*-3,4-Dihydro-3-hydroxy-6,7-dimethoxy-2,2-dimethyl-4-(*N*-morpholino)-2*H*-benzo[*b*]pyran (VIII) (Figure 1). I (118 mg) in dry tetrahydrofuran (15 mL) was reacted with morpholine (50 mg, added in 2 mL of dry tetrahydrofuran) with refluxing for 50 min. After cooling to room temperature, the mixture was concentrated in vacuo to give the crude product as a pale yellow oil. Pure VIII (111 mg, pale yellow crystals) was isolated from the crude mixture by column chromatography (10 g of silica gel eluted with benzene–ethyl acetate, 1:1) and recrystallization from *n*-hexane–benzene.

*trans*-3,4-Dihydro-3-hydroxy-6,7-dimethoxy-2,2-dimethyl-4-(phenylthio)-2*H*-benzo[*b*]pyran (IX) (Figure 1). Thiophenol (52 μL) in dry tetrahydrofuran (5 mL) was added to I (120 mg) in tetrahydrofuran (20 mL). After stirring for 30 min at room temperature, the mixture was concentrated in vacuo and the resulting product fractionated by column chromatography (5 g of silica gel). Elution with benzene followed by benzene–ethyl acetate (20:1) gave pure IX as a colorless oil (79.5 mg).

*trans*-3,4-Dihydro-3-hydroxy-6,7-dimethoxy-2,2-dimethyl-4-[*S*-(*O*-methylcysteinyl)]-2*H*-benzo[*b*]pyran (X) (Figure 1). L-Cysteine methyl ester (140 mg) in 2 mL of dry tetrahydrofuran was added to I (the crude product of the epoxidation of 220 mg of precocene II) dissolved in 40 mL of the same solvent. The mixture was stirred for 2.5 h at room temperature and then concentrated to give the crude product. Analysis of this mixture by TLC (A, E, G) revealed a large number of products. X was isolated from a portion of this mixture by preparative TLC (G) and purified by a second preparative TLC step (C) and acetylation (see below) to give the purified acetylated product (8 mg, colorless oil).

**Acetate Derivatives.** Acetates of adducts and epoxide degradation products were prepared by treatment with acetic anhydride–pyridine (1:1) overnight at room tem-

**Table II. Spectral Data for the Adducts of I with Nucleophiles and Their Acetate Derivatives**

| compd | MS, m/e[a] M+ | MS, m/e[a] base | NMR, ppm (J, Hz)[b] | IR, cm⁻¹[c] |
|---|---|---|---|---|
| II | | | 1.26, 1.45 (Me × 2), 3.32 (OH × 2), 3.56 (3-H, d, 8.2), 3.81 (OMe × 2), 4.50 (4-H, d, 8.2), 6.34 (8-H), 6.90 (5-H) | 3420 (OH), film |
| II-Ac₂ | 338 | 221 | 1.34, 1.40 (Me × 2), 2.10 (OAc × 2), 3.79, 3.83 (OMe × 2), 5.18 (3-H, d, 5.0), 5.92 (4-H, d, 5.0), 6.41 (8-H), 6.66 (5-H) | 1722 (OAc), CHCl₃ |
| III | | | 1.26, 1.45 (Me × 2), 2.27 (OH × 2), 3.64 (3-H, d, 4.0), 3.80 (OMe × 2), 4.70 (4-H, d, 4.0), 6.36 (8-H), 6.97 (5-H) | 3384 (OH), KBr |
| III-Ac₂ | 338 | 183 | 1.38 (Me × 2), 2.08, 2.10 (OAc × 2), 3.81, 3.84 (OMe × 2), 5.28 (3-H, d, 4.0), 6.13 (4-H, d, 4.0), 6.41 (8-H), 6.61 (5-H) | 1722 (OAc), CHCl₃ |
| IV | | | 1.28, 1.44 (Me × 2), 3.47 (4-OMe), 3.68 (3-H, d, 9.0), 3.82, 3.84 (6,7-(OMe)₂), 4.32 (4-H, d, 9.0), 4.42 (OH), 6.36 (8-H), 6.79 (5-H) | 3420 (OH), KBr |
| IV-Ac | 310 | 235 | 1.32, 1.37 (Me × 2), 2.10 (3-OAc), 3.46 (4-OMe), 3.83 (6,7-(OMe)₂), 4.24 (4-H, d, 4.5), 5.22 (3-H, d, 4.5), 6.39 (8-H), 6.76 (5-H) | 1740 (OAc), KBr |
| V | | | 1.26, 1.49 (Me × 2), 3.60 (4-OMe), 3.83 (6,7-(OMe)₂), 3.83 (3-H, d, 5.9), 4.39 (4-H, d, 5.9), 6.39 (8-H), 6.87 (5-H) | 3410 (OH), KBr |
| V-Ac | 310 | 181 | 1.32, 1.37 (Me × 2), 2.08 (3-OAc), 3.54 (4-OMe), 3.84 (6,7-(OMe)₂), 4.50 (4-H, d, 3.9), 5.34 (3-H, d, 3.9), 6.38 (8-H), 6.88 (5-H) | 1730 (OAc), KBr |
| VI | | | 1.37, 1.56 (Me × 2), 3.56, 3.84 (OMe × 2), 4.01 (3-H, d, 8.6), 5.62 (4-H, d, 8.6), 6.08 (8-H), 6.45 (5-H), 7.65 (2'-H), 8.22 (8'-H) | 3370 (OH), KBr |
| VI-Ac₂ | 455 | 380 | 1.43 (Me × 2), 1.79 (3-OAc), 2.59 (7'-NAc), 3.58, 3.86 (OMe × 2), 5.42 (4-H, d, 9.2), 5.89 (3-H, d, 9.2), 6.08 (8-H), 6.51 (5-H), 7.87 (2'-H), 8.75 (8'-H), 8.92 (6-NH) | 1750 (OAc), CHCl₃ |
| VII | | | 1.46, 1.57 (Me × 2), 3.60, 3.85 (OMe × 2), 3.80 (3-OH), 5.50 (4-H), 6.18 (8-H), 6.51 (5-H), 7.76 (2'-H), 8.26 (8'-H) | 3430 (OH), KBr |
| VII-Ac₂ | 455 | 380 | 1.37, 1.51 (Me × 2), 1.90 (3-OAc), 2.63 (7'-NAc), 3.64, 3.89 (OMe × 2), 6.25 (8-H), 6.53 (5-H), 7.76 (2'-H), 8.25 (8'-H) | 1750 (OAc), CHCl₃ |
| VIII | | | 1.19, 1.47 (Me × 2), 2.51 (3-OH), 2.83 (3',5'-H₄, t, 4.7), 3.70 (3,4-H₂), 3.72 (2,6'-H₄, t, 4.7), 3.80, 3.83 (OMe × 2), 6.35 (8-H), 6.95 (5-H) | 3390 (OH), KBr |
| VIII-Ac | 365 | 251 | 1.21, 1.30 (Me × 2), 2.12 (3-OAc), 2.66 (3',5'-H₄), 3.63 (2',6'-H₄, t, 4.0), 3.70 (4-H), 3.81 (OMe × 2), 5.28 (3-H, d, 9.3), 6.34 (8-H), 7.07 (5-H) | 1735 (OAc), KBr |
| IX | | | 1.22, 1.45 (Me × 2), 2.75 (3-OH), 3.57 (3-H, d, 9.2), 3.82 (OMe × 2), 4.06 (4-H, d, 9.2), 6.32 (8-H), 7.18 (5-H), 7.28 (thiophenyl-H₅) | 3640 (OH), film |
| IX-Ac | 388 | 219 | 1.24, 1.37 (Me × 2), 1.97 (3-OAc), 3.82 (OMe × 2), 4.24 (4-H, d, 7.8), 5.27 (3-H, d, 7.8), 6.35 (8-H), 7.10 (5-H), 7.31 (thiophenyl-H₅) | 1729 (OAc), CHCl₃ |
| X-Ac₂ | 455.1631[d] | | 1.23, 1.37 (Me × 2), 1.99, 2.07[e] (3-OAc, diastereomers), 2.71[e] (2'-NAc), 2.79[e] (3'-H₂, d, 5.5), 3.73[e] (CO₂Me), 3.82 (OMe × 2), 4.71 (2'-H, m), 5.09 (3-H, d, 8.0), 6.36 (8-H), 7.00 (5-H) | 3350 (NH), 1730 (CO), film |

**Table III. Spectral Data for Degradation Products of I Formed in Aqueous Media**

| compd | MS (M+, m/e)[a] | NMR, ppm (J, Hz)[b] | IR, cm⁻¹[c] |
|---|---|---|---|
| XI | | 1.21, 1.62 (Me × 2), 3.86, 3.89 (OMe × 2), 4.35 (3-H), 6.39 (8-H), 7.18 (5-H) | 3410 (OH), 1650 (CO), film |
| XI-Ac | | 1.34, 1.51 (Me × 2), 2.22 (3-OAc), 3.85, 3.89 (OMe × 2), 5.56 (3-H), 6.39 (8-H), 7.20 (5-H) | 1755 (OAc), 1695 (CO), film |
| XII | 472.2121[d] | 1.06, 1.21, 1.49[g] (Me × 4), 3.83,[g] 3.91[g] (OMe × 4), 3.36–3.52 (3,3',4,4'-H₄, unresolved signals), 6.45[g] (8,8'-H₂), 6.73, 6.79 (5,5'-H₂) | e |
| XIII | 488.2058[f] | 0.91, 1.30, 1.45, 1.69 (Me × 4), 3.35 (3-H, d, 4.2), 3.69, 3.78, 3.84 (OMe × 4), 4.30 (3'-H), 4.34 (4-H, d, 4.2), 6.11[g] (8,8'-H₂), 6.80, 6.94 (5,5'-H₂) | 3430 (OH), 1670 (CO), KBr |

[a] MS data obtained for acetate derivatives only. [b] NMR data in CHCl₃-d; all signals were singlets except where noted; single protons were singlets except where noted; all protons were not assignable in some spectra. [c] Measured absorption frequencies that confirm the presence of key functional groups. [d] High-resolution MS; calculated M, 455.1613 for C₂₁H₂₉O₈NS. [e] These protons gave pairs of signals for the two diastereomers in benzene-d₆/acetone-d₆.

[a] Molecular ion from high resolution MS. [b] NMR data in CHCl₃-d; all signals were singlets except where noted; all protons were not assignable in some spectra. [c] Measured absorption frequencies that confirm the presence of key functional groups. [d] Calculated M, 472.2097 for C₂₆H₃₂O₈. The following fragments (relative intensities) were observed at 20 eV: 472 (67), 439 (31), 237 (38), 236 (43), 235 (25), 221 (72), 220 (31), 219 (22), 205 (100), 167 (25). [e] IR spectrum did not show the presence of hydroxyl or carbonyl groups. [f] Calculated M, 488.2046 for C₂₆H₃₂O₉. The following fragments (relative intensities) were observed at 20 eV: 488 (6), 470 (5), 371 (12), 237 (100), 221 (21), 220 (22), 195 (55), 181 (20), 180 (38), 179 (20), 167 (32). [g] Two singlet signals were observed in benzene-d₆/acetone-d₆.
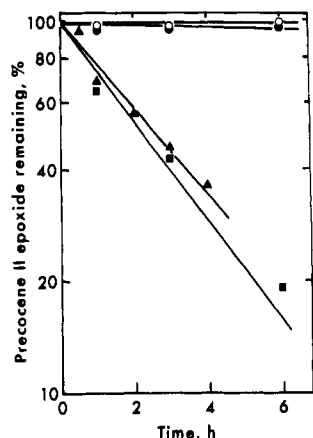
**Figure 2.** Disappearance curves for the reaction of I (1.34 × 10⁻²
M) with nucleophiles in tetrahydrofuran: (●) water, 1.34 M; (O)
methanol, 1.34 M; (■) morpholine, 1.34 M; (▲) thiophenol, 1.34
× 10⁻² M.



**Figure 3.** Disappearance curves for the reaction of I (1.34 × 10⁻²
M) with L-cysteine methyl ester at several concentrations: (●)
1.34 × 10⁻² M; (■) 1 × 10⁻² M; (▲) 6.7 × 10⁻³ M; (O) 1.34 × 10⁻³
M.



**Figure 4.** Disappearance curves for the reaction of I (1.34 × 10⁻²
M) with water at several concentrations: (●) 1.34 M; (■) 5.55 M;
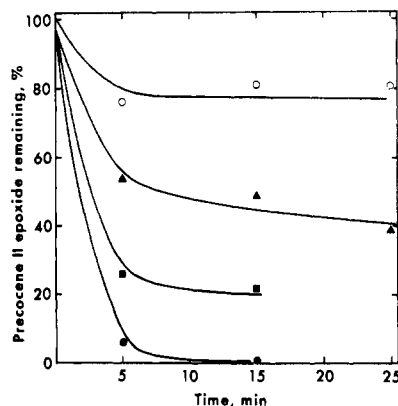(▲) 11.1 M; (O) 27.8 M.

perature. Acetates were recovered by evaporation of the
reagent and were analyzed directly except for the acetate
of **X**, which was purified by preparative TLC (K × 2, E).
In the text, acetates are indicated by the Roman numeral
designation of the parent compound and the suffix Ac or
Ac₂; thus, the diacetate derivative of **X** is identified as
**X**-Ac₂.

**Measurement of Rates of Epoxide Degradation.** I
(final concentration, 1.34 × 10⁻² M) was incubated with
nucleophiles in tetrahydrofuran (final nucleophile con-
centrations, 1.34 × 10⁻² M to 27.8 M) at room temperature
with occasional stirring. The extent of epoxide reaction
at various times up to 6 h after initiation of the reaction
was determined by GLC analysis of the remaining epoxide
with precocene II as the internal standard (see Table I).
The recovery of I in the absence of added nucleophile was
>98% for all reaction times. Results presented here are
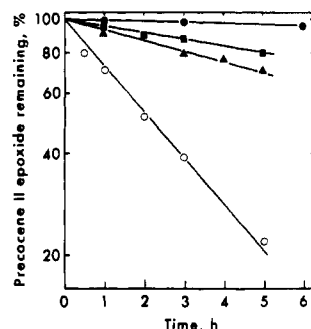means of two separate experiments under each set of re-
action conditions.

**Isolation of Degradation Products Formed in
Aqueous Media.** Larger scale reactions for the isolation
of additional degradation products of I were performed
as follows. For reaction under neutral conditions, I (330
mg) was stirred in water–tetrahydrofuran (1:1, 5 mL) for
8 h at room temperature and then left to stand overnight.
The mixture was concentrated in vacuo and the concen-
trate extracted with ethyl acetate. Both the extract and
the aqueous layer were concentrated to dryness and the
resulting product mixtures analyzed by TLC as described
below. For reaction under basic conditions, I (65 mg) was
stirred in a mixture of 0.2 N NaOH (2 mL) and tetra-
hydrofuran (2 mL) in an ice bath for 3 h. The mixture was
extracted with diethyl ether and the extract dried (Na₂S-
O₄), concentrated, and analyzed by TLC as described be-
low.

RESULTS

**Rates of Degradation of I in the Presence of Model
Nucleophiles.** We initially studied the rates of reaction
of I with model nucleophiles by using a nucleophile–
epoxide ratio of 100:1. Under these conditions, the reac-
tions of I with water and methanol were barely detectable
over a 6-h period (Figure 2), and there was no detectable
degradation of I in the presence of adenine. The reaction
of I with morpholine was much more rapid and followed
apparent first-order kinetics with a half-life for I of 2.6 h
(Figure 2). Under these conditions, the reaction of I with
thiophenol proceeded very rapidly, so that less than 10%
of the initial epoxide was recoverable after 5 min. Re-

duction of the nucleophile concentration to give a nu-
cleophile–epoxide ratio of 1:1 gave a reaction rate similar
to that measured with a 100-fold excess of morpholine
(Figure 2).

The most rapid reactions of I were detected with L-
cysteine methyl ester as the nucleophile (Figure 3). This
reaction was too fast to be measured by GLC techniques
when performed with a nucleophile–epoxide ratio of 1:1.
At this ratio, more than 90% of I reacted within 5 min,
the shortest reaction time used in this study. At reduced
nucleophile concentrations, we observed the rapid and
complete reaction of all available nucleophile, and in
contrast to the results with other nucleophiles, little or no
subsequent degradation of the remaining epoxide (Figure
3).

Although the shapes of the epoxide recovery curves in
several of these studies suggested that these reactions
followed first-order kinetics, we observed that the rates of
reaction of I were dependent on nucleophile concentration.
Figure 4 shows the rates of reaction of I with water at
constant epoxide concentrations and nucleophile–epoxide
ratios ranging from 100:1 to 2000:1. Similar effects of
nucleophile concentration were observed with all other
nucleophiles for which rates of reaction were measured.

**Products of the Reactions of I with Model Nu-
cleophiles.** GLC and two-dimensional TLC analysis (H,
I) of product mixtures obtained in the measurement of
epoxide degradation rates showed the presence of nu-
merous degradation products in addition to the adducts
described below. These products were observed with all
nucleophiles but were most numerous in the reactions of
I with methanol and thiophenol. Products detected by
TLC analysis also included those arising from the degra-
dation of unreacted I, which is unstable under TLC con-
ditions (Soderlund et al., 1980). From these mixtures, we

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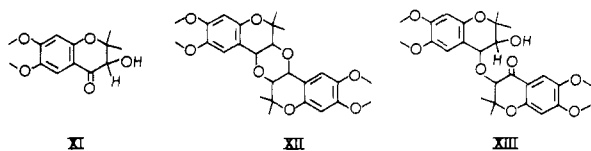


**Figure 5.** Structures of new degradation products of **I** isolated from neutral and basic aqueous media.



**Figure 6.** Proposed mechanisms for the reaction of **I** with nucleophiles.

isolated and identified the primary adducts formed in the reactions of **I** with the model nucleophiles used in the studies of degradation rates described above. The structures of the adducts formed in these reactions are shown in Figure 1. The assignment of these structures is based on spectroscopic data for the isolated adducts or their acetylated derivatives (Table II).

Reaction of **I** with water produced the *trans-* and *cis-*dihydrodiols **II** and **III** described in several previous studies. Similarly, reaction of **I** with methanol produced the *trans-* and *cis-*methoxyhydrins **IV** and **V**. Although no reaction of **I** with adenine was observed under the conditions used for measuring reaction rates, we were able to isolate small amounts of the trans- and cis-substituted adenyl adducts **VI** and **VII** when the reaction was carried out with excess nucleophile at elevated temperature. In contrast, reaction of **I** with morpholine, thiophenol, and L-cysteine methyl ester produced only the corresponding trans-substituted adducts **VIII–X**.

**Products of the Degradation of I in Aqueous Media.** Three additional products were identified from the reactions of **I** in neutral or basic aqueous media (Figure 5). These structures are assigned on the basis of spectral data for the isolated products and their acetylated derivatives summarized in Table III. The concentrated ethyl acetate extract of the reaction of **I** in neutral aqueous medium (290 mg) was initially fractionated by column chromatography on $SiO_2$ (15 g), eluting with benzene–ethyl acetate (10:1), ethyl acetate, ethyl acetate–methanol (6:1), and ethyl acetate–methanol (3:1). Preparative TLC (E) of the benzene–ethyl acetate fraction gave two compounds at $R_f$ 0.70 and 0.40. The less polar compound (9.8 mg) was identified as the 4-ketochroman-3-ol derivative **XI** on the basis of its NMR and IR spectra. **XI** was easily recognized on TLC by its mobility and characteristic intense fluorescence at 366 nm and was detected as a degradation product of **I** under both neutral and basic aqueous conditions and in the product mixtures obtained from the reaction of **I** with several of the nucleophiles in this study. The more polar component from the above open column fraction (10.1 mg) could not be acetylated, and the IR spectrum indicated that the compound lacked carbonyl and hydroxyl groups. This compound was recognized as a dimer of **I** by its mass spectrum, which showed a molecular ion at *m/e* 472. The structure (Figure 5, **XII**) was assigned on the basis of the NMR and mass spectra.

The reaction of **I** in basic aqueous medium gave **II**, **III**, **XI**, and a fourth product separable by TLC ($R_f$ 0.20, E). The mass spectrum of this compound showed a molecular ion at *m/e* 488, suggesting an oxygenated dimer of **I**, and the IR spectrum showed both hydroxyl (3430 cm⁻¹) and carbonyl (1670 cm⁻¹) absorption bands. These data and the NMR and mass spectra of the isolated material support the assignment of the structure shown in Figure 5 (**XIII**) for this compound.

DISCUSSION

Prior studies of the reactivity of precocene epoxides have been restricted to precocene I epoxide and the use of water as the nucleophile (Hamnett et al., 1981). Our findings provide the first data on the reactions of precocene II
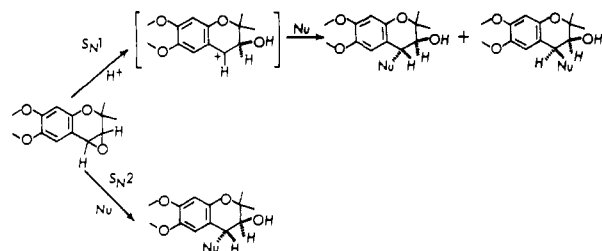
epoxide and on the relative ease with which this epoxide is attacked by a variety of nucleophiles. We found large differences in the rate of degradation of **I** by different nucleophiles in this study. In particular, **I** reacted much more rapidly with thiols than with water or methanol. This finding suggests that tissue thiols may compete successfully with tissue water for **I** formed by oxidative activation in target and nontarget tissues and that reactions with thiols may be critical in the macromolecular labeling observed with precocene-derived tritium in CA preparations (Pratt et al., 1980; Soderlund et al., 1981; Hamnett and Pratt, 1983).

One limitation of the method used in these studies is that it measures epoxide degradation rather than adduct formation. Although the adducts formed were detectable by GLC in most cases (Table I), these analyses also revealed the formation of several other products in addition to the primary nucleophile adducts of **I**. These additional products are nucleophile dependent and may result from further attack of the primary adduct on the remaining epoxide, nucleophile dependent side reactions of the epoxide, or degradation of the primary adduct by oxygen or peroxides in the reaction solvent.

The structures of the adducts identified in this study suggest that two mechanisms may be involved in the nucleophilic attack on **I**. Hamnett et al. (1981) proposed that the reaction of water with precocene I epoxide to give the *cis-* and *trans-*dihydrodiols proceeds via an $S_N1$ mechanism involving a trigonal carbonium ion (Figure 6). Our finding that the reactions of **I** with water, methanol, and adenine produce both the *cis-* and *trans-*substituted adducts suggests that these reactions involve the $S_N1$ mechanism as well. In contrast, we found only the trans-substituted adducts in the reactions of **I** with morpholine, thiophenol, and L-cysteine methyl ester. These results suggest that nucleophilic attack on **I** can also proceed by an $S_N2$ mechanism (Figure 6), a finding consistent with the extensive literature on the mechanisms of nucleophilic attack on a wide variety of epoxides (Long and Pritchard, 1956; Pritchard and Long, 1956; Parker and Isaacs, 1959; Buchanan and Sable, 1972). From our limited sample of nucleophiles, it appears that the $S_N2$ mechanism is restricted to highly reactive nucleophiles.

Prior to this study, the isomeric dihydrodiols were the only known chemical degradation products of precocene epoxides. Our studies demonstrate the ubiquitous occurence of the 4-ketochroman-3-ol **XI** as an additional degradation product. However, the immediate precusor of this compound and its mechanism of formation during the $S_N1$ and $S_N2$ nucleophilic substitution reactions of the epoxide are not clear from our studies and remain to be determined. To date, this product has not been identified as a metabolite in biological systems, but its presence should be investigated in future studies since it might be formed from one or both diols by enzymatic oxidation at the 4-position. We have also shown that **I** can react with itself or with its degradation products to produce dimeric com-

pounds. Since these arise in aqueous media, it is likely that they may result from attack of the $S_n1$ carbonium ion intermediate on **I** (to form **XII**) or reaction of this intermediate with **XI** (to form **XIII**). Recent studies (Camps et al., 1985) have identified a thermal dimerization product of **I** having properties similar to **XII** in which the two monomers are joined to form a tetrahydrofuran ring and one of the monomeric units bears a hydroxyl group at the 3-position. Although the NMR data for the thermal dimer and **XII** are similar, we found no spectral evidence for the presence of a hydroxyl group in **XII** (Table III), which suggests that these compounds are not the same.

**Registry No.** I, 62471-06-1; II, 74094-54-5; II-Ac$_2$, 74094-47-6; III, 74094-53-4; III-Ac$_2$, 74094-48-7; IV, 95421-28-6; IV-Ac, 95421-29-7; V, 95421-30-0; V-Ac, 95421-31-1; VI, 95464-50-9; VI-Ac$_2$, 95421-32-2; VII, 95421-33-3; VII-Ac$_2$, 95421-34-4; VIII, 95421-35-5; VIII-Ac, 95421-36-6; IX, 95421-37-7; IX-Ac, 95421-38-8; X (isomer I), 95421-39-9; X (isomer II), 95529-70-7; X-Ac$_2$ (isomer I), 95421-40-2; X-Ac$_2$ (isomer II), 95529-71-8; XI, 95421-41-3; XI-Ac, 95421-42-4; XII, 95421-43-5; XIII, 95421-44-6; XIII-Ac, 95421-45-7; precocene II, 644-06-4; adenine, 73-24-5; morpholine, 110-91-8; thiophenol, 108-98-5; L-cysteine methyl ester, 2485-62-3; methanol, 67-56-1; water, 7732-18-5.

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# Volatile Compounds Inhibiting *Aspergillus flavus*

Richard C. Gueldner,* David M. Wilson, and Amy R. Heidt

The volatile compounds, *trans*-2-hexenal, 2,4-hexadienal, furfural, β-ionone, and 1-nonanol, found to occur naturally in corn ears, inhibit the growth of *Aspergillus flavus*. The order of activity for these natural compounds and synthetic analogues was found to be aldehydes > ketones > alcohols. Reduction of active aldehydes and ketones to the corresponding alcohols reduced inhibitory activity. Selected methyl ketones, terpene hydrocarbons, and terpene hydrocarbon oxides were not active.

Moldy food toxicoses affect animal and human health worldwide. Aflatoxin, in particular, has been identified in many foods as a result of fungal infection with certain members of the *Aspergillus flavus* group, notably *A. flavus* Link and *A. parasiticus* Speare.

Species of the *A. flavus* group often infect corn in the hot, and at times, semiarid summers of the southeastern United States. In this ecological niche species of the *Aspergillus flavus* group compete relatively well with other microorganisms and aflatoxin may contaminate food and feed supplies.

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Corn in the field is most susceptible to spore contamination and growth of *Aspergillus flavus* just after pollination when the silks are yellow/brown in color (Payne 1983; Jones et al., 1980). In subsequent storage following harvest the growth of *Aspergillus flavus* and production of aflatoxin may increase.

Since aflatoxin is not manifest during early stages of corn kernel development and silking, when volatile metabolites would be at peak concentration, Wilson et al. (1981) screened volatile compounds reported by Flath et al. (1978), Buttery et al. (1978), and Cantelo and Jacobson (1979) to be present in corn ears for growth suppression of *A. flavus* and *A. parasiticus*. Among the volatile compounds found the conjugated methyl ketone, β-ionone was observed to induce profound effects on the morphology and development of the conidia of *A. flavus* (CP-22). Further, when 100 ppm β-ionone was added to a shake culture of *A. parasiticus* (NRRL 2999) aflatoxin accumu-