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# 3,4-EPOXYPRECOCENE AS A MODEL OF CYTOTOXIC EPOXIDES: SYNTHESIS OF THE TRANS ADDUCTS OCCURRING IN THE GLUTATHIONE METABOLIC PATHWAY[1]

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## ABSTRACT

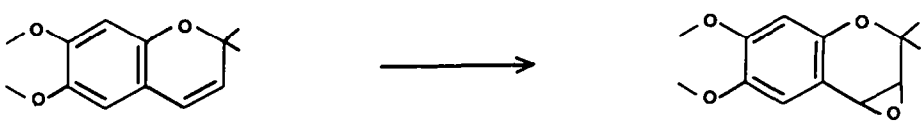
The adducts of 3,4-epoxyprecocene II (2) with N-acetylcysteine, cysteine and glutathione have been synthesized. Under the basic reaction conditions employed, the resulting products were originated from a trans opening of the oxirane ring with the sulfur atom linked to the C-4 benzylic position. In addition, the stereochemical correlation between the two sets of diastereomeric trans adducts 5a-c has been unambiguously established by $^1$H NMR, reversed-phase HPLC and enzymatic means.

## INTRODUCTION

Bioactive epoxides generated from olefins by action of microsomal monoxygenases can play different roles, either as intermediates or final products in complex biosynthetic pathways. Alternatively, these epoxides can also be formed in detoxification processes of xenobiotics, leading in some cases to undesirable toxins. The epoxides of polycyclic aromatic hydrocarbons[2] and aflatoxins[3] are relevant examples of these toxic bioactivated xenobiotics.

Our interest in bioactive epoxides is derived from our work on precocenes (1)[4], insect juvenile hormone antagonists which suppress the secretion of juvenile hormones in sensitive insect species by destruction of the corpora allata, the glands where the hormones are biosynthesized[5]. Previous studies have suggested that precocenes are converted by the allatal monoxygenases into the corresponding 3,4-epoxy derivatives 2[6,7]. These highly reactive intermediates[8] are assumed to be the responsible of the cytotoxic reaction on the corpora allata[9]. Moreover, this cytotoxicity is not restricted to invertebrates since it has been recently reported that precocenes are also hepato-[10,11] and nephrotoxic[12].

Considering this general toxicity, we anticipated that 3,4-epoxyprecocenes could be appropriate models for the study of the reactivity of bioactive epoxides towards selected nucleophiles related to those potentially found in cellular compartments. In this context, the reactivity of 3,4-epoxyprecocenes with acid reagents and oxygen nucleophiles has already been investigated[13-15].



1 2

On the other hand, preliminary studies of the reaction of 2 with the methyl ester of cysteine have suggested that this epoxide reacts faster with sulfur nucleophiles than with water or methanol; this selectivity may be critical in the macromolecular alkylation observed in corpora allata preparations[16]. In addition, a mercapturic acid derivative of precocene II has been tentatively detected among the metabolites resulting from the administration of this compound to rats[17]. Likewise, the synergistic effect caused by diethyl maleate, a known glutathione depletor, in the biological activity of precocenes on immature stages of the seed bug, Oxycarenus lavaterae[18], and the extended depletion of endogenous glutathione levels that we have observed in adults of Blattella germanica after topical application of precocene II, both confirm the intervention of sulfur nucleophiles in the metabolism of these toxins.

In the present paper, we report on the preparation and unambiguous structural elucidation of the sulfur conjugates of 3,4-epoxyprecocene II, related to the glutathione detoxification pathway[19], in which a trans addition of the thiol moiety to the benzylic position of the epoxide has taken place. This particular reaction model was chosen in view of the regio- and stereoselectivity previously found in the conjugation of different bioactive epoxides catalyzed by S-glutathione transferases[20-23].

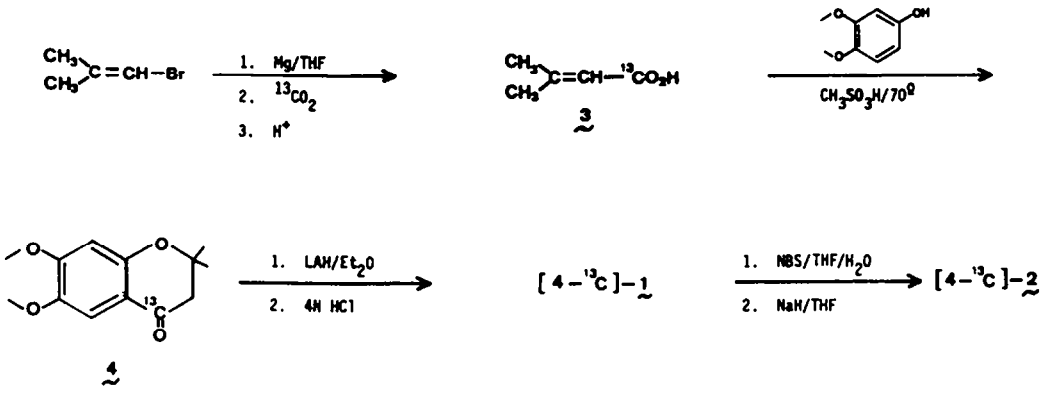
## RESULTS AND DISCUSSION

[4- $^{13}C$] -3,4-Epoxyprecocene II (2). Although the reactivity of epoxide 2 towards oxygen nucleophiles, such as alcohols and carboxylic acids, showed that the major compounds obtained in this reaction are derived from the regioselective opening of the oxirane ring through the benzylic position (C-4), we anticipated that being the thiol reagents softer nucleophiles than those oxygen moieties, its corresponding addition to the epoxide could change the regioselectivity observed in this case[24]. Moreover, a different chemoselectivity might be expected with thiol nucleophiles containing also free carboxylic acid groups.

In order to elucidate easily the regioselectivity of the nucleophilic attack by $^{13}C$ NMR, we prepared the epoxide 2 labelled with $^{13}C$ at C-4. For this synthesis, the $^{13}C$ was incorporated into the 3-methylbut-2-enoic acid and this labelled compound was used as prenyl synthon according to a general procedure previously applied in this laboratory (Scheme 1)[25]. A similar strategy had also been used in investigations of the S-conjugates of benzo[a]pyrene 4,5-oxide[26] and styrene oxide[27,28]. In these compounds, it was found that a $^{13}C$ NMR absorption appeared within the 65-75 ppm range when the corresponding carbon atom was linked to an oxygen atom, whereas this absorption was shifted upfield (45-55 ppm) when a C-S bond was formed.

Reaction of 2 with N-acetylcysteine and with cysteine. As anticipated, the addition of epoxide 2 to the sodium salt of N-acetylcysteine in basic medium (0.1 N NaOH) took place rapidly leading to the formation of two major compounds which accounted for over 80% of the starting substrate. The reaction course was monitored by HPLC[29]. Filtration through C-18 Sep-Pack cartridges allowed an easy purification of the reaction crude and the major compounds were separated by fractionated

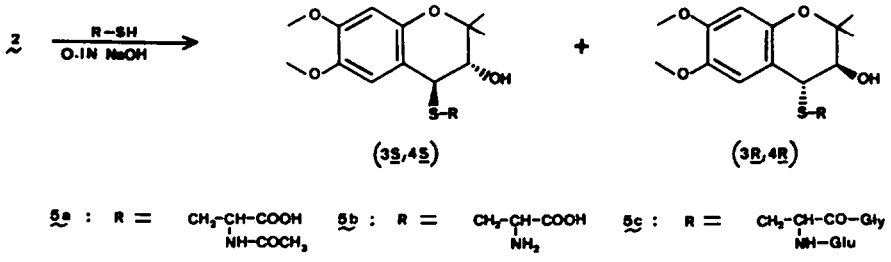
crystallization.




**Scheme 1**

When the reaction was carried out with a sample of the labelled epoxide 2, the $^{13}C$ NMR spectrum of the purified reaction mixture showed absorptions at 49.60 and 48.49 ppm, which confirmed that the addition of the thiol reagent had been both chemoselective for the sulfur atom and regioselective on the benzylic position[30].

On the other hand, mass spectral data of the pure compounds showed peaks at m/z 381 ($M^+$-18) and 236, the latter being characteristic of the precocene oxybenzopyranyl fragment. The $^1H$ NMR spectra revealed some interesting features. First, the value observed for the coupling constant between protons at C-3 and C-4 (9.7 Hz) agreed with the expected trans stereochemistry of the ring opening under the basic reaction conditions employed[27,28,31]. It was also confirmed that the slight broadening observed for the peak assigned to the hydrogen at C-5 was due to a small coupling with the benzylic hydrogen. This coupling has been very useful for the identification of benzylic protons from the complex patterns appearing in the 3-4 ppm region of the spectra of these derivatives. Finally, the selective irradiation of the methine of the acetylcysteinyl residue led to the appearance of a different pattern for the contiguous methylene group absorption in both isomers. This behaviour, which was also present in the case of the corresponding cysteine and glutathione adducts, will be discussed later. From all these spectral features, the crude reaction mixture was identified as a diastereomeric pair of the trans S(N-acetylcysteinyl) adducts at C-4, 5a (Scheme 2).




**Scheme 2**

The absolute configuration of these adducts was determined by X-ray diffraction analysis of the adduct with the highest retention time in HPLC[32]. In fact, obtention of appropriate crystals was a tedious work involving successive recrystallizations. Unfortunately, the test crystal resulted to be centrosymmetric and it included four molecules which were enantiomeric by pairs. However, since the starting N-acetylcysteine was confirmed to be R, we could discard the enantiomeric structures with the cysteinyl fragment presenting the S configuration. These forms would probably have emerged from isomerization during the successive recrystallization procedures. Thus, absolute configuration of this diastereomer resulted to be 3S, 4S (cf. Scheme 2) while the opposite configuration 3R, 4R was then assigned to the other diastereomer.

Similarly, reaction of cysteine with epoxide 2 under basic conditions occurred rapidly affording a 87% yield of the corresponding mixture of trans adducts 5b. Structural correlation of these compounds with the corresponding mercapturates 5a was carried out by acetylation under mild conditions and HPLC comparison with authentic standards.

Reaction of 2 with glutathione. Likewise, reaction of glutathione with epoxide 2 under the same reaction conditions afforded a 80% yield of the diastereomeric mixture of adducts 5c which were separated by semi-preparative HPLC. In this case, FAB-MS spectra of both compounds, in the positive fragmentation mode, showed the molecular ion peak (m/z 544) and a peak corresponding to the oxybenzopyranyl fragment (m/z 237), whereas a peak at m/z 306, due to the glutathione fragment, was present in the negative ion spectra along with those at m/z 542 and 543. Additionally, the $^1$H NMR spectra of both diastereomers were in agreement with the presence of a S-linked glutathione residue at C-4 with a trans relative stereochemistry between the hydrogen atoms at C-3 and C-4.

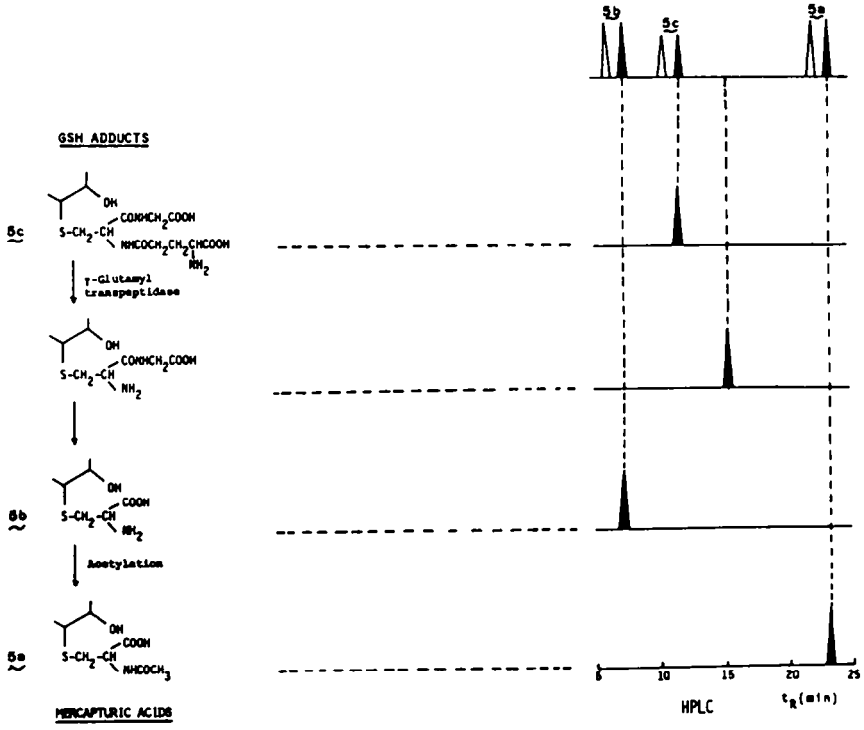



Figure 1. Stereochemical correlation among the diastereomers of the trans adducts of 3,4-epoxyprecocene II with glutathione, cysteine and N-acetylcysteine (5a-c). White and black peaks correspond to the 3R,4R and 3S,4S configuration sets, respectively. For chromatographic conditions see Experimental Section.

The stereochemical correlation between the pair of glutathione adducts 5c and the corresponding cysteine and N-acetylcysteine pairs 5b and 5a was carried out by the sequence depicted in Fig. 1. Thus, when the diastereomer 5c with the highest retention time in HPLC was incubated with γ-glutamyl transpeptidase (E.C. 2.3.2.2.), it was rapidly converted into a compound eluting later in HPLC. This intermediate, assumed to be the corresponding glycylcysteinyl adduct, was not isolated and the enzymatic incubation was pursued under the same reaction conditions to lead to the corresponding cysteine adduct 5b. This second cleavage occurred at a slower rate and it was attributed to the non specific protease activity exhibited by the enzyme used. The stereochemical correlation was completed by acetylation of the 5b adduct obtained which afforded the 5a (3S,4S) diastereomer. Likewise, the exclusive formation of the corresponding 5a (3R,4R) derivative was detected when the other glutathione adduct was independently subjected to the same process.

Once the stereochemical correlation between the different diastereomeric trans adducts 5a-c had been unambiguously established, the complementary correlations by reversed phase HPLC and $^1$H NMR observed for these adducts deserve some comment. First, as shown in Fig. 2, it was found that for each set of diastereomers 5a,b,c, that with R configuration at the benzylic position eluted earlier than the corresponding S isomer in HPLC. These results agree with those reported in the case of styrene oxide glutathione adducts[33], suggesting the potential value of HPLC for the stereochemical assignment of related compounds.

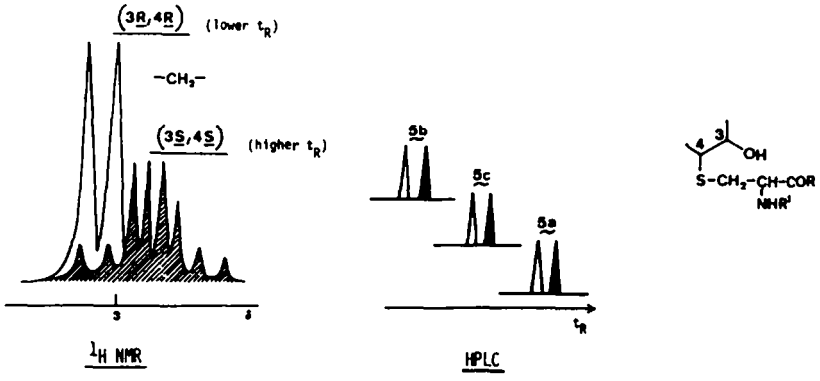



Figure 2. Spectroscopic ($^1$H NMR) and chromatographic (HPLC) correlations among the trans adducts of 3,4-epoxyprecocene II with glutathione, cysteine and N-acetylcysteine (5a-c).

The second distinctive feature for each set of diastereomers is the $^1$H NMR absorption pattern of the cysteinyl methylene group (Fig. 2). As shown, this absorption in diastereomers with the lowest HPLC retention time (i.e., the 3R,4R set) appears as a doublet. On the other hand, the (3S,4S) derivatives exhibit a more complex system which reveals the diastereotopic character of the hydrogen atoms in this configuration. In fact, upon selective irradiation of the neighboring methine this system collapsed into a doublet (J = 13.3 Hz) in contrast to the singlet obtained for 3R,4R diastereomers.

In conclusion, the adducts of 3,4-epoxyprecocene II (2) with N-acetylcysteine, cysteine and glutathione obtained under basic conditions have been identified as those derived from a trans opening of the oxirane ring by the thiol moiety with the sulfur atom linked at the benzylic centre. These compounds are quite stable (no isomerization has been detected when stored in pure form) and constitute useful models for the study of the role of S-conjugation in the detoxification of precocenes which is now progress in our laboratory.

## EXPERIMENTAL SECTION

The HPLC analyses were carried out with a Waters modular system provided with two Model 510 pumps, an automated gradient controller and a Model 481 UV detector. Melting points (uncorrected) were determined with a Koffler apparatus. The infrared spectra were recorded on a Perkin Elmer 399B instrument. The nuclear magnetic resonance spectra were recorded on a Bruker WP-80 SY apparatus operating at 80.13 MHz for $^1H$ and 20.15 for $^{13}C$ in the Fourier transform mode. All chemical shifts are given in ppm downfield from internal tetramethylsilane. Elemental analyses were performed with a Carlo Erba Model 1106 instrument. Optical rotations were determined in methanol solutions at 22° with a Perkin Elmer Model 141 polarimeter. The chemical ionization mass spectra were obtained on an updated AEI MS-902 instrument with a PDP 11/24 computer for data processing and using ammonia as reagent gas. Samples were introduced into the ion source through the solids probe. The FAB mass spectra were obtained on a VG-ZAB HF instrument using xenon as bombarding gas at 8 KV energy. The samples were applied in a glycerol matrix.

**Synthesis of [4-$^{13}C$]-6,7-dimethoxy-2,2-dimethyl-3,4-epoxy-3,4-dihydro-2H 1-benzopyran (2).** **[1-$^{13}C$]-3-Methylbut-2-enoic acid (3).** Dry $^{13}CO_2$, generated from $Ba^{13}CO_3$ (2.10 g, 10.6 mmol) and 6N HCl, was bubbled through a suspension of 2-methylbutenyl magnesium bromide (8.9 mmol), maintained at 0°C. Conventional work-up procedure afforded 0.463 g of the acid 3 (52% yield). $^1H$ NMR ($CDCl_3$) 1.93 (d,3H,J= 0.9 Hz), 2.18 (s,3H) and 5.71 (m,1H); $^{13}C$ NMR ($CDCl_3$) 171.91 ($CO_2H$).

Labelled epoxide 2 was prepared following a previously described general procedure[25]. Accordingly, 3,4-dimethoxyphenol (0,35 g, 2.3 mmol) and the acid 3 (0.23 g, 2.3 mmol) were caused to react in methanesulfonic acid (10 mL) for 30 min at 70°C. After usual work-up, the crude reaction mixture afforded 0.477 g of crystalline labelled chromanone 4 (89% yield). 4: $^1H$ NMR ($CDCl_3$) 1.44 (s,6H), 2.65 (d,2H,$J_{CH}$= 6.3 Hz), 3.86 (s,3H), 3.89 (s,3H), 6.40 (d,1H,$J_{CH}$= 2Hz, ArH) and 7.27 (d,1H,$J_{CH}$= 3.9 Hz); $^{13}C$ NMR ($CDCl_3$) 190.98 (CO); MS, m/z (relative intensity) 237 ($M^+$), 222 ($M^+$-15, 100%).

Reduction of chromanone 4 (0.285 g, 1.2 mmol) with lithium aluminium hydride (0.060 g, 1.8 mmol) in dry ether (20 mL) led to the intermediate alcohol which was dehydrated by treatment in situ with 6N HCl for 1 h at 20°C. The residue obtained after usual work-up was distilled bulb-to-bulb affording 0.206 g of the labelled chromene 1 (78% overall yield). 1: $^1H$ NMR ($CDCl_3$) 1.41 (s,6H), 3.82 (s,6H), 6.22 (dd,1H,$J_{CH}$= 164 Hz, J= 9.7 Hz, HC-4), 5.48 (d,1H,J= 9.7 Hz), 6.41 (s,1H) and 6.53 (d,1H,$J_{CH}$= 5.5 Hz, ArH); $^{13}C$ NMR ($CDCl_3$) 122.11 (HC-4).

Finally, a solution of N-bromosuccinimide (0.11 g, 0.6 mmol) in THF (15 mL) was added dropwise to a solution of labelled chromene 1 (0.12 g, 0.5 mmol) in 1:1 THF:$H_2O$ (25 mL). After the addition was completed, treatment of the crude reaction mixture afforded the corresponding bromohydrin which was redissolved in dry THF (20 mL) and added to a suspension of sodium hydride (0.018 g, 0.8 mmol) in the same solvent (15 mL). The mixture was stirred for 24 h at room temperature. After filtration of salts, the crude labelled epoxide 2 obtained was stored in THF solution at 0°. The yield, estimated by $^1H$ NMR with tetrachloroethane as internal standard, was over 90%. 2: $^1H$ NMR ($CDCl_3$) 1.25 (s,3H), 1.56 (s,3H), 3.45 (dd,1H,$J_{CH}$= 4.5 Hz, J= 2 Hz, HC-3), 3.82 (s,3H), 3.83 (dd,1H,$J_{CH}$= 180 Hz, J= 4.5 Hz, HC-4), 3.85 (s,3H), 6.41 (s,1H) and 6.84 (d,1H, $J_{CH}$= 5.9 Hz); $^{13}C$ NMR ($CDCl_3$) 51.01 (HC-4).

Reaction of epoxide 2 with N-acetylcysteine. A solution of the epoxide 2 (0.24 g, 1 mmol) in anhydrous THF (25 mL) was added dropwise to a solution of N-acetylcysteine (0.33 g, 2 mmol) in 0.1 N sodium hydroxide (40 mL). When the addition was completed HPLC monitoring revealed the total conversion of the starting epoxide (10 x 0.46 cm Hypersil ODS column, 5μ, eluting with 60:40 $CH_3OH$:0.05 M formic acid/triethylamine buffer at pH 5) . After removing the THF under reduced pressure, the residue was washed with ether, acidified with 2N HCl (10 mL) and extracted with ethyl acetate (2 x 10 mL). The joined extracts were washed with water, brine and dried over magnesium sulfate. The residue obtained after solvent elimination was redissolved in $H_2O$ (5 mL), the pH was adjusted to 6.5 and the solution was diluted to a final 10 mL volume. Aliquots of 2 mL were filtered through a system of 3 interconnected C-18 Sep-Pak cartridges (previously washed with $CH_3CN$, $CH_3OH$ and $H_2O$). Washing with $H_2O$ (10 mL) and elution with $CH_3OH$ (5 mL), followed by evaporation, led to the isolation of a 1:1 mixture of pure diastereomeric trans adducts 5a(3R,4R) and 5a(3S,4S) (0.326 g, 82% overall yield). Separation of these diastereomers was achieved by fractionated crystallyzation in chloroform/hexane/ether mixtures. Diastereomeric purity of crystals was over 99% (HPLC). 5a(3R,4R): mp 171-4°C; IR (KBr) 3300, 2970, 2930, 1730, 1660, 1620, 1510, 1445, 1410, 1265, 1220, 1205, 1240, 1120, 1010 and 920 $cm^{-1}$; $^1H$ NMR ($CDCl_3$ + 10% $CD_3COCD_3$) 1.19 (s,3H), 1.49 (s,3H), 2.04 (s,3H, $COCH_3$), 3,09 (d,2H,J = 5.3 Hz, $SCH_2$), 3.63 (d,1H,J = 9.7 Hz, HC-3), 3.82 (s,3H), 3.85 (s,3H), 3.88 (d,1H,J= 9.7 Hz, HC-4), 4.92 (m,1H, CH-NH), 6.33 (s,1H, ArH), 6.98 (d,1H,J = 8.3 Hz, NH) and 7.10 (s,1H, ArH); MS, m/z 381 ($M^+$-18) and 236; $[\alpha]_D$ +15.9° (c= 0.8). Anal. Calcd. for $C_{18}H_{25}O_7NS$: C, 54.07; H, 6.30; N, 3.50; S, 8.03. Found: C, 54.13; H, 6.59; N, 3.26; S, 8.00.
5a(3S,4S): mp 180-2°C; IR (KBr) 3380, 2970, 2930, 1725, 1595, 1515, 1440, 1405, 1265, 1205, 1120, 1010, 925 and 785 $cm^{-1}$; $^1H$ NMR ($CDCl_3$+ 10% $CD_3COCD_3$) 1.18 (s,3H), 1.51 (s,3H), 2.09 (s,3H,$COCH_3$), 2.88 (m,2H, $SCH_2$), 3.66 (d,1H, J= 9.7 Hz, HC-3), 3.79 (d,1H, J = 9.7 Hz, HC-4), 3.82 (s,3H), 3.84 (s,3H), 4.84 (m,1H, CHNH), 6.35 (s,1H, ArH), 6.69 (d,1H,J= 8.0 Hz, NH) and 7.10 (s,1H, ArH); MS, m/z 381 ($M^+$-18) and 236; $[\alpha]_D$ -3.7° (c= 0.8).

In a similar fashion, reaction of N-acetylcysteine with [4-$^{13}C$]-3,4-epoxyprecocene II (0.028 g, 0.12 mmol), afforded 0.032 g (68% yield) of the corresponding $^{13}C$ labelled diastereomeric mixture of adducts 5a. $^1H$ NMR ($CDCl_3$) 3.83 (dd,2H, J= 10 Hz, $J_{CH}$= 128 Hz, HC-4), 7.08 (d,2H,$J_{CH}$= 4.0 Hz,

ArH); $^{13}C$ NMR ($CDCl_3$) 49.60 and 48.49.

**Reaction of epoxide 2 with cysteine.** By using the same procedure as that described for the case of N-acetylcysteine, reaction of cysteine (0.12 g, 1 mmol) with the epoxide 2 (0.12 g, 0.5 mmol) in a 0.1 N NaOH/THF mixture afforded, after usual work-up, 0.145 g of a diastereomeric mixture of the trans cysteine adducts 5b (87% yield). In this case, all efforts for separating the diastereomers by fractionated crystallization were unsuccesful and only enriched samples of each compound were obtained. $^1H$ NMR ($D_2O$) 1.11 (s,6H), 1.41 (s,6H), 2.70-3.10 (4H,$CH_2S$), 3.60-3.85 (16H, $CH_3O$, HC-3, NHCH), 3.90 (d,2H, J= 9.5 Hz, HC-4), 6.42 (s,2H, ArH), 7.17 (s,1H, ArH) and 7.20 (s,1H, ArH); FAB-MS, m/z (relative intensity) 358 ($M^+$, 10%), 237 ($M^+$-cysteine residue, 100%).

**Reaction of epoxide 2 with glutathione.** A solution of the epoxide 2 (0.12 g, 0.5 mmol) in THF (20 mL) was added dropwise to a solution of glutathione (0.31 g, 1 mmol) in 0.1 N NaOH (30 mL). When the addition was completed, HPLC monitoring revealed the total conversion of the starting epoxide. Separation of lipophilic by-products and elimination of non reacted glutathione was carried out as described for the N-acetylcysteine adducts except that pH was adjusted to 7.0. Evaporation of the corresponding $CH_3OH$ eluate yielded 0.22 g of a 1:1 diastereomeric mixture of trans glutathione adducts 5c (80% yield). Separation of these compounds was performed by semipreparative HPLC with a 15 x 0.1 cm ODS-2 Spherisorb column, 5 µ, using a 80:20 mixture of buffer (0.075 M trifluoroacetic acid:triethylamine, pH 3.1) and organic modifier ($H_2O$:$CH_3CN$:THF 3:1:1) at 4.3 mL/min. By this procedure, compounds 5c(3R,4R) and 5c(3S,4S) were collected over 97% pure. Organic solvents from the joined eluates were evaporated under reduced pressure to give a residue which was neutralized and filtered through Sep-Pak C-18 cartridges. Addition of base to the filtrate up to pH 10, followed by elimination of triethylamine under reduced pressure, reneutralization and final filtration through Sep-Pak afforded the desired compounds. 5c(3R,4R): mp 93-8°; IR (KBr) 3325 (br), 2960, 2920, 1625 (br), 1510, 1440, 1400, 1260, 1200, 1160, 1120 and 1005 $cm^{-1}$; $^1H$ NMR ($D_2O$) 1.15 (s,3H), 1.46 (s,3H), 1.90-2.30 (m,2H, β-$CH_2$ glutamyl), 2.30-2.70 (m,2H, γ-$CH_2$ glutamyl), 2.93 (d,2H, J= 6.3, $CH_2S$), 3.82 (s,6H), 3.70-4.10 (5H, glutamyl and glycyl CH-N, HC-3, HC-4), 4.35 (m,1H, cysteinyl CH-N), 6.47 (s,1H, ArH) and 7.20 (s,1H, ArH); $[\alpha]_D$ +19.5 (c= 0.6); FAB-MS, m/z (relative intensity) positive ions: 544 ($M^+$, 16%), 237 ($M^+$-glutathione residue, 100%); negative ions: 543 ($M^-$-1, 28%), 542 ($M^-$-2, 100%), 306 ($M^-$-benzopyranyl residue, 17%).
5c(3S,4S): mp 191-5°C; IR (KBr) 3325 (br), 2960, 2920, 1630 (br), 1510, 1440, 1400, 1260, 1200, 1160, 1120 and 1005 $cm^{-1}$; $^1H$ NMR ($D_2O$) 1.16 (s,3H), 1.46 (s,3H), 1.90-2.30 (m,2H, β-$CH_2$ glutamyl), 2.30-2.70 (m,2H, γ-$CH_2$ glutamyl), 2.70-3.40 (m,2H, $CH_2S$), 3.82 (s,6H), 3.70-4.10 (5H, glutamyl and glycyl CH-N, HC-3, HC-4), 4.35 (m,1H, cysteinyl CH-N), 6.47 (s,1H, ArH) and 7.15 (s,1H, ArH); $[\alpha]_D$-48.9° (c= 0.8). FAB-MS, m/z (relative intensity) positive ions: 544 ($M^+$, 15%), 237 ($M^+$-glutathione residue, 100%); negative ions: 543 ($M^-$-1, 27%), 542 ($M^-$-2, 100%), 306 ($M^-$-benzopyranyl residue, 13%).

**Stereochemical correlation of adducts 5a-c.** A solution of γ-glutamyl transpeptidase (0.50 mg, 7.65 units, Sigma) in 0.5 mL of a 40 mM Tris/HCl buffer at pH 8.0 containing $MgCl_2$ was added to a solution of the corresponding glutathione adduct 5c (0.55 mg, 1 µmol) in the same buffer (6 mL), which had been warmed up for 30 min at 37°C. The final concentration of the glutathione adduct was 110 µM. The progress of successive degradations was monitored by HPLC, Hypersil ODS, 5 µ, eluting with a 80:20 buffer (0.05 M formic acid/triethylamine at pH 3.0): solvent mixture ($H_2O$:$CH_3CN$:THF 3:1:1) at 1.8 mL/min. When all the starting adduct had been converted into the corresponding cysteine adduct 5b(3R,4R) or 5b(3S,4S), an aliquot of the crude reaction mixture (containing ca. 0.16 µmol of the cysteine adduct) was filtered through a Sep-Pak C-18 minicolumn. After washing with $H_2O$ (10 mL), elution with $CH_3OH$ (5 mL) and further evaporation of the solvent under $N_2$ rendered a residue which was treated with acetic anhydride (5 µl) in 50% NaOAc (1 mL) for 30 min at 0°. In both cases, HPLC analyses showed the formation of the corresponding mercapturic acid derivative 5c(3R3R) and 5c(3S,4S) in almost quantitative yield.

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## REFERENCES

1.- Presented in part at The Sixth International Congress of Pesticide Chemistry IUPAC, Ottawa (Canada), 1986.
2.- Thakker, D.R.; Yagi, H.; Levin, W.; Wood, A.W.; Conney, A.H.; Jerina, D.M. In Bioactivation of Foreign Compounds; M.W. Anders, Ed.; Academic Press: London, 1985; pp 178-242.
3.- Garner, R.C.; Martin, C.N.; Smith, J.R.L.; Coles, B.F.; Tolson M.R. Chem.-Biol. Interact. 1979, 26, 57-73.
4.- Camps, F. In Biorregulators for Pest Control; P.A. Hedin, Ed.; ACS Symposium Series Nº 276, 1985, pp 237-243.
5.- Bowers, W.S.. In Comprehensive Insect Physiology, Biochemistry and Pharmacology; G.A. Kerkut and L.I. Gilbert, Eds.; Pergamon Press: London, 1985; Vol 8, pp 551-564.
6.- Soderlund, D.M.; Messeguer, A.; Bowers, W.S. J. Agric. Food Chem. 1980, 28, 724-728.
7.- Hamnett, A.F.; Pratt, G.E. Life Sci., 1983, 32, 2747-2753.
8.- Camps, F.; Coll, J.; Conchillo, A.; Messeguer, A. Tetrahedron 1985, 41, 5169-5174.
9.- Pratt, G.E.. In Natural Products for Innovative Pest Management; T.R. Odhiambo, Ed.; Pergamon Press: London, 1980; pp 325-337.

10.- Halpin, R.A.; Vyas, K.P.; El Naggar, S.F.; Jerina, D.M. Chem.-Biol. Interact. 1984, 31, 297-315.
11.- Hsia, M.T.S.; Kreamer, B.L.; Duddy, S.K. Cancer Letters 1985, 26, 311-318.
12.- Schrankel, K.R.; Grossman, S.J.; Hsia, M.T.S. Toxicology Letters 1982, 12, 95-100.
13.- Jennings, R.C. Tetrahedron Letters 1982, 2693-2696.
14.- Halpin, R.A.; El Naggar, S.F.; McCombe, K.M.; Yas, K.P.; Boyd, D.R.; Jerina , D.M. Tetrahedron Letters 1982, 1655-1658.
15.- Jennings, R.C.; Ottridge, A.P. J.C.S. Perkin Trans 1984, 1733-1738.
16.- Aizawa, H.; Bowers, W.S.; Soderlund, D.M. J. Agric. Food Chem. 1985, 33, 406-411.
17.- Grossmann, S.J.; Hsia, M.T.S. Toxicology 1982, 25, 293-298.
18.- Bellés, X.; Baldellou, M.; Messeguer, A. Arch. Insect Biochem. Physiol 1987, 4, 107-112.
19.- Meister, A.; Andersen, M.E. Ann. Rev. Biochem. 1983, 52, 711-760.
20.- Watabe, T.; Ozawa, N; Hiratsuka, A.; Saitoh, Y.; Tsurumori, T. Biochem. Pharmacol. 1984, 33, 2687-2690.
21.- Watabe, T.; Hiratsuka, A.; Tsurumori, T. Biochem. Biophys. Res. Commun. 1985, 130, 65-70.
22.- Moss, E.J.; Neal, G.E.; Judah, D.J. Chem.-Biol. Interact. 1985, 55, 139-155.
23.- Cobb, D.; Boehlert, C; Lewis, D.; Armstrong, R.N. Biochemistry 1983, 22, 805-12.
24.- Kayser, M.M.; Morand, P. Can. J. Chem. 1980, 58, 302-306.
25.- Camps, F.; Conchillo, A.; Messeguer, A. Z. Naturforsch. 1985, 40b, 556-558.
26.- Hylarides, M.D.; Lyk, T.A.; Daub, G.H.; Vanderjogt, D.L. J. Org. Chem. 1979, 44, 4652-4657.
27.- Yagen, B.; Hernández, O.; Bend, J.R.; Cox , R.H. Bioorg. Chem. 1981, 10 299-310.
28.- Yagen, B.; Hernández, O.; Bend, J.R.; Cox , R.H. Chem.-Biol. Interact. 1981, 34, 57-67.
29.- As it occurs with TLC and normal phase HPLC, under reversed-phase HPLC conditions epoxide 2 is also unstable and only products coming from its reaction with the eluent mixture, are detected, i.e., the cis/trans mixture of diols or the corresponding methoxyhydrins when methanol is used as major component of the organic modifier. Actually, the methoxyhydrins have been used as chromatographic tracers for unreacted epoxide along this work.
30.- This assignation was further confirmed by comparison with the corresponding $^{13}C$ NMR absorptions of the cis and trans 3,4-dihydrodiols obtained by acid hydrolysis of the labelled epoxide 2, which appeared at 65.42 and 70.11 ppm, respectively.
31.- Belaud, F.; Harvey, R.G. J. Amer. Chem. Soc. 1976, 98, 4963-4970.
32.- Camps, F.; Conchillo, A.; Messeguer, A.; Molins, E.; Miravitlles , C. J. Cryst. Spect. Res. 1987, in the press.
33.- Hernández, O.; Bhatia, A.V.; Walker. M. J. Liquid Chromatog. 1983, 6, 1475-1489.