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Metabolism Studies of Chlormethiazole by Gas Chromatography-Mass Spectrometry

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Summary. The metabolic products of chlormethiazole were extracted from liver and isolated by thin layer chromatography. Chlormethiazole and three other products were obtained. One of these was identified earlier as being 4-methyl-5-β-hydroxy-ethylthiazole and now structures are proposed for the other two. A fourth metabolite, 4-methyl-5-thiazoleacetic acid, was formd in urine. The samples were analyzed by gas chromatography, gas chromatography-mass spectrometry and by high resolution mass spectrometry.

Zusammenfassung. Die Metabolitprodukte von Chlormethiazol wurden aus der Leber extrahiert und mittels Dünnschichtchromatographie isoliert. Es wurden Chlormethiazol und drei andere Produkte nachgewiesen, wobei ein Produkt schon vorher als 4-Methyl-5-β-hydroxyethylthiazol identifiziert wurde. Die Strukturformeln für die zwei anderen Produkte wurden vorgeschlagen. Ein vierter Metabolit (4-Methyl-5-thiazolessigsäure) wurde aus Urin erhalten. Die Proben wurden durch Gaschromatographie, Gaschromatographie-Massenspektrometrie und durch hochauflösende Massenspektrometrie analysiert.

Key words: Chlormethiazole, metabolites — Gas chromatography — Mass spectrometry, chlormethiazole.

Introduction

Allgén *et al.* studied tissue distribution, excretion and metabolism of ^{35}S-labelled chlormethiazole in mice and rats [1]. The metabolites were separated by electrophoresis and the activity was measured. Spectrophotometry was also used. Traces of chlormethiazole was found in urine but the main metabolites were 4-methyl-5-thiazoleacetic acid and a conjugate of 4-methyl-5-thiazoleacetic acid and glutamine. Lindberg discussed seven papers covering studies on hypnotic and anticonvulsant agents related to the thiazole part of thiamine [2]. Several compounds related to chlormethiazole were synthesized and tested pharmacologically.

The mass spectra of 14 thiazoles were reported and discussed by Clarke *et al.* [3]. The fragmentations were determined from metastable transitions. No high resolution mass spectrometry was used, and none of the compounds in this research were studied. Hesse *et al.* studied the mass spectra of vitamin B_1 and some model compounds [4] and found that vitamin B_1 decomposes thermally in the mass spectrometer, mainly into 2-methyl-4-amino-5-chloromethyl-pyrimidine and 4-methyl-5-β-hydroxy-ethylthiazole. The fragmentations of these products were elucidated with the aid of metastable ions and high resolution mass spectrometry.

Herbertz *et al.* studied the metabolism of chlormethiazole in rats [5]. The urinary excretion products were isolated by high voltage electrophoresis and thin layer chromatography. Five excretion products were identified, of which 2-hydroxy-4-methylthiazole-acetyl-5-glycine was found to be the main metabolite. Mass spectra were published for two of the substances.

From our routine work we selected specimens from a suicide case which contained several unknown components related to chlormethiazole.

Experimental

Ground liver, 10—20 g of liver was grounded with 50 ml of water, and extracted three times with 100 ml of chloroform at pH 8.5. The chloroform extract was evaporated to a small volume, and extracted twice with a smaller volume of 1N sulfuric acid. The acid was brought to pH 8.5 and extracted three times with the double volume of chloroform.

Urine was extracted four times with twice the volume of chloroform at pH 3, and evaporated to a small volume. The chloroform was extracted twice with 1N sulfuric acid, which was adjusted to pH 3 and extracted with chloroform. After evaporation to dryness the residue was dissolved in ethanol. The compounds were separated by thin layer chromatography and eluted with ethanol. After evaporation to dryness the residue was dissolved in ether.

The samples were analyzed by a Perkin-Elmer F11 gas chromatograph using a 5% Carbowax 5% KOH column (3 m × 3 mm i.d.) run at 160°C and by the LKB 9000 GC-MS instrument with a 1% SE-30 column (3 m × 2 mm i.d.) at 95°C. Helium was used as carrier gas at a flow rate of 30 ml per minute. Mass spectra were continuously registered by an off line computer system and evaluated by an IBM 1800 computer. The 4-methyl-5-thiazoleacetic acid was analyzed using the direct inlet system of the LKB 9000. Chlormethiazole and one of the unknown metabolites were also analyzed by the double focussing mass spectrometer SM1. To study the fragmentation of one of the hydroxyl compounds more in detail, it was labelled by a simple method. A few micrograms of compound 2 were dissolved in 20 µl of D_2O and from this solution 3 µl were injected into the GC-MS instrument.

Results and Discussion

To obtain maximum information about chlormethiazole and its metabolites, it was appropriate to use different analytical methods. The concentration of chlormethiazole and metabolites absorption according to UV-spectrometry was 4.4 mg% in blood and 6.9 mg% in liver. Thin layer chromatography gave four separated spots as is shown in Fig. 1. Spot 5 was obtained from a urine extract, and is shown only for comparison.

The gas chromatographic results given in Fig. 2 show five components from the liver extract and of these compounds 1 and 4 were identified as chlormethiazole and 4-methyl-5-β-hydroxy-ethylthiazole respectively by comparing the retention times of the components with reference compounds. Component 1 and 3 have been found in all examined cases of chlormethiazole intoxication. Only in this particular suicide case have all of the components been found. To determine the

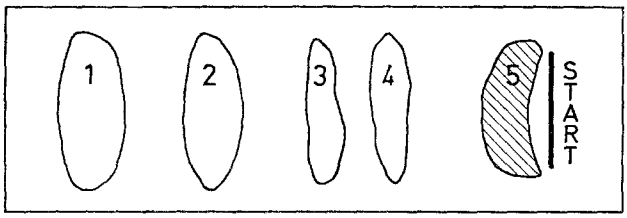


Fig. 1. Thin layer chromatography of extract from liver. Spot *1* is chlormethiazole, *2* and *3* are unknown compounds, *4* is 4-methyl-5-β-hydroxy-ethylthiazole and spot *5* is 4-methyl-5-thiazoleacetic acid. The latter was obtained from a urine extract. Silica gel GF 254. Solvent: n-hexan-methylethylketon (butanone 2) 1:1

Table 1. Metastable decomposition of the compounds separated by thin layer chromatography

Spot No.	Characteristic ions
1 Chlormethiazole	$M(161)^+ \xrightarrow{77.8} 112^+ \xrightarrow{64.4} 85^+ \xrightarrow{23.8} 45^+$
2	$(177)^+ \xrightarrow{92.5} 128^+ \xrightarrow{78.2} 100^+ \xrightarrow{53.3} 73^+ \xrightarrow{27.8} 45^+$
	$128^+ \xrightarrow{41.2} 73^+$
	$(177)^+ \longrightarrow 141^+ \longrightarrow 112^+ \xrightarrow{64.2} 85^+$
3	$(143)^+ \xrightarrow{114.5} 128^+ \xrightarrow{78.2} 100^+ \xrightarrow{53.3} 73^+$
	$128^+ \xrightarrow{41.2} 73^+$
4 4-methyl-5-β-hydroxy-ethylthiazole	$M(143)^+ \xrightarrow{89.5} 113^+$
	$112^+ \xrightarrow{64.5} 85^+ \xrightarrow{23.8} 45^+$
5 4-methyl-5-thiazoleacetic acid	$M(157)^+ \xrightarrow{79.9} 112^+ \xrightarrow{64.4} 85^+ \xrightarrow{23.9} 45^+$
	*m (metastable ions)

Table 2. Exact mass determination of characteristic ions of chlormethiazole and compound 2

Compound	Nominal mass	Mass determined	Mass calculated	Elemental composition	Error p.p.m.
Chlormethiazole	112	112.0212	112.0220	C_5H_6NS	— 7.1
	85	85.0119	85.0110	C_4H_5S	+ 10.5
	59	58.9962	58.9954	C_2H_3S	+ 13.7
	58	57.9881	57.9876	C_2H_2S	+ 8.7
Compound 2	177	177.0008	177.0014	C_6H_8NOSCl	— 3.3
	141	141.0261	141.0274	C_6H_7NOS	— 9.2
	128	128.0169	128.0169	C_5H_6NOS	± 0.0
	112	112.0224	112.0220	C_5H_6NS	+ 3.5
	100	100.0217	100.0220	C_4H_6NS	— 3.0
	73	73.0103	73.0111	C_3H_5S	— 11.0

structure of the three unknown components 2a, 2b and 3 (shown in Fig. 2) it was of great benefit to study the cracking patterns of the mass spectra for all six compounds. In the identification process the fragmentation was calculated

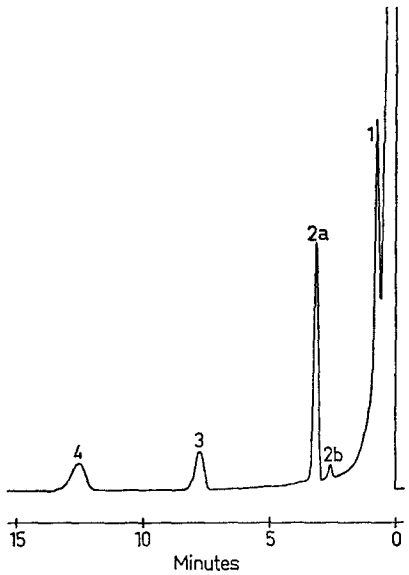


Fig. 2. Gas chromatographic diagram of chlormethiazole and metabolites obtained from a 5% Carbowax 5% KOH column at 160°C (compounds 1 to 4)

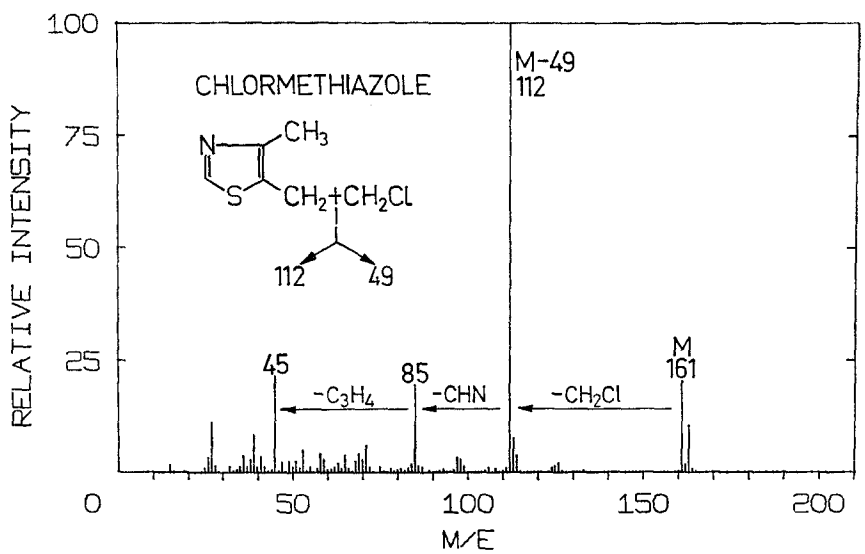


Fig. 3. Mass spectrum of chlormethiazole (compound 1)

from the metastable ions given in Table 1 and from the exact mass determination of characteristic fragment ions of chlormethiazole and of spot 2 (from the thin layer chromatography) given in Table 2.

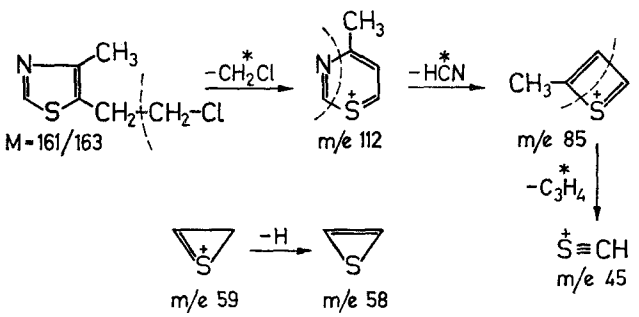
The mass spectrum of chlormethiazole (Fig. 3) was studied in detail for comparison with the three unknown compounds.

The results from the exact mass determination and of the metastable transitions defined the fragmentation paths of chlormethiazole are given in scheme 1. The loss of HCN from m/e 112 is confirmed both by the metastable transition

at *m = 64.4 and from accurate mass measurement and it was possible to follow the decomposition down to m/e 45. No evidence was found indicating from which ion the peaks at m/e 58 and m/e 59 arise. The mass spectrum of chlormethiazole has been studied by several authors but the loss of HCN from m/e 112 has been described only for thiazole and for 2- and 4-methylthiazoles [3].

As expected the mass spectrum of chlormethiazole (Fig. 3) shows a similar cracking pattern to 4-methyl-5-thiazoleacetic acid (Fig. 4) up to m/e 112 or after the loss of —CH_2Cl and —COOH respectively, which is shown in Table 1. Fig. 5 shows the mass spectrum of compound 4 (4-methyl-5-β-hydroxyethylthiazole) which also illustrates the same decomposition as the spectrum of chlormethiazole except that the m/e 112 ion did not arise directly from the molecular ion by a metastable transition. A strong metastable ion was found for the decomposition $M\,(143)^+ \rightarrow 113^+ + CH_2O$.

The 4-methyl-5-β-hydroxy-ethylthiazole is a part of the thiamine chloride molecule (vitamin B_1) which has been studied also using mass spectrometry [4].



Scheme 1. The fragmentation paths of chlormethiazole established by exact mass determination and by metastable transitions

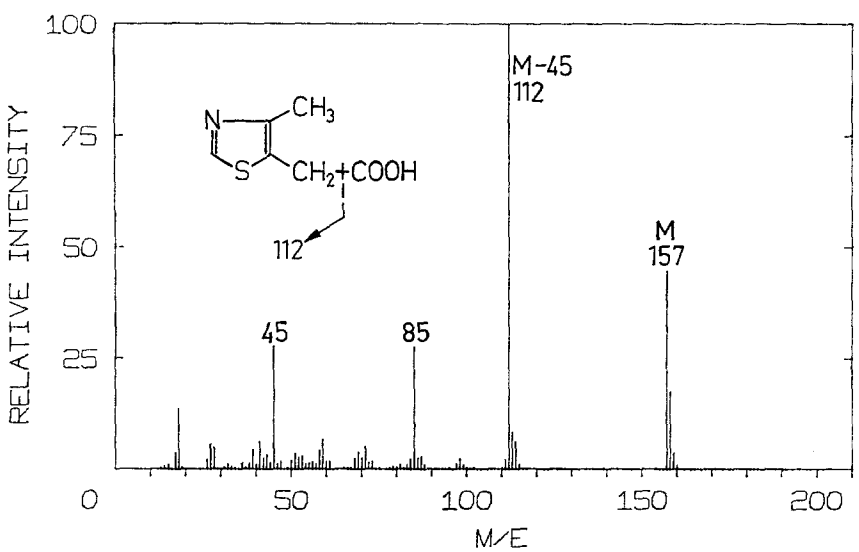


Fig. 4. Mass spectrum of 4-methyl-5-thiazoleacetic acid (compound 5)

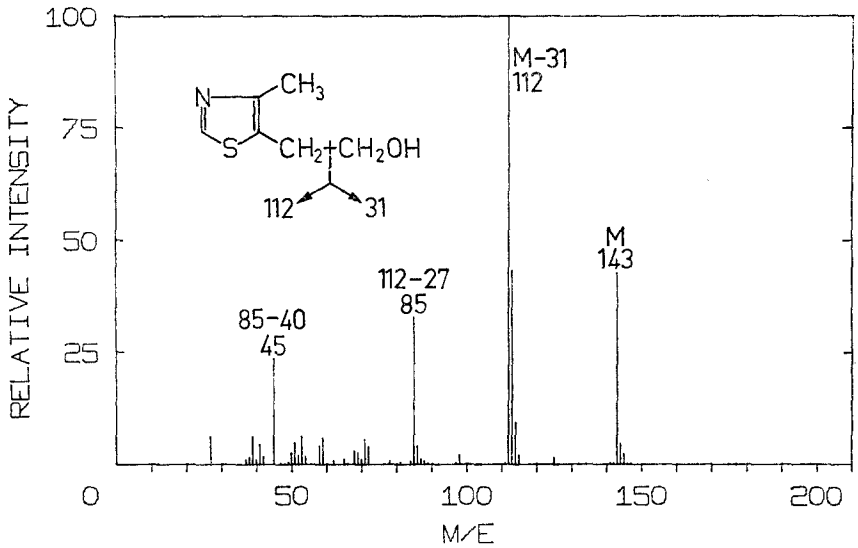


Fig. 5. Mass spectrum of 4-methyl-5-β-hydroxy-ethylthiazole (compound 4)

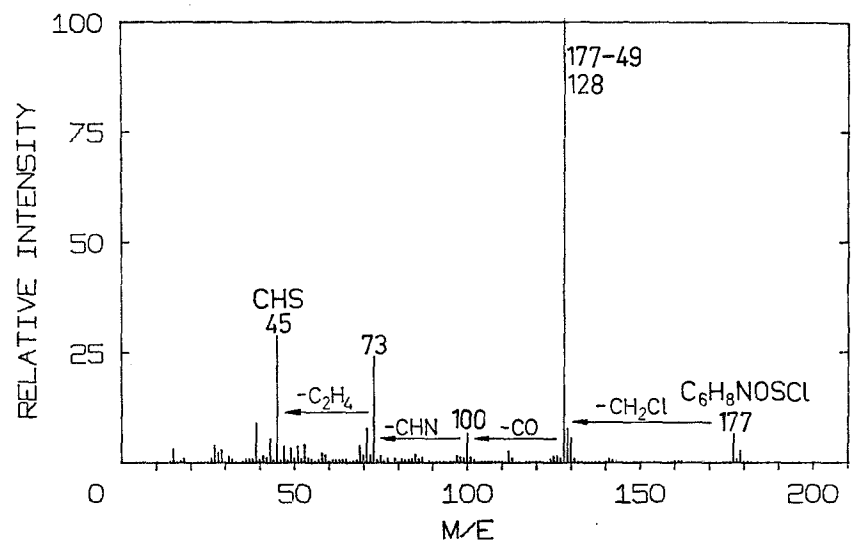
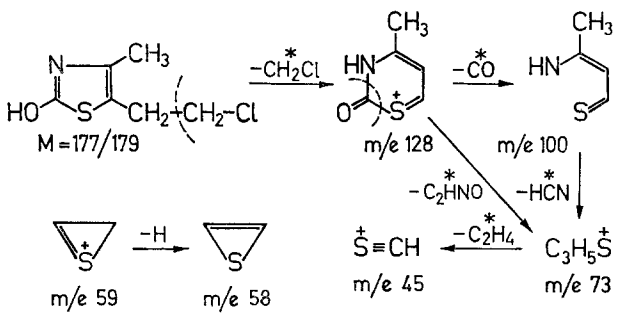


Fig. 6. Mass spectrum of compound 2 identified as 2-hydroxy-4-methyl-5-β-chloro-ethylthiazole

Characteristic fragments from thiamine chloride were obtained at m/e 143, 112, 85 and 45 or a similar pattern as for Fig. 5.

Fig. 6 shows the mass spectrum of compound 2 with parent peaks at mass numbers 177 and 179 with intensities typical for compounds with one chlorine atom. All characteristic peaks in the spectrum were determined by metastable transitions and of accurate mass measurements which are given in Tables 1 and 2. The reaction m/e $177^+ \rightarrow 141^+ \rightarrow 112^+ \rightarrow 85^+$ is independent of the fragmentation of m/e $177^+ \rightarrow 128^+ \rightarrow 100^+ \rightarrow 73^+$ and of m/e $177^+ \rightarrow 128^+ \rightarrow 73^+$ but varies in intensity

from time to time depending upon the temperature of the inlet system and no metastable reaction is indicated for m/e $177^+ \rightarrow 141^+ + HCl$. Therefore the loss of HCl can be explained as thermal degradation and m/e 141 will be the new parent ion. Scheme 2 shows a proposed decomposition of compound 2. The characteristic m/e 73 fragment is only observed in the mass spectra of compounds 2 and 3. The fragmentation m/e $128^+ \rightarrow 100^+ + CO$ gives evidence of a hydroxyl group being connected to the thiazole ring. From the decomposition of m/e $100^+ \rightarrow 73^+ + HCN$ and of m/e $128^+ \rightarrow 73^+ + C_2HNO$ it is difficult to know from which position the carbon is taken when HCN is lost, since a methyl group is connected to neighbouring carbon. To be able to clarify the fragmentation path of compound 2 the hydrogen in the hydroxyl group was exchanged with deuterium by dissolving the compound in 99% D_2O before the sample was introduced into the GC-MS instrument. Fig. 7 shows the mass spectrum of the labelled compound 2 and from the M+1 peak and the m/e 128+1 peak the percentage H→D exchange can be calculated to about 50%.



Scheme 2. The proposed fragmentation of compound 2 established by exact mass determination and by metastable transitions

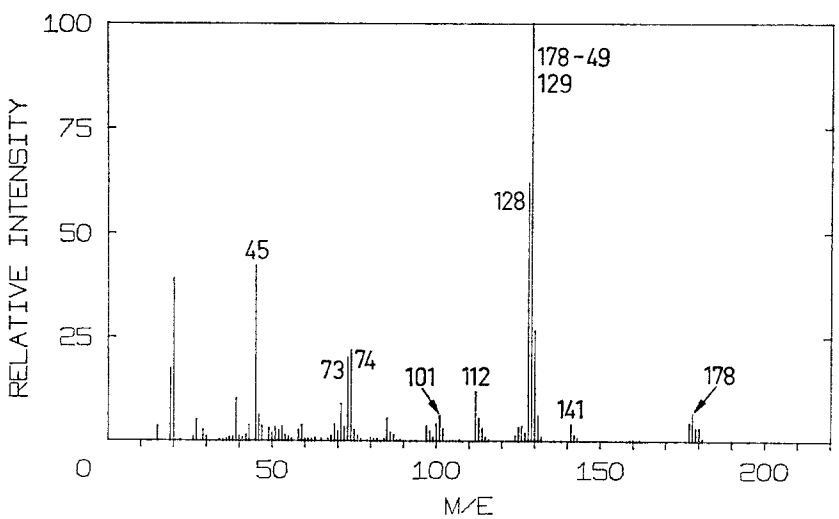


Fig. 7. Mass spectrum of the deuterium labelled compound 2

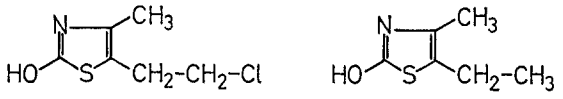


Fig. 8a and b. Proposed structures of compound 2 (a) and compound 3 (b)

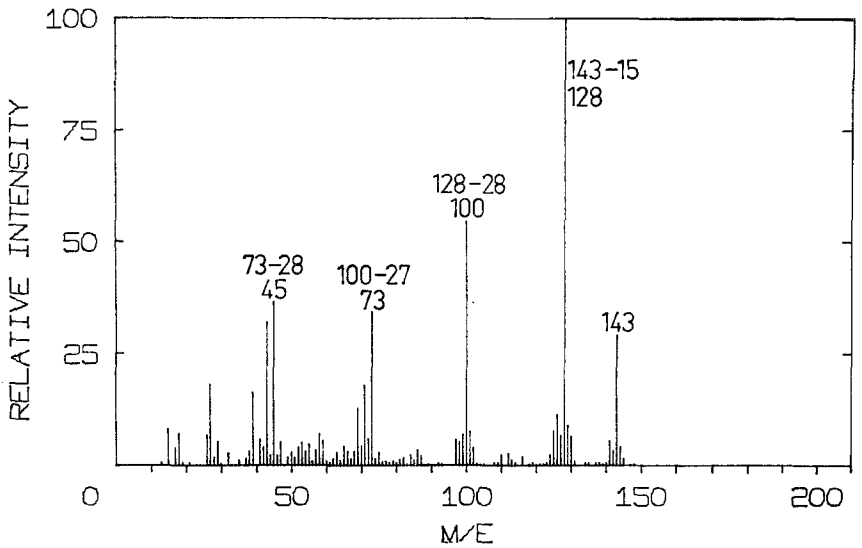


Fig. 9. Mass spectrum of compound 3 identified as 2-hydroxy-4-methyl-5-ethylthiazole

Using the information from the metastable transitions, the exact mass measurement and the mass spectrum of the labelled compound, the following decomposition is established. It is still difficult, however, to determine which carbon atom is lost in the fragment HCN.

$C_6H_7DNOSCl$ (m/e 178)$^+$ $\rightarrow$ C_5H_5DNOS (m/e 129)$^+$ + CH_2Cl
C_5H_5DNOS (m/e 129)$^+$ $\rightarrow$ C_4H_5DNS (m/e 101)$^+$ + CO
C_5H_5DNOS (m/e 129)$^+$ $\rightarrow$ C_3H_4DS (m/e 74)$^+$ + C_2HNO
C_4H_5DNS (m/e 101)$^+$ $\rightarrow$ C_3H_4DS (m/e 74)$^+$ + HCN
C_3H_4DS (m/e 74)$^+$ $\rightarrow$ CHS (m/e 45)$^+$ + C_2H_3D

According to the structure for chlormethiazole the structure of compound 2 is 2-hydroxy-4-methyl-5-β-chloro-ethylthiazole (Fig. 8a).

However, the fragmentation of these types of compounds will be further studied, since it is important that the fragments from the mass spectrometric analyses can be unmistakably identified.

Fig. 9 shows the mass spectrum of the eluted spot 3 with a parent ion at m/e 143 and from a metastable transition for *m = 114.5 decomposition m/e $143^+ \rightarrow 128^+ + 15$ is determined. The m/e 128 ion undergoes cleavage in the same manner as compound 2, which is shown in Table 1. This indicates it has the same structure with the exception of the side chain which consists of an ethyl group instead of the chloroethyl group. The methyl group lost at M—15 results from

cleavage between the carbons in the ethyl group. Thus the chemical name should be 2-hydroxy-4-methyl-5-ethylthiazole (Fig. 8b). The component 2b in Fig. 2 gave rise to a mass spectrum with a base peak at m/e 85 and other high intensity peaks at m/e 86, 58, 57, 45, 43 and 29. The component is probably not a metabolite and may be regarded as an impurity.

Conclusion

Two new human metabolites of chlormethiazole have been isolated and identified as 2-hydroxy-4-methyl-5-β-chloro-ethylthiazole and 2-hydroxy-4-methyl-5-ethylthiazole. Further studies are planned to confirm the fragmentation of different thiazole compounds.

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