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UNEXPECTED METABOLITES PRODUCED FROM CLOMETHIAZOLE*

ALBRECHT GRUPE and GERHARD SPITELLER*

Lehrstuhl für Organische Chemie I der Universität Bayreuth, Postfach 3008, 8580 Bayreuth (G.F.R.)

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SUMMARY

The heterocyclic drug clomethiazole is metabolized by body passage partly to small aliphatic molecules. The occurrence of such unexpected metabolites is usually overlooked. An appropriate method for their detection is the comparison of urine profiles during drug intake and after withdrawal of the drug.

INTRODUCTION

We have compared urine profiles of healthy individuals and patients suffering from chronic diseases [1–3] to establish whether or not there are any metabolic changes. In the course of these studies we obtained a urinary acid profile of a patient, hospitalized for misuse of alcohol (Fig. 1). This profile showed some peaks (Nos. 2, 6, 8, 9, 12, 23), not present in the urine of healthy individuals. A gas chromatographic—mass spectrometric (GC—MS) run revealed one of the unknown compounds (No. 8) to be 4-methylthiazole-5-acetic acid methyl ester (1a). The corresponding acid (1b) is a known metabolite of clomethiazole (2) [5-(2-chloroethyl)-4-methylthiazole; trademarks are Distraneurin® and Hemineurin®] [4].

1a: R = CH_3 2
1b: R = H

Inquiry in the clinical department where the patient was hospitalized assured us that the individual was treated with clomethiazole to repress withdrawal symptoms. So we suspected that the other unknown peaks in the GC run might

*Dedicated to Professor H. Schildknecht on the occasion of his 60th birthday.

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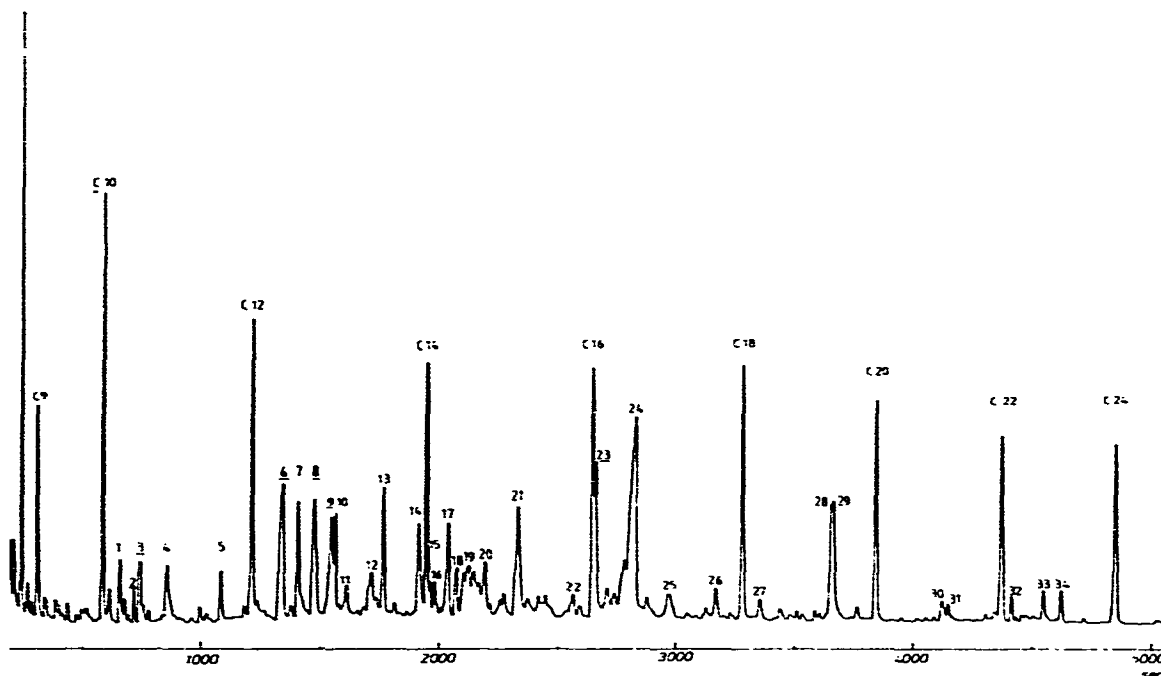


Fig. 1. Profile of the acid fraction obtained from the urine of a patient during treatment with clomethiazole. Peaks: 1 = succinic acid dimethyl ester; 2 = unknown metabolite of clomethiazole (mol.wt. 130); 3 = 3-methyl-mercapto-2,4-pentadione; 4 = unknown; 5 = methylglutaconic acid methyl ester; 6 = thiodiacetic acid dimethyl ester; 7 = 3-methyladipic acid dimethyl ester; 8 = 4-methylthiazole-5-acetic acid methyl ester; 9 = unknown metabolite of clomethiazole; 10 = unknown; 11 = pimelic acid dimethyl ester; 12 = 4-carboxymethoxy-5-vinylthiazole [6]; 13 = phenylbutyric acid methyl ester (internal standard); 14 = 4-methoxyphenylacetic acid methyl ester; 15 = unknown; 16 = unknown dicarboxylic acid dimethyl ester; 17 = decanoic acid dimethyl ester (branched); 18 = decanoic acid dimethyl ester (branched); 19 = unknown; 20 = decanoic acid dimethyl ester (branched); 21 = homovanillic acid methyl ester; 22 = 3,4-dimethoxyphenylacetic acid methyl ester; 23 = unknown metabolite of clomethiazole (mol.wt. 213); 24 = hippuric acid methyl ester; 25 = unknown (mixture); 26 = indoleacetic acid methyl ester; 27 = unknown (mol.wt. 240); 28 = pentylurofuranic acid dimethyl ester; 29 = 4-methoxyhippuric acid methyl ester; 30 = stearic acid methyl ester; 31 = unknown urofuranic acid dimethyl ester; 32 = pimaric acid methyl ester; 33 = isopimaric acid methyl ester; 34 = dehydroabietic acid methyl ester.

also be metabolites of clomethiazole. To verify this hypothesis, another urine sample of the same patient was investigated several days after the withdrawal of the drug. The GC run (Fig. 2) did not show peaks 2, 6, 8, 9, 12 and 23. Some other peaks which had disappeared (32, 33, 34) originated from an impurity present in the Extrelut® columns used [5]; some other peaks (e.g. 16, 21) which are rather small in the GC run reproduced in Fig. 2 compared to Fig. 1, were identified by MS to be common metabolites produced in very different proportions depending on nutritional conditons. Similar investigations carried out with other patients during and after intake of clomethiazole confirmed the differences in the GC runs.

Clomethiazole is used in high doses (2—6 g/day) for the treatment of

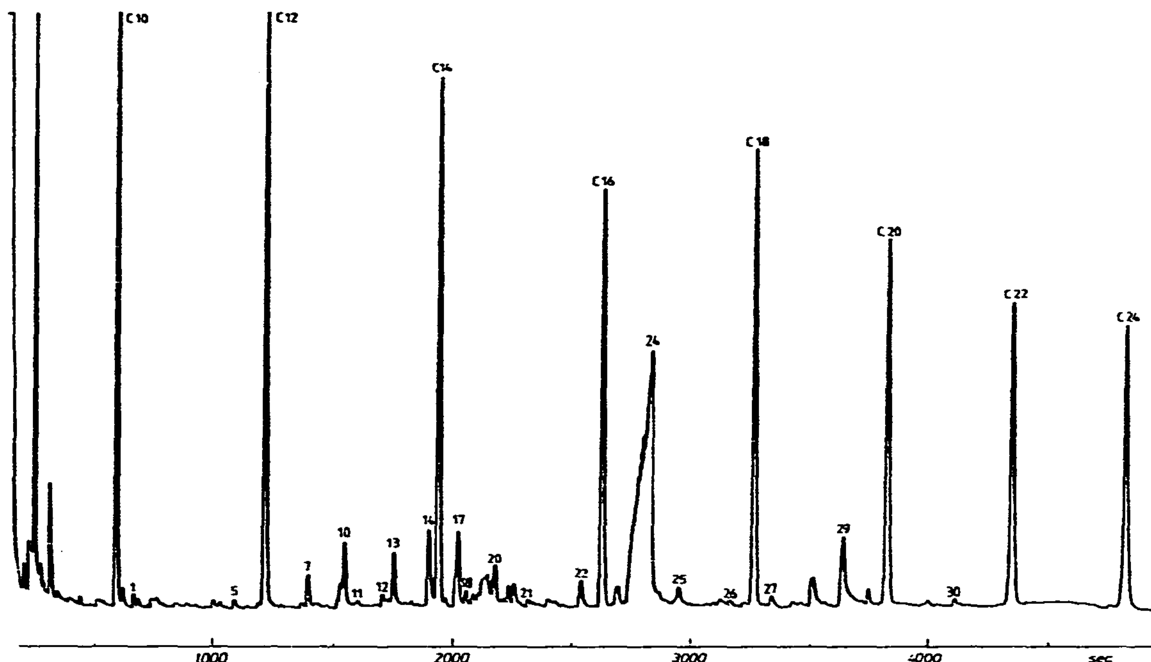


Fig. 2. Profile of the same urine fraction of the same individual as in Fig. 1, three days after treatment with clomethiazole was stopped.

delirium tremens and general withdrawal symptoms [7]. A great number of its metabolites have been detected. They all are oxidation products of the side-chain carbon atoms in position 3 and 4 [8—11]. Nevertheless, and in spite of radioactive labelling at C-2, only a small percentage of (2) was recovered in the form of metabolites. The fate of most of the drug after body passage remains unknown.

Thus the unexpected peaks offered the possibility of obtaining an insight into the still obscure pathway of metabolism.

This paper deals with the structure elucidation of some of the unknown metabolites indicated in the GC runs. It deals further with some conclusions that must be drawn from this investigation.

EXPERIMENTAL

Instruments

The GC apparatus and conditions were as follows: Carlo-Erba gas chromatograph 2900; hydrogen flow-rate: 2 ml/min; 25-m thin-film glass capillary coated with OV-101; injector, 275°C; oven, 80—300°C; temperature program, 2°C/min; flame ionization detector.

For MS measurements an LKB 2091 mass spectrometer was used with electron-impact (EI) ion source, an electron energy of 70 eV, and registration of the total ion current signal at 20 eV. The mass spectrometer was combined with a Pye-Unicam gas chromatograph, a 25-m thin-film glass capillary coated

with OV-101; temperature program as above; helium flow-rate, 2 ml/min. The instrument was combined with an LKB 2130 data system, using a PDP 11 computer.

High-resolution data were obtained with a Varian 312 mass spectrometer with a chemical ionization (CI)/EI ion source, combined with a Varian Model 3700 gas chromatograph with open split; helium flow-rate, 2 ml/min, 25-m thin-film glass capillary column coated with OV-101, conditions as above. Data system: MAT 200, PDP 11/34.

^{1}H-NMR measurements were carried out with a Bruker WM 250 instrument.

Work-up procedure for profiling

To 20 ml of urine 1 ml of standard solution containing 0.2 mg/ml phenylbutyric acid in water were added and then 2 N H_2SO_4 was added drop by drop until pH 1 was reached. This solution was made up with water to 22 ml. This sample was poured on an Extrelut® column (E. Merck, Darmstadt, G.F.R.) and eluted with 60 ml of ethyl acetate.

The extract was evaporated nearly to dryness in vacuo, 1 ml of methanol was added followed by ethereal diazomethane solution until the yellow colour remained. After 5 min the excess diazomethane was evaporated by a nitrogen stream. The solution was evaporated to 0.5 ml; 0.2—1.0 μl of this solution was injected into the gas chromatograph.

Work-up procedure for the separation of clomethiazole netabolites

A 150-ml urine sample from a patient treated with 2 g clomethiazole per day (after the second day of intake) was acidified to pH 1 by the addition of concentrated sulphuric acid (diluted 1:1). The acidified urine was extracted three times with 100 ml of ethyl acetate. The organic extract was dried over sodium sulphate and evaporated to dryness in vacuo. The residue (1.2 g) was dissolved in 1 ml of methanol. Ethereal diazomethane solution was added until the solution remained yellow. The excess diazomethane was evaporated by a nitrogen stream.

The residue was separated on home-made thin-layer chromatography (TLC) plates 20 X 20 cm (1 mm silica-gel layer) using a mobile phase of diethyl ether—cyclohexane (5:3). The zone with an R_F value of 0.42—0.48 was scratched off and eluted with ether. After evaporation to dryness, 14.5 mg of a viscous fluid remained. A glass capillary gas chromatogram revealed that this fraction consisted of 56% of this thiodiacetic acid dimethyl ester (3a).

Semiquantitative measurements

Urine specimens (24 h) of patients treated with known amounts of clomethiazole were collected. After the addition of 2 ml of the standard solution to 20 ml of the urine the sample was worked-up as described above.

Peak areas were measured with an integrator (Spectra-Physics Autolab System I). The amounts of the acetic acid dimethyl ester were put in relation to the standard compound. Recovery of the standard was estimated to be about 100%.

In Table I data of thiodiacetic acid dimethyl ester obtained from two

TABLE I

EXCRETION OF THIODIACETIC ACID DIMETHYL ESTER

	Interval (h)	Amount of drug consumption (g)	Amount of thiodiacetic dimethyl ester (mg)
Patient 1	48	11	462
	24	4	142
	24	2	80
	24	2	68
	24	0	16
Patient 2	48	8	44
	24	4	49
	24	2	29
	24	1.5	34

patients are recorded to show typical examples and the differences in excretion rates.

Sources of reference compounds

(1) Thiodiacetic acid (3b) and mercaptoacetic acid (4b) were obtained from EGA-Chemie, Steinheim, G.F.R.

(2) S-(2-Oxopropyl)-mercaptoacetic acid (5a). One gram (0.01 mol) of mercaptoacetic acid (4b) was dissolved in a saturated $NaHCO_3$ solution; under vigorous stirring 2 g of chloroacetone were added. After 15 min the mixture was filtered and the filtrate washed three times with diethyl ether. The aqueous solution was brought to pH 1 by adding concentrated hydrochloric acid and extracted three times with ether. The ethereal solution was dried with sodium sulphate and evaporated. A viscous resin remained (0.69 g). Distillation (0.2 Torr/150°C) gave a colourless waxy liquid. ^{1}H-NMR (C^2HCl_3): δ = 2.31 ppm (s, 3H, $-C\underline{H}_3$); 3.31 (s, 2H, $-S-C\underline{H}_2-COOH$); 3.5 (s, 2H, $-CO-C\underline{H}_2-S-$); 10.25 (s, 1H, $-O\underline{H}$).

(3) 3-Methylmercapto-2,4-pentadione (6a). (A) 1-Bromo-2,4-pentadione (7). Ten grams (0.1 mole) of freshly distilled acetylacetone were dissolved in dry carbon tetrachloride (dried over P_4O_{10}); 17.8 g (0.01 mole) of N-bromosuccinimide and 200 mg of azobisisobutyronitrile were added and heated under reflux for 2 h. After cooling the precipitated succinimide was filtered off and the remaining solution was concentrated in vacuo. GC—MS analysis showed that 1-bromo-2,4-pentadione (7) was the main product. (B) 3-Methylmercapto-2,4-pentadione (6a). The rough carbon tetrachloride solution of the above reaction was cooled to −10°C. Gaseous mercaptomethane (from S-methylthiourea and potassium hydroxide) was introduced and condensed by cooling. After 10 min at room temperature the solution was washed three times with $NaHCO_3$ solution, dried and evaporated. The mixture was purified by preparative gas chromatography which rendered enough sample to determine an NMR spectrum. ^{1}H-NMR (C^2HCl_3): δ = 2.13 ppm (s, 3H, $-SC\underline{H}_3$); 2.44 (s, 6H, $-COC\underline{H}_3$); 16.97 (s, 1H, $-O\underline{H}$). ^{13}C-NMR (C^2HCl_3): δ = 20.0 ppm (q, $-S-\underline{C}H_3$); 24.3 (q, $-CO-\underline{C}H_3$); 116.4 (s, $-\underline{C}=$); 197.2 (s, $-\underline{C}O$) ppm.

RESULTS

The structures of the unknown compounds represented by peaks 3 and 6 in the glass capillary chromatogram represented in Fig. 1 were clarified by interpretation of the spectra, synthesis of comparison compounds and determination of their identity with the compounds found in the GC run 1.

The mass spectrum of peak 6 (Fig. 3) showed a molecular ion of mass 178. Peaks at M−32, M−59, M−60 and at mass 59 indicated the presence of at least one carbomethoxy group. The isotope ratio of these peaks suggested the presence of sulphur. This assumption was confirmed by a high-resolution measurement which showed the molecular formula to be $C_6H_{10}O_4S$.

Since it was not possible to deduce further structural information from the spectrum, we tried to accumulate sufficient pure material for an NMR investigation. For this purpose the whole acid fraction was methylated and each zone subjected to GC analysis. The zone containing the compound of mol. wt. 178 was eluted. The GC run of this zone showed an enrichment of 56%, sufficient for NMR analysis. The NMR spectrum was very simple. It showed — besides the small peaks of impurities — only two signals in the ratio 2:3 at δ = 3.40 and δ = 3.78 ppm.

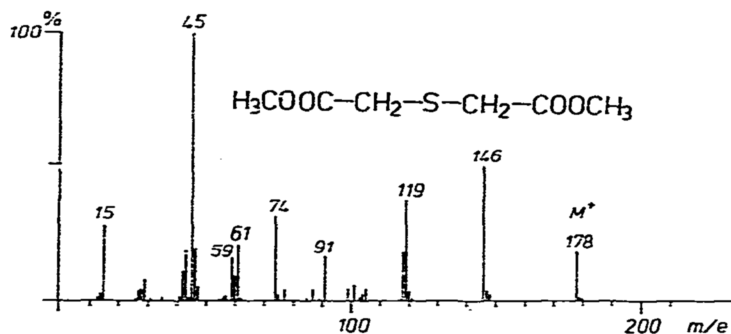
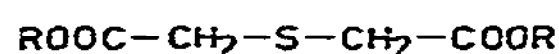


Fig. 3. Mass spectrum of thiodiacetic acid dimethyl ester (3a) (synthetic sample).

Since the compound contained ten hydrogen atoms, the presence of two CH_3− and two CH_2— groups each in a similar surrounding was established. The two methyl groups could be only carbomethoxy groups according to the MS fragmentation pattern. The remaining two CH_2— groups and the sulphur atom consequently must have been present as a CH_2—S—CH_2 group. Therefore the structure of the molecule was derived to be 3a.



3a: R = CH_3

3b: R = H

The mass spectrum (Fig. 4) of peak 3 in Fig. 1 corresponded to a compound of mol. wt. 146. By high-resolution measurement the molecular formula was

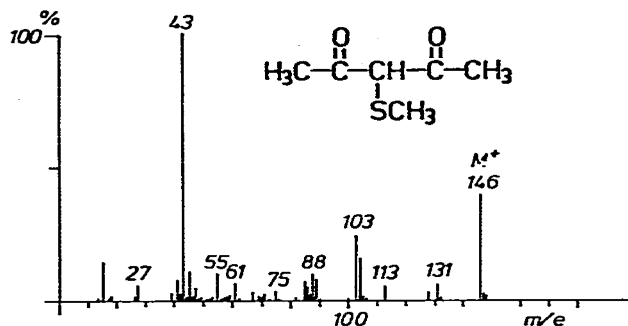
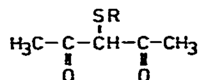


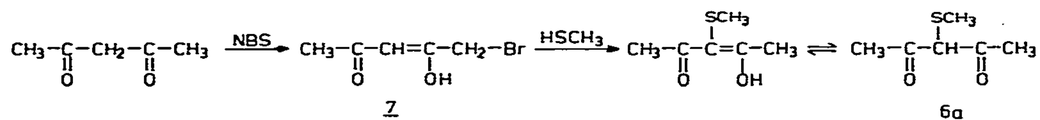
Fig. 4. Mass spectrum of 3-methylmercapto-2,4-pentadione (6a) (synthetic sample).

determined to be $C_6H_{10}O_2S$. The ion of mass 103 (C_4H_7OS) corresponded to the loss of CH_3CO. The presence of such a group was further indicated by the base peak of mass 43. Since the molecule contained only one more double bond equivalent and the spectrum showed no indication of the presence of an OH— or an SH-- group (peaks at M−18 and M−34 were missing), the presence of a further CO- -CH_3 was assumed, rendering compound 6a as the most probable structure for the molecule.



6a: R = CH_3
6b: R = H

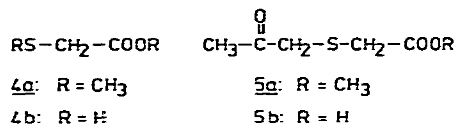
Compound 6a was synthesized by bromation of acetylacetone to compound 7 and reaction with $HSCH_3$ to compound 6a.



Thus in the sample compound 6b was originally present and is methylated by treatment with diazomethane to compound 6a. It proved to be identical in all respects to the metabolite.

It should be mentioned that trace amounts of two other sulphur-containing compounds were detected by a careful inspection of all mass spectra of the dif-

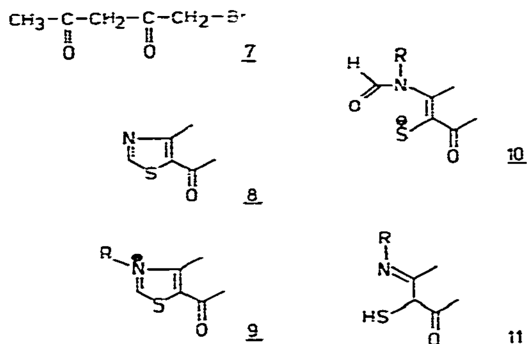
ferent TLC fractions. One metabolite was identified, using the eight-peak index [12], as methylmercaptoacetic acid methyl ester (4a); the other was found to be S-(2-oxopropyl)-mercaptoacetic acid methyl ester (5a) with the aid of reference spectra indicating that the metabolites originally present must have structures 4b and 5b.



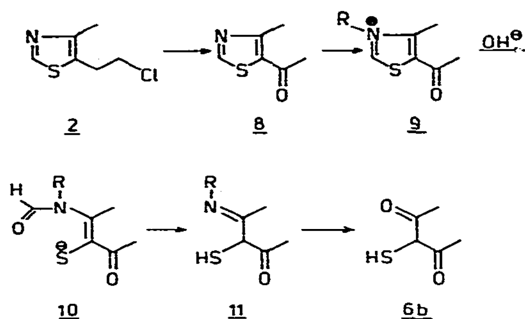
DISCUSSION

Thiodiacetic acid (3b) was found to be a metabolite of vinyl chloride [13]. In addition, some drugs containing a N—CH_2—CH_2—Cl chain (e.g. ifosfamide, manomustine) were found to be also metabolized to thiodiacetic acid [14]. It is assumed that the chlorine atom is substituted in a nucleophilic reaction by the sulphur atom of cysteine, and then further degraded by desamination, decarboxylation and oxidation to the final metabolite, thiodiacetic acid (3b).

Since clomethiazole (2) contains a CH_2—CH_2—Cl side-chain, degradation in a similar way by loss of the heterocyclic ring can be envisaged. This in turn leads to the assumption of a primary or secondary attack on the heterocyclic ring. This hypothesis is strengthened by the occurrence of metabolite 6b which is probably produced from the already known metabolite 5-acetyl-4-methylthiazole (8). We suppose that metabolite 8 is alkylated at the nitrogen atom to quaternary compound 9.



Such compounds suffer cleavage — a well-known reaction of vitamin B_1 [15]. In our case we assume formyl derivative 10 to be formed, which could be degraded further by hydrolysis to the imine 11 and to 3-mercapto-2,4-pentadione (6b).



The SH— group is methylated by treatment with diazomethane to form compound 6a. Thus the presence of compound 6b is a strong hint for the already mentioned postulate that clomethiazole is metabolized not only by side-chain oxidation but also by cleavage of the heterocyclic ring.

In addition, Dr. Pal in our laboratory detected recently several metabolites of clomethiazole oxidized at C-2 [6], obviously missing links in the formation of aliphatic degradation products.

The occurrence of compounds 4b and 5b not yet found in the urine of healthy people gives further hints for the total cleavage of the heterocyclic ring of clomethiazole in the body.

While the amount of compound 6a is rather low, thiodiacetic acid (3b) must be one of the major metabolites. A precise quantitative determination of compound 3b was not possible due to lack of an appropriate standard (HOOC—C^2H_2—S—C^2H_2—COOH easily exchanges the deuterium atoms against hydrogens and can not therefore be used as standard). Thus we were only able to estimate the daily excretion by use of an internal standard (phenylbutyric acid). It turned out that the amount of compound 3b produced from compound 2 is very much dependent on individual parameters (see Table I), but at least in one case intake of 11 g of clomethiazole within two days caused a production of about 460 mg of compound 3b. Assuming that the production of 1 mole of compound 3b requires 1 mole of cysteine (if the side-chain is attacked as in other compounds with a CH_2—CH_2—Cl side-chain), the metabolism of compound 2 to compound 3b needs at least the presence of 370 mg of cysteine. This amount of cysteine is lost with the urine. Since the daily excretion of cysteine in healthy adult individuals is about 6—66 mg/day [16], treatment with clomethiazole must cause a decrease of cysteine in the body. This phenomenon must be investigated in the future. It might well be connected with the side-effects observed by continuous treatment with clomethiazole.

The finding of aliphatic metabolites of clomethiazole seems to us important also in an other respect. Usually the metabolic pathway of a drug is evaluated by labelling experiments. The label is introduced into the drug molecule at a position where one can expect that it will not be lost by body passage, e.g. in an aromatic or a heterocyclic ring. The detection of aliphatic major products in the metabolism of clomethiazole demonstrates the danger of such labelling

experiments. For reasons of synthesis C-2 was labelled and this atom is lost — as we now know — preferentially by biochemical degradation. Thus the label is lost and consequently the degradation products escaped detection.

Profiling of all fractions (basic, neutral, acid) obtained by work-up of biological fluids after drug intake followed by MS identification of the same fractions after withdrawal of the drug should therefore be applied as an appropriate and supplementary method to radioactive labelling experiments.

ACKNOWLEDGEMENTS

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