Journal of Chromatography, 530 (1990) 283–294
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5384

Biotransformation of diethenylbenzenes

II. Metabolic pattern of 1,4-diethenylbenzene

IGOR LINHART[a,*]

Institute of Hygiene and Epidemiology, Centre of Industrial Hygiene and Occupational Diseases, Prague (Czechoslovakia)

JAROMÍR NOVÁK

Institute of Microbiology, Czechoslovak Academy of Sciences, Prague (Czechoslovakia)

(First received November 23rd, 1989; revised manuscript received April 28th, 1990)

ABSTRACT

The metabolism of 1,4-diethenylbenzene in the rat was followed by gas chromatographic–mass spectrometric analysis of urine using three different derivatization procedures: (i) methylation–acetylation; (ii) methylation–trimethylsilylation; (iii) methylation followed by conversion into trimethylsilyloximes. Fifteen metabolites were found in the urine of rats dosed with a single intraperitoneal injection of 1,4-diethenylbenzene (300 mg/kg). Nine of them were identified in our previous study [I. Lindhart *et al.*, *Xenobiotica*, 19 (1989) 645], but the other six have not previously been reported. New metabolites, namely, 1-ethenyl-4-(1-hydroxyethyl)benzene, 4-(1,2-dihydroxyethyl)benzoic acid, (4-carboxymethylphenyl)acetylglycine, N-acetyl-S-[2-carboxy-1-(4-ethenylphenyl)ethyl]-L-cysteine, and two isomeric β-D-glucosiduronates derived from 1-(4-ethenylphenyl)ethane-1,2-diol, were identified by mass spectrometry of their derivatives and comparison of them with the spectra of analogous metabolites of styrene and 4-methylstyrene. Acetylation of methylated urine extracts seems to be the most suitable derivatization procedure, but a combination of at least two procedures is needed if the virtually complete metabolic pattern of diethenylbenzene is to be obtained. Possible routes of biotransformation leading to the newly identified metabolites are discussed.

INTRODUCTION

1,4-Diethenylbenzene (1,4-DEB) is a bifunctional monomer, which is used along with 1,3-diethenylbenzene as a cross-linking agent in co-polymerization with styrene. In a previous paper we investigated the main routes in the biotransformation of 1,4-DEB. As many as ten metabolites have been identified in urine extracts treated with diazomethane [1]. This preliminary analysis did not allow the identification of all the metabolites detected. Moreover, many polar groups, such as hydroxyl and amino groups, remained underivatized after diazomethane

[a] Temporary address (till end of October 1990): c/o Pharmacy, Institute of Pharmaceutical Sciences, Aston University, AstonTriangle, Birmingham B4 7ET, U.K.

0378-4347/90/$03.50

treatment so that a further derivatization seems to be necessary to improve separation and also to avoid formation of chromatographic artifacts.

The aim of the present study was to find a suitable derivatization procedure that would allow the elucidation of the virtually complete metabolic pattern of 1,4-DEB and the identification of new metabolites that might be important for the evaluation of health hazards arising from exposure to DEB. Although identification of a new metabolite based solely on its mass spectrum must always be considered tentative, it is often the only way to get some insight into the complex metabolism of a xenobiotic.

EXPERIMENTAL

Chemicals

1,4-DEB (>99.9% pure) was prepared in our laboratory [2]. Acetic anhydride and ethyl acetate (Lachema, Brno, Czechoslovakia) were distilled before use. Bis-(trimethylsilyl)trifluoroacetamide (BSTFA) was from Pierce (Beijerland, The Netherlands). Pyridine A.R. (Avondale Labs., Brinny, U.K.) was dried over potassium hydroxide, distilled and stored over a molecular sieve. Hydroxylamine hydrochloride was from Lachema.

Apparatus

Gas chromatography–mass spectrometry (GS–MS) was performed with a Finnigan Mat 90 mass spectrometer coupled to a Varian 3400 gas chromatograph. Electron impact (EI) spectra were measured at 70 eV at either ordinary or high resolution (6000). Perfluorokerosene was used as calibration standard for high-resolution measurements. Chemical ionization (CI) spectra were taken using ammonia as reagent gas: the mass range was 50–600 a.u. Metabolites were separated on a 20 m × 0.2 mm I.D. fused-silica capillary column coated with cross-linked OV-1 (Hewlett-Packard). The flow-rate of helium carrier gas was 0.5 ml/min. The injector port temperature was 250°C, the column oven temperature was held at 35°C for 1 min with the splitter closed and thereafter programmed from 35 to 55°C at 30°C/min and from 55 to 250°C at 5°C/min with the splitter open. Alternatively, a Varian 3400 gas chromatograph equipped with a Model 700 ion trap detector (Finnigan Mat) and a 30 m × 0.2 mm I.D. fused-silica column coated with cross-linked SE-54 (Hewlett-Packard) were used. The chromatograph was operated in the open split mode. The injector port temperature was 250°C, the column oven temperature was held at 35°C for 1 min and thereafter programmed from 35 to 100°C at 30°C/min and from 100 to 250°C at 8°C/min.

Animals

Adult female Wistar rats (Velaz, Prague, Czechoslovakia) weighing 200–260 g were dosed intraperitoneally with a single injection of 300 mg/kg 1,4-DEB in sunflower oil; the control group received sunflower oil only. Each group consisted

of five animals. Animals were handled as described previously in greater detail [1]. Individual urine samples were collected 24 h after dosing, diluted to 15 ml or more, if needed, with distilled water and stored at −18°C.

Sample preparation

Average samples were prepared by pooling aliquots of individual urine samples from both the exposed and the control group. They were derivatized in three different ways:

(i) Acetylation. Urine samples were filtered through 0.45-μm Durapore membrane filters (Millipore, Bedford, U.S.A.). To 1 ml of filtered urine, 4 ml of water, 1 g of sodium bicarbonate and 250 μl of acetic anhydride were added. After the evolution of carbon dioxide stopped, samples were acidified to pH 2 with dilute hydrochloric acid (1:1) and extracted with two 5-ml portions of ethyl acetate. Extracts were treated with a slight excess of a diethyl ether solution of diazomethane, dried with magnesium sulphate, filtered and concentrated to 2 ml . Thin-layer chromatography (TLC) on Merck aluminium sheets coated with silica gel $60F_{254}$ (layer thickness 0.2 mm) using chloroform–acetone (7:3, v/v) as eluent indicated that acetylation was not complete at this stage. Spots of metabolites that were expected to acetylate, *e.g.* mercapturates at R_F 0.39 and 0.49, had not disappeared completely. Therefore, extracts were treated with an additional portion of 0.5 ml of acetic anhydride and 20 μl of pyridine. After 3 h at room temperature samples were diluted with 3 ml of ethyl acetate, washed with three 1-ml portions of saturated sodium bicarbonate solution and with 1 ml of water. Finally, they were dried over anhydrous magnesium sulphate, filtered and concentrated to *ca.* 0.5 ml by evaporation of the solvent *in vacuo*.

(ii) Trimethylsilylation. Extracts obtained from 5 ml of urine were treated with diazomethane [1] and concentrated to 2 ml. Aliquots (200 μl) were mixed with 40 μl of BSTFA in screw-cap vials with a PTFE valve and allowed to react for 40 min at room temperature. Aliquots of 1 μl were injected into the GC apparatus.

(iii) Conversion of ketones into trimethylsilyloximes (SOX). Diazomethane-treated extracts obtained from 5 ml urine were evaporated to dryness *in vacuo*. The residues were dessicated by adding 1 ml of toluene, which was thereafter evaporated *in vacuo*, and were dissolved in 0.5 ml of pyridine containing 5 mg of hydroxylamine hydrochloride. After 1 h at room temperature, 5 ml of ethyl acetate were added and the solutions were washed with two 3-ml portions of dilute hydrochloric acid followed by water. Samples were dried over magnesium sulphate and filtered. Aliquots were treated with BSTFA as described above.

RESULTS

Mass chromatograms of urine extracts treated with acetic anhydride and diazomethane (procedure i), with diazomethane and BSTFA (procedure ii) or with diazomethane, hydroxylamine and BSTFA (procedure iii) are shown in the Figs.

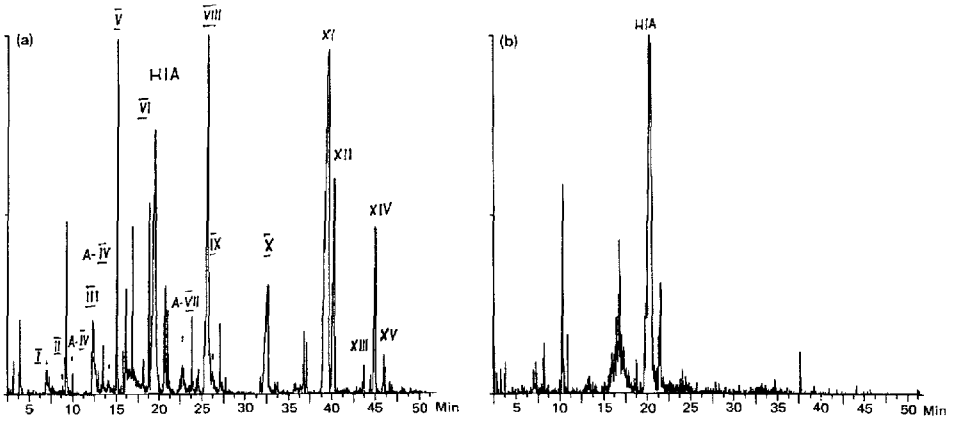

Fig. 1. Mass chromatograms of urine extracts treated with diazomethane and acetic anhydride (procedure i). (a) Rats exposed to 300 mg/kg 1,4-DEB; (b) control rats. HIA is hippuric acid, and the letter "A" followed by the number of a certain metabolite denotes artifacts that are likely to be formed from corresponding metabolite.

1, 2 and 3, respectively. Metabolites could be recognized by the comparison of urine extracts from exposed rats with those from control rats. Additionally, characteristic fragmentation of metabolites containing the 4-ethenylphenyl moiety, *i.e.* $(CH_2=CH-C_6H_4)^+$ at *m/z* 103, $(CH_2=CH-C_6H_4CO)^+$ at *m/z* 131 and/or $(CH_2=CH-C_7H_7O)^+$ at *m/z* 133 was observed. These fragments were only to be expected in metabolites containing an intact ethenyl group. Nine metabolites reported here were identified in the first part of this series, another six metabolites are newly identified. They are numbered in the order of elution from the GC column (Fig. 1). Mass spectra are listed in Table I. Only spectra not reported previously are given. The following metabolites were identified:

4-Ethenylbenzoic acid (I). This compound was isolated from urine [1]; the mass spectrum of its methyl ester was previously reported [3].

4-Ethenylphenylacetic acid (II). This compound was identified by GC–MS as a minor metabolite [1].

1-Ethenyl-4-(1-hydroxyethyl)benzene (III). 1-Phenylethanol and 2-phenylethanol were identified as styrene metabolites by Bakke and Scheline [4]. In the acetylated extract a compound with M = 190 showing the main fragment at *m/z* 131 ($[M-COOCH_3]^+$) but no ion at *m/z* 117 ($[M-CH_2OOCH_3]^+$) was found. Its fragmentation is in agreement with the structure of a 1-phenylethanol analogue but not with that of a 2-phenylethanol analogue [5]. The trimethylsilyl (TMS) derivative of III could not be found, probably because of its thermal degradation in the injector and/or on column.

1-(4-Ethenylphenyl)ethane-1,2-diol (IV). This metabolite was isolated from urine [1]. It does not appear on the chromatogram unless hydroxyl groups are converted into less polar TMS or acetyl derivatives. The TMS derivative shows a

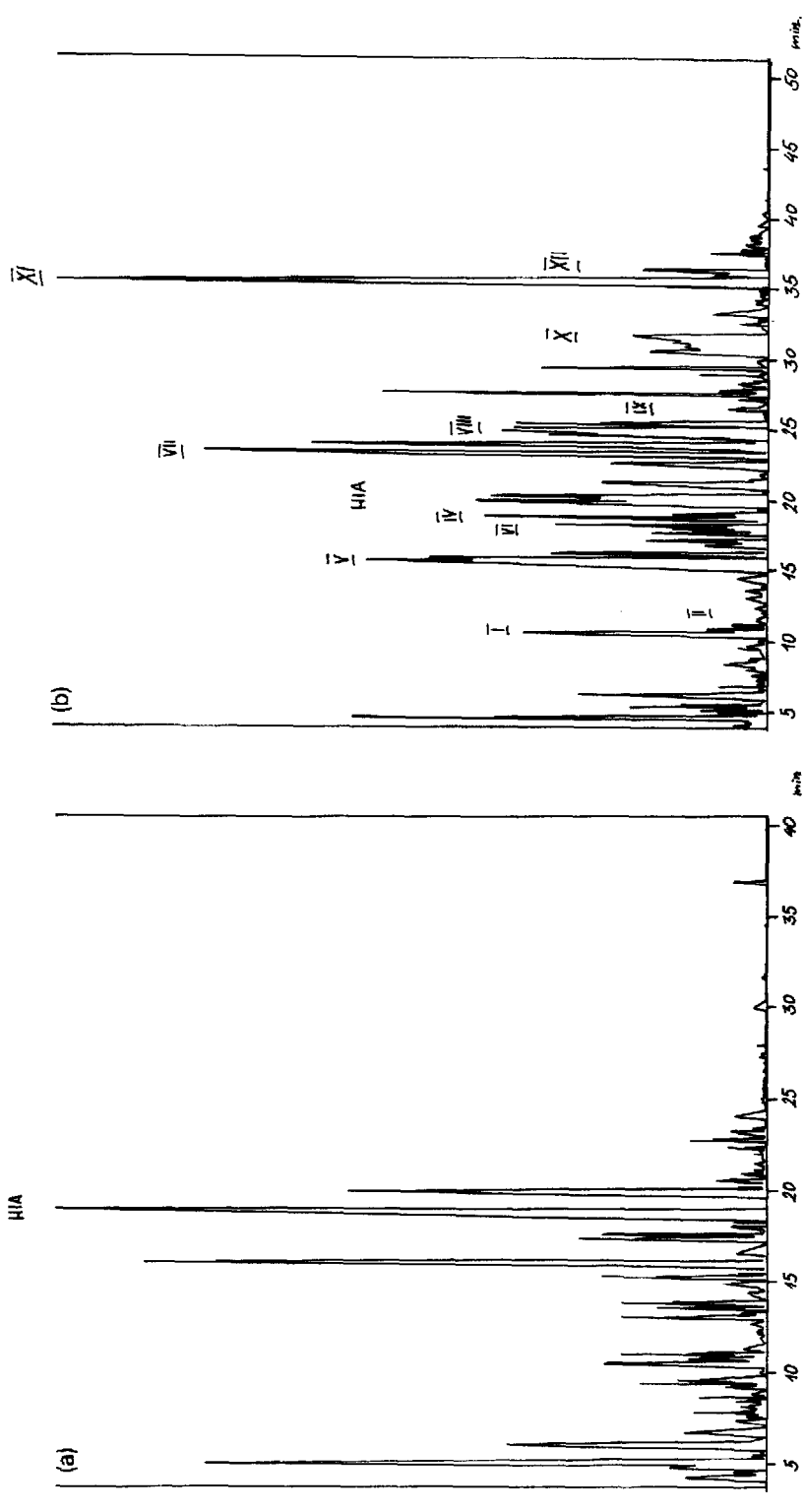

Fig. 2. Mass chromatograms of urine extracts treated with diazomethane and BSTFA (procedure ii). (a) Control rats; (b) rats exposed to a single intraperitoneal dose of 300 mg/kg 1,4-DEB.

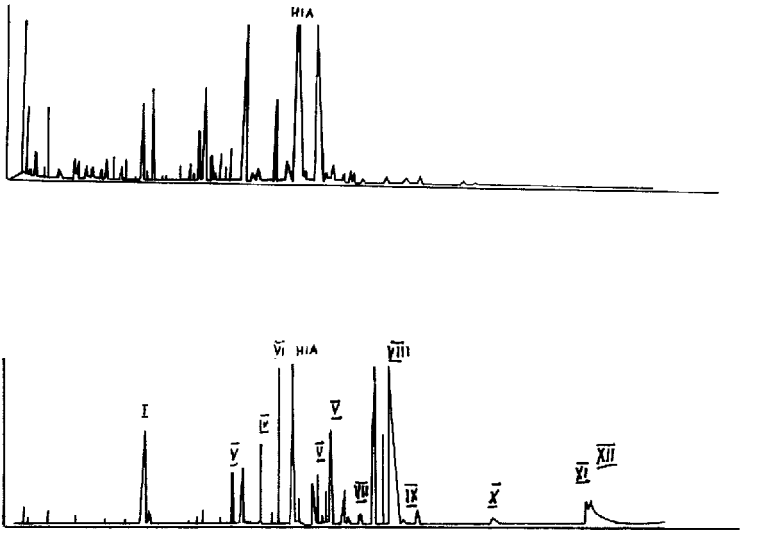

Fig. 3. Mass chromatograms of urine extracts treated with diazomethane, hydroxylamine and BSTFA (procedure iii). (Top) Control rats; (bottom) rats exposed to a single intraperitoneal dose of 300 mg/kg 1,4-DEB.

molecular ion at *m/z* 308 and characteristic fragments ($[M-CH_3]^+$ and $[M-CH_2OTMS]^+$). The observed mass spectrum is in good agreement with the data on the TMS derivative of 1-(4-methylphenyl)ethane-1,2-diol, a metabolite of 4-methylstyrene [6]. The diacetyl derivative of IV was not detected directly, but at least two compounds with M = 188 and elution times of 10 and 14 min (Fig. 1) were detected. These compounds could arise artificially by thermally induced elimination of acetic acid from the diacetyl derivative of IV (Fig. 4).

4-Ethenylphenylglyoxylic acid (V). This metabolite was identified by GC–MS of its methyl ester [1]. It can be converted into the trimethylsilyloxime of the methyl ester by procedure iii. Two peaks on Fig. 3 show virtually identical mass spectra and could be assigned to the *E*- and *Z*-isomers. The major peak is probably that of the thermodynamically more stable isomer. Isomer *E*, with the two most bulky groups in *trans*-positions, should be the more stable one in the absence of hydrogen-bonding stabilizing other *Z*-oximes. Conversion into the SOX derivative was incomplete under the conditions used. A considerable amount of the methyl ester of V was detected in the same run. MS fragmentation of the methyl ester of V is closely similar to that reported for methyl phenylglyoxylate [7].

4-Ethenylmandelic acid (VI). When derivatized only with diazomethane, VI was not completely separated from V. Conversion of the hydroxyl group into the TMS ether or acetate ester led to a good separation of VI. Neither the TMS nor the acetyl derivative of VI showed molecular ions in EI-induced MS. In contrast, an apparent MH^+ ion at *m/z* 235 was observed in the CI-induced MS of the

TABLE I

MASS SPECTRA OF 1,4-DEB METABOLITES

Metabolite	Derivative		Main ionic species (m/z)/relative intensities (%)
III	Ac	CI:	191/12 (MH^+); 131/100 ($[M-CH_3COO]^+$); 103/5 ($C_6H_5CH=CH_2^+$)
IV	Di-TMS	EI:	308/0.5 (M^+); 293/1 ($[M-CH_3]^+$); 205/100 ($[M-CH_2OTMS]^+$)
V	Me ester-SOX[a]	EI:	277/55 (M^+); 262/60 ($[M-CH_3]^+$); 234/20 ($[M-CH_3-CO]^+$); 218/8 ($[M-COOCH_3]^+$); 129/12 ($[M-COOCH_3-TMSO]^+$); 89/100 ($TMSO^+$)
VI	Me ester-TMS	EI:	249/5 ($[M-CH_3]^+$); 205/100 ($[M-COOCH_3]^+$); 73/41 (TMS^+)
	Me ester-Ac	EI:	202/48 ($[M-CH_3OH]^+$); 192/15 ($[M-CH_2CO]^+$); 133/100 ($[M-CH_2COCOOCH_3]^+$)
		CI:	235/5 (MH^+); 202/61; 192/38; 175/100 ($[M-COOCH_3]^+$)
X	Di-Me ester	EI[b]:	279/19 (−1.4; M^+); 220/17 (−3.7; $C_{12}H_{14}NO_3$); 190/40 (+2.3; $C_{11}H_{10}O_3$); 164/58 (+4.4; $C_{10}H_{12}O_2$); 104/100 (+0.9; C_8H_8).
		CI:	280/100 (MH^+); 190/95 ($[M-NH_2CH_2COOCH_3)]^+$); 164/66 ($[M-CONHCHCOOCH_3]^+$); 104/100 ($C_8H_8^+$)
XI	Me ester-Ac	EI:	246/19 ($[M-CH_3CONH_2-CH_3COOH]^+$); 162/17 ($C_{10}H_{10}S^+$); 189/11 ($[M-SCH_2CH(NHCOCH_3)COOCH_3]^+$); 130/100 ($C_{10}H_{10}^+$)
	Me ester-TMS	EI:	380/2 ($[M-CH_3]^+$); 305/4 ($[M-TMSOH]^+$); 250/38 ($[M-CH_2OTMS-CH_2CO]^+$); 144/98 ($CH_2C(NHCOCH_3)-COOCH_3^+$); 292/35 ($[M-CH_2OTMS]^+$); 219/45 ($[M-SCH_2CH-(NHCOCH_3)COOCH_3]^+$); 73/100 ($TMS^+$)
XII	Me ester-Ac	EI:	305 ($[M-CH_3COOH]^+$); 130/100 ($C_{10}H_{10}^+$)
		CI:	366/11 (MH^+); 306/63 ($[M-COOCH_3]^+$); 246/22 ($[M-CH_3CONH_2-CH_3COOH]^+$); 189/78 ($[M-SCH_2CH-(NHCOCH_3)COOCH_3]^+$); 162/39 ($C_{10}H_{10}S^+$); 144/40; 130/100
	Me ester-TMS	EI:	191/22 ($CH_3SCH_2CH(NHCOCH_3)COOCH_3^+$); 133/41 ($CH_2=CHC_7H_6O^+$); 132/100 ($CH_3SCH=CHCOOCH_3^+$)
XIII	Di-Me ester	CI:	352/22 (MH^+); 292/18 ($[M-COOCH_3]^+$); 176/77 ($SCH_2CH-(NHCOCH_3)COOCH_3^+$); 144/100 ($CH_2C(NHCOCH_3)COOCH_3^+$); 117/78 ($CH_2=CHC_7H_6^+$); 91/20 ($C_7H_7^+$)
XIV	Me ester-Ac	CI:	317/7 ($[M-CH_2=CHC_6H_6CH(OAc)CH_2O]^+$); 257/2; 189/100 ($CH_2=CHC_6H_4CHCH_2OAc^+$); 154/30; 129/30
XV	Me ester-Ac	CI:	317/100; 257/33; 197/25; 188/40; 189/28; 155/35; 146/20

[a] Mass spectra of *E*- and *Z*-isomers are almost identical; the spectrum of the major isomer is reported here.

[b] High-resolution MS; errors (found–calculated in parenthesis) given in 10^{-3} mass unit.

O-acetyl methyl ester. Ionic species at m/z 202 and 192 could be assigned to fragments $[M-CH_3OH]^+$ and $[M-CH_2CO]^+$, respectively. Analogous fragmentation was reported for mandelic acid derivatives [6,8].

4-(1,2-Dihydroxyethyl)benzoic acid (VII). This compound was reported to be a metabolite of 4-methylstyrene [6]. Its identification was therefore made by direct comparison of the mass spectrum of the di-TMS ether methyl ester derivative with published data. In the acetylated sample, no spectrum consistent with the

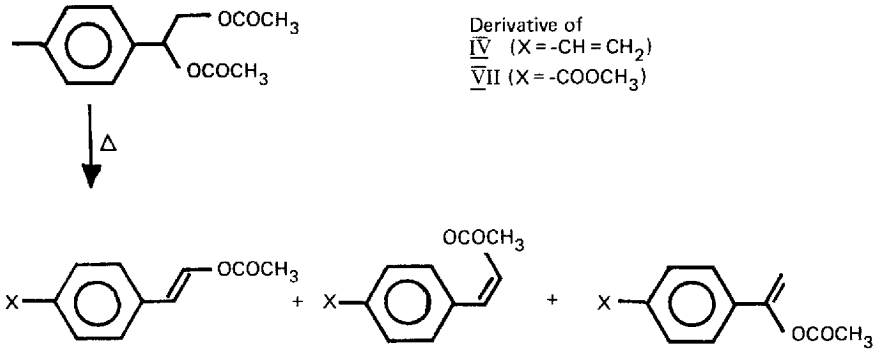

Fig. 4. Possible route of thermal degradation of glycol diacetates (IV an VII).

structure of a diacetyl methyl ester of VII was found. However, two acetylated compounds were detected (M = 220), which could possibly arise from a derivative of VII by elimination of acetic acid in the GC injector (Fig. 4).

4-Ethenylbenzoylglycine (VIII). This compound was isolated from urine and identified as its methyl ester [1]. It is one of the main metabolites of 1,4-DEB.

4-Ethenylphenylacetylglycine (IX). This compound is a major metabolite identified by GC–MS [1]. Peaks of its methyl ester are apparent in chromatograms in Figs. 1–3.

(4-Carboxymethylphenyl)acetylglycine (X). This compound is one of the major metabolites: it was not identified earlier, and shows a strong molecular ion at *m/z* 279 irrespective of the derivatization procedure used. Its mass spectrum is consistent with the structure of X; however, an alternative structure of 4-carboxycarbonylbenzoylglycine may come into account. To decide between these two structures high-resolution MS was used in GC–MS mode to reveal the elemental composition of the main ionic species. The exact masses obtained were in good agreement with the elemental composition of major peaks shown in Table I, but not with possible fragmentation of the alternative structure, 4-carboxycarbonylbenzoylglycine. Therefore, the structure of X seems to be confirmed.

N-Acetyl-S-[1-(4-ethenylphenyl)-2-hydroxyethyl]-L-cysteine (XI). Mercapturic acid XI was isolated and identified previously [1]. Significant degradation was observed when the compound chromatographed as its methyl ester. An additional derivatization with BSTFA or with acetic anhydride eliminated the formation of chromatographic artifacts from XI. Spectra of both the TMS ether methyl ester and the O-acetyl methyl ester derivatives are in good agreement with the structure of XI (Table I).

N-Acetyl-S-[2-(4-ethenylphenyl)-2-hydroxyethyl]-L-cysteine (XII). Formation of minor mercapturic acid was also reported [1]. Trimethylsilylation of the methyl ester of XII led to a derivative that is probably less stable than the TMS ether of the methyl ester of XI. Only a minor peak, which did not correspond to

the reported amount of XII formed [1], was detected. In contrast, acetylation led to a stable derivative. Its MH^+ ion was found in CI-induced MS.

N-Acetyl-S-[2-carboxy-1-(4-ethenylphenyl)ethyl]-L-cysteine (XIII). A minor peak eluting after mercapturic acids XI and XII (Fig. 1) was identified as the dimethyl ester of XIII. Its spectrum contains characteristic ions of mercapturic acid methyl esters at *m/z* 176 ($[CH_3OOCCH(NHCOCH_3)CH_2S]^+$) and 144 ($[CH_2=C(NHCOCH_3)COOCH_3]^+$) and fragments at *m/z* 352 (MH^+) and 292 ($[M-COOCH_3]^+$). The formation of XIII by metabolic oxidation of either XI or its precursors (glutathione, cysteinylglycine and cysteine adducts) seems to be highly probable.

β-D-Glucosiduronates of glycol IV: major (XIV) and minor (XV). A glucosiduronate was detected in the urine of DEB-treated rats by TLC using naphthoresorcinol spraying reagent [1]. By GC–MS of an acetylated extract two compounds were detected, which contained characteristic fragments of an acetylated glucuronyl moiety, *i.e.* *m/z* 317 and 257, as well as intensive ions at *m/z* 189 and 188, corresponding to acetylated glycol IV. This observation may be accounted for by the formation of two isomeric β-D-glucosiduronates containing glycol IV as an aglycone. Their structures are shown on Fig. 5. Without further structural information it is not possible to assign the structures of the metabolites XIV and XV unequivocally. No glucosiduronates were detected using procedures ii and iii, which rely on extraction and subsequent derivatization. Apparently, a derivatization in aqueous phase is required to obtain extractable derivatives.

The main routes in the biotransformation of 1,4-DEB were discussed in the previous study. Therefore, possible routes of formation are shown here only for the newly identified metabolites (Figs. 6–8).

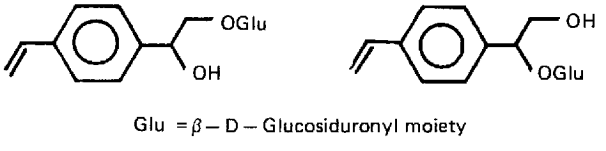

Fig. 5. Proposed structures of β-D-glucosiduronates.

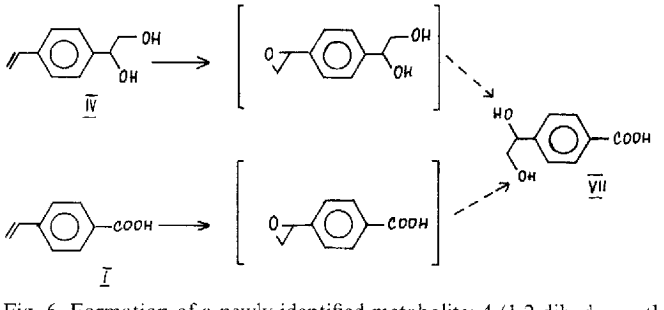

Fig. 6. Formation of a newly identified metabolite: 4-(1,2-dihydroxyethyl)benzoate (VII).

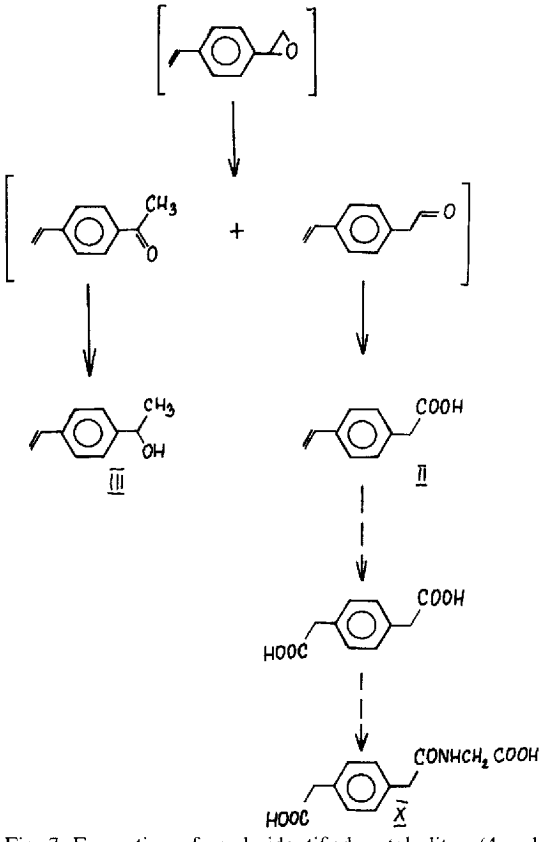

Fig. 7. Formation of newly identified metabolites: (4-carboxymethylphenyl)acetylglycine (X) and l-ethenyl-4-(l-hydroxyethyl)benzene (III).

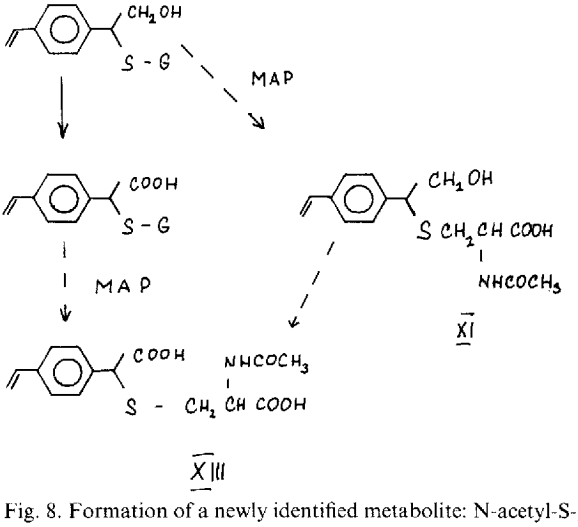

Fig. 8. Formation of a newly identified metabolite: N-acetyl-S-[2-carboxy-1-(4-ethenylphenyl)ethyl]-L-cysteine (XIII).

DISCUSSION

Biotransformation of 1,4-DEB in the rat seems to be closely similar to that of styrene. 4-Ethenyl analogues were found for all the reported styrene metabolites, except that of a minor mercapturic acid reported by Seutter-Berlage *et al.* [9] and 2-phenylethanol reported by Bakke and Scheline [4]. Nevertheless, the metabolic profile of 1,4-DEB seems to be more complicated than that of styrene. At least two compounds, VII and X, arose from biotransformation of both ethenyl groups in the DEB molecule. Although identification of VII and X should be considered as tentative (compounds were not isolated), it may be concluded from their MS fragmentation that they have not retained any ethenylphenyl moiety intact. All metabolites that were identified previously were also found during this study, except for N-acetyl-S-[1-(4-formylphenyl)-2-hydroxyethyl]-L-cysteine (XVI). This mercapturic acid, which is derived from metabolite XI by conversion of the remaining ethenyl group into an aldehyde, was isolated as its methyl ester but was not detected directly in the urine extract. This may indicate formation of XVI during the isolation procedure as a product of oxidation of XI by air. The formation of glycine adduct X, which is one of the major metabolites, from a minor urinary metabolite II (Fig. 7) seems to be surprising. However, it may be explained by the lipophilicity of II and its glycine adduct IX. These compounds are probably reabsorbed in kidneys and may undergo further oxidative metabolism resulting in the formation of X as an end-product of biotransformation, which is readily excreted in urine.

The results of this study indicate that GC–MS analysis of urine extracts derivatized with diazomethane and acetic anhydride (procedure i) as well as with diazomethane and BSTFA (procedure ii) is an efficient tool for studies on biotransformation of DEB and related compounds. Two-step acetylation with acetic anhydride seems to be more efficient then single acetylation of extracted material. The first step, acetylation in aqueous phase, enhanced the extraction of polar metabolites, such as glucosiduronates. The second step, acetylation of the extracted material, is necessary to complete the conversion into acetates. However, none of these derivatization procedures eliminated the formation of chromatographic artifacts completely. Therefore, parallel use of two or more derivatization procedures is required if a virtually complete metabolic pattern is to be obtained. SOX derivatization (procedure iii) was used to prevent possible formation of enol ethers from ketones and aldehydes and also to get more information on DEB metabolites. Only phenylglyoxylate V was found to form oximes during procedure (iii). However, its conversion into the SOX derivative was incomplete, and two isomers (*E* and *Z*) were formed. Therefore, SOX derivatization does not seem to be suitable for the analysis of DEB metabolites.

ACKNOWLEDGEMENT

The authors thank Mrs. Rút Uzlová for her skilful technical assistance and for preparing the figures.

REFERENCES

1 I. Linhart, V. Hanus, J. Novák, J. Šmejkal and P. Pech, *Xenobiotica*, 19 (1989) 645.
2 J. Šmejkal, J. Kopecký and I. Linhart, *Z.* Chem., 26 (1986) 397.
3 F. W. McLafferty and D. B. Stauffer, *The Wiley/NBS Registery of Mass Spectral Data, 490-3-10*, Wiley, New York, 1989.
4 O. M. Bakke and R. R. Scheline, *Toxicol. Appl. Pharmacol.*, 16 (1970) 691.
5 F. W. McLafferty and D. B. Stauffer, *The Wiley/NBS Registry of Mass Spectral Data, 514-2-6*, Wiley, New York, 1989.
6 K. Bergemalm Rynell and G. Steen, *Toxicol. Appl. Pharmacol.*, 62 (1982) 19.
7 F. W. McLafferty and D. B. Stauffer, *The Wiley/NBS Registry of Mass Spectral Data, 512-2-4*, Wiley, New York, 1989.
8 F. W. McLafferty and D. B. Stauffer, *The Wiley/NBS Registry of Mass Spectral Data, 539-1-11, 1209-3-11 and 1746-2-2*, Wiley, New York, 1989.
9 F. Seutter-Berlage, L. P. C. Delbressine, F. L. M. Smeets and H. C. J. Ketelaars, *Xenobiotica*, 8 (1978) 413.