Synthesis of Glutathione Adducts of K-Region Arene Oxides

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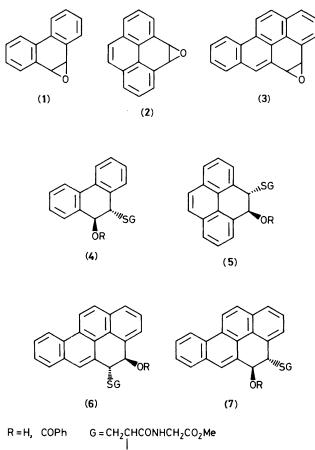
The K-region oxides of phenanthrene, pyrene, and benzo[a]pyrene react with *N*-trifluoroacetylglutathione dimethyl ester to give diastereoisomeric conjugates amenable to separation by chromatography.

The enzymatic reaction of glutathione (γ -glutamylcysteinylglycine, GSH) with epoxides is an important metabolic pathway in a general detoxication scheme leading to the eventual elimination of these reactive intermediates.¹ The use of K-region oxides of polynuclear aromatic hydrocarbons as substrates has provided valuable information relevant to the stereochemistry of this enzymatic transformation.²

Further mechanistic studies have been hampered by the lack of suitable procedures for the preparation of diastereoisomerically pure GSH adducts of K-region oxides. The separation of isomeric sulphide derivatives of arene oxides, including those from simple thiols,³ has been traditionally difficult; this difficulty is magnified when attempting purifications on a preparative scale. In the present communication

GSH adduct ^a	Configuration	Chemical shift (δ)		Coupling
		-CHSG	-CHOCOPh	constant (Hz)
(4 a)	[9S, 10S]	4.76	6.32	$J_{9,10}$ 1.4
(4b)	[9R, 10R]	4.57	6.30	$J_{9,10}2.4$
(5a)	[4S, 5S]	5.09	6.64	$J_{4.5}$ 1.1
(5b)	[4R, 5R]	5.06	6.59	$J_{4.5}$ 2.2
(6a)	[4S, 5S]	5.22	6.74	$J_{4,5}$ 1.63
(7a)	[4S, 5S]	5.20	6.79	$J_{4,5}$ 1.62
(6b)	[4R, 5R]	5.02	6.70	$J_{4,5}$ 1.96
(7b)	[4R, 5R]	4.99	6.76	J _{4,5} 2.22

^a R = COPh; compounds listed in order of elution from Zorbax-Sil column, *i.e.*, (a) is first eluting in series.



R = H, COPH G = CH2CHCONHCH2CO2Me | NHCOCH2CH2CHCO2Me | NHCOCF3

we describe procedures for the synthesis and purification of GSH adducts of phenanthrene 9,10-epoxide (1), pyrene 4,5-epoxide (2), and (\pm) -benzo[a]pyrene 4,5-epoxide (3).

Phenanthrene 9,10-epoxide (1) (1 mmol), N-trifluoroacetylglutathione dimethyl ester⁴ (2 mmol), and triethylamine (6 mmol) in dry methanol (25 ml) were stirred under argon at 50—55 °C. After 2 h the solvent was removed and the crude reaction mixture purified by h.p.l.c. (Zorbax-Sil column 21.2 \times 250 mm, C₆H₁₂: CH₂Cl₂, 3:2/5% propan-2-ol) to provide the pure hydroxysulphide (4; R = H) (93%) as a mixture of diastereoisomers. Similarly, the diastereoisomeric adducts of (2) and (3) were prepared in 85 and 79% isolated yields [(5), (6), and (7), R = H]. The sulphide diastereoisomers were more conveniently separated as the benzoates (4 equiv. benzoyl chloride. 4 equiv. 4-dimethylaminopyridine,⁵ CH₂Cl₂, 25 °C, 10 min); percolation of crude benzoate mixture through Florisil (ethyl acetate) followed by h.p.l.c. (Zorbax-Sil column, CHCl₃; 1% propan-2-ol) gave (4a; R = COPh), m.p. 104–105 °C, $[\alpha]_{\rm D}$ + 237.6 ° (c = 0.12, MeOH), λ_{max} 270 nm ($\epsilon = 16\,000\,\text{dm}^3\,\text{mol}^{-1}\,\text{cm}^{-1}$, MeOH), and (4b; R = COPh), m.p. 97—100°C, $[\alpha]_D$ - 301.1° (c = 0.10, MeOH), λ_{max} 270 nm (ε = 15 000 dm³ mol⁻¹ cm⁻¹, MeOH). The diastereoisomeric GSH adducts of (2) and (3) were also separated as their benzoates (R = COPh) on a Zorbax-Sil column using 1.5% propan-2-ol-CHCl₃ for (5) and EtOAc- C_6H_{12} -CHCl₃ (4:5:1) for (6) and (7). The stereochemistry of the sulphide adducts was assigned as trans as expected from an $S_{\rm N}2$ type addition reaction which requires an antiperiplanar arrangement of epoxide and thiol nucleophile.⁶ The ¹H n.m.r. spectra of the conjugates supported this assignment; the small J values (Table 1) were in agreement with values reported for similar trans systems and suggested a strong conformational preference for diaxial substituents.^{2,3,6,7} The absolute stereochemistry of the adducts was determined by comparison of their circular dichroism (c.d.) spectra with published spectra of free conjugates.² There are no observable differences in the c.d. spectra of protected vs. unprotected conjugates. † A small but discernible difference was found in the J values (Table 1), with the coupling constants for the S-diastereoisomers being consistently smaller than the corresponding values for the *R*-diestereoisomers. In addition, the order of elution correlated with the configuration of the sulphur-bearing carbon and, as established for reversed-phase h.p.l.c.,⁸ for each regioisomer the diastereoisomer with S-configuration eluted ahead of the R-diastereoisomer.

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 $[\]dagger$ The protected conjugates were hydrolysed (0.1 \star K2CO3-MeOH), purified by reversed-phase h.p.l.c. (ref. 8), and their c.d. spectra recorded.