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Short Communications

Identification of Two Urine Metabolites of Vinyl Chloride by GC-MS-Investigations

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Summary. Thiodiacetic acid and S-(carboxymethyl)cysteine are found in the urine of rats after a 48 h exposure to 1000 ppm vinyl chloride. The structure of both compounds could be clarified by GC-MS investigations. Chloroethylene oxide, chloroacetaldehyde and chloroacetic acid are assumed to be intermediates in vinyl chloride metabolism. Compounds which can be transformed to one of these alkylating agents in vivo should also lead to renal excretion of thiodiacetic acid and S-(carboxymethyl)cysteine.

Key words: Vinyl chloride - Thiodiacetic acid - S-(carboxymethyl)cysteine - Urinary metabolites.

Recently we described the determination of thiodiacetic acid and S-(carboxymethyl)cysteine in urine specimens after vinyl chloride (VC) exposure [12]. The structure of these VC metabolites was clarified by GC-MS investigations on the dimethyl ester of thiodiacetic acid and on the N-trifluoroacetyl-n-butyl ester of S-(carboxymethyl)cysteine. The results of our investigations and the methods used are reported here.

MATERIALS AND METHODS

Animals

Male SPF Wistar rats of the AF/Han strain of the Zentralinstitut für Versuchstierzucht, D-3000 Hannover-Linden, were used. Their weight was 130-150 g.

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Chemicals

Vinyl chloride of 99.99% purity was purchased from Messer-Griesheim GmbH, D-4600 Dortmund 1, thiodiacetic acid (98% purity) and S-(carboxymethyl)cysteine from Janssen Pharmaceutica, Dpt. Aldrich Europe, B-2340 Beerse, Belgium. Diazomethane was generated from N-methyl-N-nitroso-p-toluene sulfonamide according to de Boer and Backer [1]. All other substances including trifluoroacetic acid anhydride were purchased at analytical grade from E. Merck AG, D-6100 Darmstadt.

Procedures

Inhalation Experiments. Several rats were exposed over 48 h to 1000 ppm vinyl chloride in the air in an inhalation chamber of 60 × 50 × 40 cm. The concentration of vinyl chloride was adjusted by GC determinations. During the inhalation experiments the urine was collected and then prepared for analytical procedures.

Urine Preparations. Five ml samples of the collected urines were subjected to ion exchange column chromatography using 20 ml of Dowex 50 WX3, 50-100 mesh, $H^{\oplus}$, of Serva Feinbiochemica GmbH & Co, D-6900 Heidelberg. To obtain a fraction containing thiodiacetic acid the column was then washed using distilled water until neutral reaction was found in the eluate. The collected effluent was evaporated to dryness under reduced pressure at 40°C. The residue was dissolved in 2 ml of methanol and methylated by diazomethane according to de Boer and Backer [1]. After concentration to 1 ml this solution was injected into GC-MS. A fraction containing S-(carboxymethyl)cysteine was fixed by Dowex 50 WX8, 50-100 mesh, $H^{\oplus}$, after washing the resin with distilled water. This fraction could be eluted by 1 n acetic acid. The effluent was then evaporated to dryness and the residue was methylated in 10 ml of methanol HCl and then butylated with 10 ml of butanol HCl according to Gehrke and Stalling [3]. The solution was filtered and evaporated to dryness and then treated with 1 ml trifluoro acetic acid anhydride and 4 ml dichloromethane at room temperature (see [3]). After evaporation to dryness the residue was dissolved in 1 ml dichloromethane for GC-MS measurements.

GC-MS Analysis. A mass spectrometer MAT 112 of the Varian/MAT GmbH, D-2800 Bremen, was used in combination with a Varian 1400 gas chromatograph equipped with a steel column of 1.8 m length and 3 mm diameter containing 3% SE 30 at Supelcoport 100/120. The carrier gas was helium adjusted at a flow of 30 ml/min. The oven temperature was 135°C and the temperature of the injection

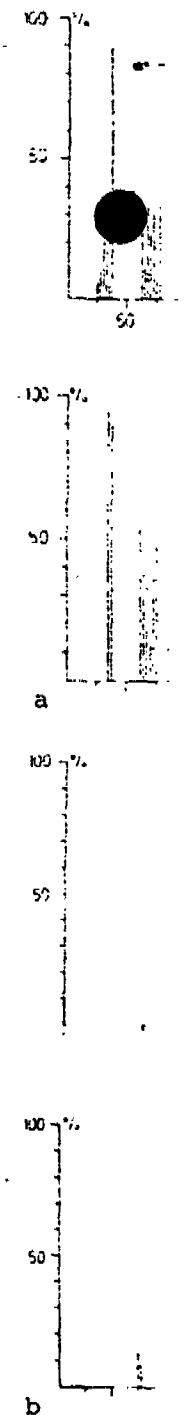

Fig. 1
(a) Mass sp
B = derivat
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R&S 114918

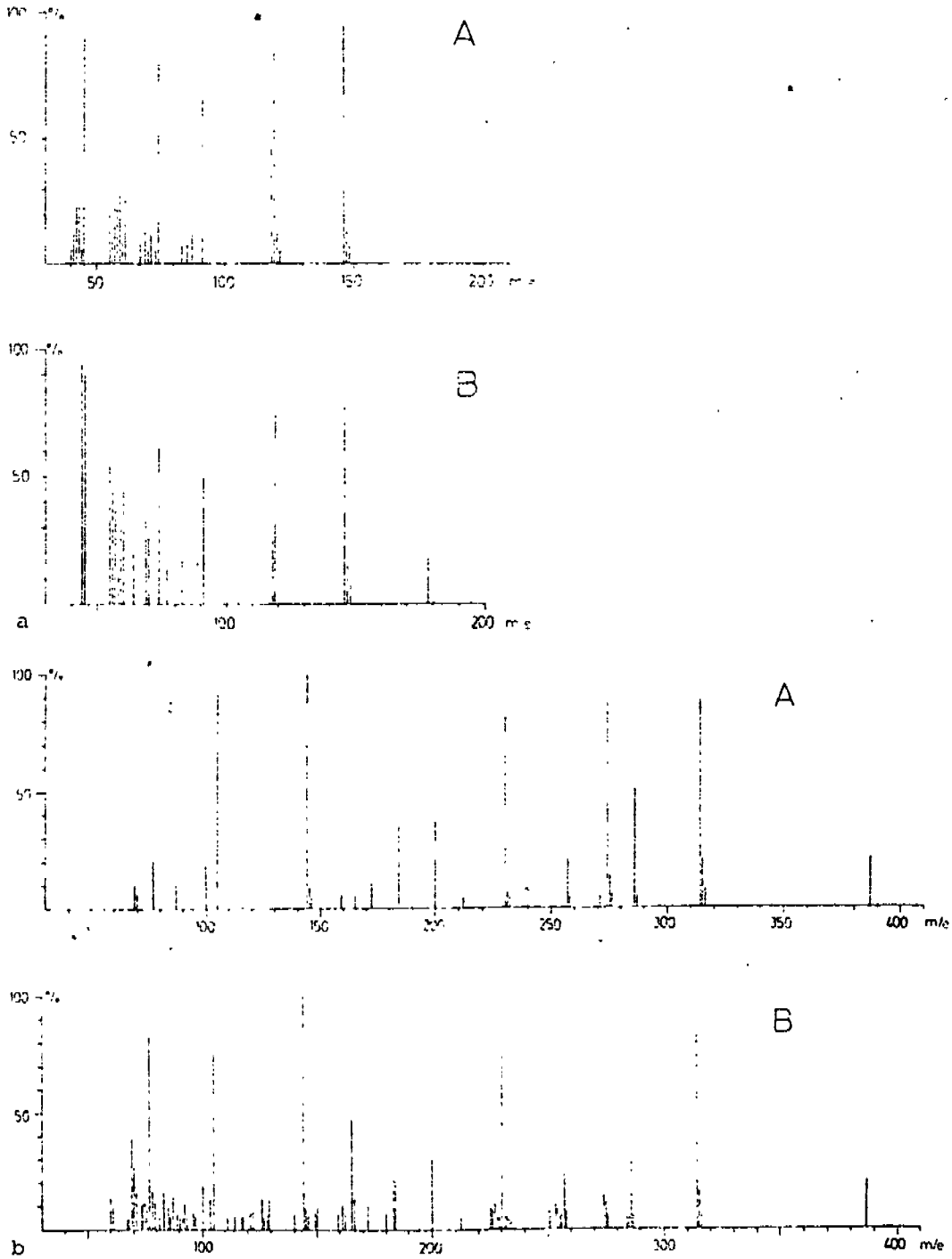

Fig.1
(a) Mass spectrograms of thiodiacetic acid diemethylester. A = standard; B = derivative of an urine metabolite of vinyl chloride. (b) Mass spectrograms of N-fluoroacetyl-S-(carboxymethyl)-L-cysteine dibutylester. A = standard; B = derivative of an urine metabolite of vinyl chloride

R&S 114919

Table 1

Proposed MS fragmentation patterns of derivatives of S-(carboxymethyl) cysteine and thiodiacetic acid found in the urine of VC-treated rats

N-Fluoroacetyl-S-(carboxymethyl)-L-cysteine-dibutylester		Thiodiacetic acid dimethylester	
Molecular	387	Molecular	178
$M-C_4H_9$	314	$M-CH_3OH$	146
$M-COOC_4H_9$	288	$M-COOCH_3$	119
$M-CF_3\ CO\ NH_2$	274	$M-HCOOCH_3$	118
$M-S-CH_2\ COOC_4H_9$	240		
$M-COC_4H_9-C_4H_8$	230		
$M-COC_4H_9-HOC_4H_9$	212		
$M-COOC_4H_9-CF_3CONH_2$	200		

port 155°C. The ion source of the mass spectrometer was adjusted by 270°C. The emission stream was 1.5 mA and the ionizing energy 70 eV.

RESULTS

In Figure 1 the mass spectra of two urine compounds obtained with rats after VC exposure (1000 ppm/8 h) are demonstrated. The molecule peaks indicate me values of 178 and 387, respectively. The fragmentation patterns (shown in Table 1) correspond to the structure of the dimethyl ester of thiodiacetic acid and the N-trifluoroacetyl-n-butyl ester of S-(carboxymethyl)cysteine, respectively. They are identical with the patterns obtained after injection of the corresponding derivatives of the authentic substances.

DISCUSSION

It is assumed by several authors that the carcinogenic effect of vinyl chloride is due to its oxidative biotransformation to chloroethylene oxide [6,10,11]. After chloroacetic acid has been found as a vinyl chloride metabolite [7,15] chloroacetaldehyde was discussed as an alkylating precursor produced by spontaneous rearrangement of chloroethylene oxide [6,11]. This compound which shows mutagenic effects [10,11], could also be detected [5]. The detection of S-(carboxymethyl)cysteine and thiodiacetic acid in the urine of rats after exposure to vinyl

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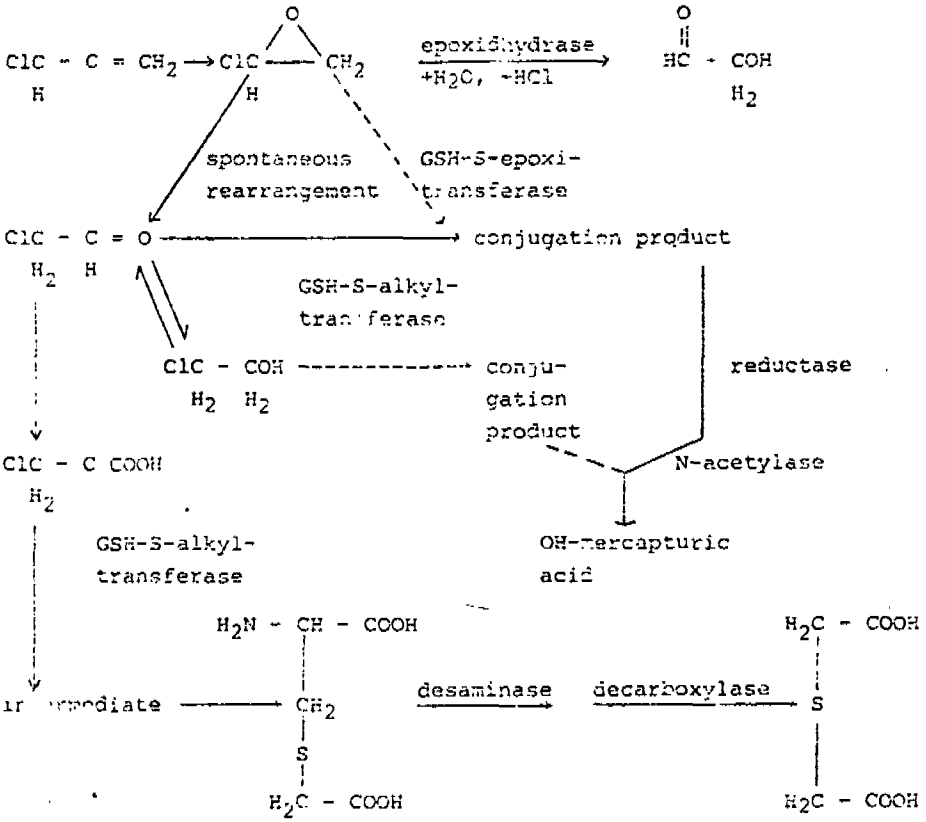
chloride supports the findings of Yliner who observed the biotransformation of chloroacetic acid to these compounds in mice [17]. Another metabolic pathway including neither the production of chloroethylene oxide nor any conjugation reaction with glutathione is proposed by Green and Hathway [11]. Supposing oxidative biotransformation of vinyl chloride to chloroethylene oxide the entire metabolism of the carcinogen can be outlined as follows:



As demonstrated by this metabolic scheme it is assumed that chloroacetaldehyde can be reduced to chloroethanol. This compound lowers for its part the liver content of glutathione [8]. Furthermore it seems probable that an epoxidhydrase is involved in the metabolism of vinyl chloride [2]. Our studies on the urine of patients treated with the cancer chemotherapeutic agent Ifosfamide[1] have shown that thiodiacetic acid and S-(carboxymethyl)cysteine appear as urine metabolites of this drug too [14]. The explanation is that halogenated acetaldehyde arises as product of the biotransformation of at least three groups of foreign compounds:

[1] Test drug of the ASTA-Werke AG, D-4812 Brackwede

1. Free ethyl halides as 1,2 dichloroethane [19] and 1,1,2 tri-chloroethane [18].
2. Free vinyl chloride, vinyl bromide and vinylidene chloride.
3. Haloethyl compounds, which can be dehaloethylated by oxidative N- and O-dealkylation, e.g. Ifosfamide[1], cyclophosphamide, BCNU, CCNU, and 2,2'-bis(chloroethyl) ether [13].

Recently Watanabe et al. came to the conclusion that S-(2-chloroethyl-)cysteine and its acetylated analogue, found by Green and Hathway [4], are not real metabolites of vinyl chloride but artifacts of the derivatization procedure [16]. The authors assume that these artifacts may arise from S-(2-hydroxyethyl)-cysteine and its acetylated derivative. The latter compound could be isolated from the urine of vinyl chloride treated rats and identified by MS investigations [16]. Thus it seems clear that vinyl chloride is metabolized via two different pathways, one of them leading to a hydroxymercapturic acid. Watanabe et al. discuss the initial formation of a chloroethyl conjugate in the animal followed by hydrolysis to the corresponding hydroxyethyl compounds before excretion [16]. The possibility, however, may be taken into consideration, that a reductive step is involved in the metabolism of a chloride-free conjugation product as it has been discussed concerning the formation of hydroxymercapturic acid from acrolein [9]. Our findings show that there is another metabolic pathway of vinyl chloride leading to thiodiacetic acid and this compound has also been found by Green and Hathway [4] and by Watanabe et al. [16]. S-(carboxymethyl)-L-cysteine, a real precursor of thiodiacetic acid, accumulates only in the case of highly dosed treatment [14]. We found neither this compound nor any thiodiacetic acid in the urine of rats not treated with vinyl chloride.

In a previous paper we discussed the possibility to develop biological exposure controls on vinyl chloride workers based on urine metabolite analysis [14]. The aspect of specificity, however, needs further investigation.

R&S 114922

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R&S 114923