THE DETECTION OF AN EPOXIDE INTERMEDIATE BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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The metabolism of allyl substituents in various drugs, particularly barbiturates (Waddell, 1965) has attracted interest in recent years. Drugs of this type exert a variety of effects on hepatic microsomal mixed function oxidases (Ioannides and Parke, 1976) and the allyl groups may be transformed to the corresponding diols via epoxide intermediates as has been demonstrated for the allyl barbiturates (Harvey and others, 1972). The anti-inflammatory drug alclofenac contains an allyl group and the diol accounts for over 50% of the urinary metabolites in the mouse (Roncucci and Lambelin, 1977). The present communication describes the detection of the corresponding epoxide.

Mice were dosed with alclofenac (100-200mg·kg^{-1}, p.o.) and the urine was collected at 5°C. The urine was acidified and extracted with chloroform and the residue methylated with diazomethane. The methyl esters were chromatographed directly using a 1% OV101 column interfaced, by a single-stage glass jet separator, to a mass spectrometer. The methyl ester of alclofenac epoxide had sufficient stability to be chromatographed directly, although the corresponding diol could not be assayed using this derivatisation technique.

The epoxide was converted to the trimethylsilyl (TMS) ether of the corresponding chlorohydrin (Harvey and others, 1972) and the diol converted to the di-TMS ether using the same derivatisation technique. Although both TMS derivatives could now be assayed simultaneously (Table 1), retention characteristics were very similar and it was found that a large excess of the diol interfered with the quantitation of trace amounts of the epoxide. The use of t-butyldimethylsilyl (tBDMS) derivatives in gas chromatography-mass spectrometry is well documented (Gaskell and Brooks, 1976) and the tBDMS ether of the chlorohydrin, derived from the epoxide, and the di-tBDMS ether of the diol were prepared. The improvement in the resolution of the epoxide and diol derivatives can be seen in Table 1. The tBDMS derivatives also have the advantage of higher sensitivity and specificity when multiple ion monitoring is utilised.

Table 1. Retention indices of the methyl esters

Derivative	1% OV101	1% OV17
Alclofenac	1820	2055
Alclofenac epoxide	1993	2331
TMS chlorohydrin	2220	2463
di-TMS diol	2289	2445
tBDMS chlorohydrin	2349	2690
di-tBDMS diol	2686	2798

Gaskell,S.J. and Brooks,C.J.W.(1976). Biochem.Soc.Trans., 4, 111-113.

Harvey,D.J., Glazener,L. and others.(1972).Res.Commun.Chem.Path.Pharm.,4,247-260.

Ioannides,C. and Parke,D.V.(1976). Chem.Biol.Interact., 14, 241-249.

Roncucci,R. and Lambelin,G.(1977). Drugs Exptl.Clin.Res., 2, 9-25.

Waddell,W.J.(1965). J.Pharmacol.Exp.Ther., 149, 23-28.

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