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THE PREPARATION OF PURE 2-THIO-THIAZOLIDINE-4-CARBOXYLIC-ACID (TTCA) AS A REFERENCE STANDARD FOR CARBON DISULFIDE EXPOSURE TESTS.

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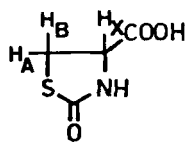
Abstract : 2-Thio-thiozalidine-4-carboxylic-acid is synthesized and subsequently purified by high-pressure liquid chromatography. The spectral properties of the title compound are described and commented upon.

2-Thio-thiazolidine-4-carboxylic-acid (1) was detected in urine of rats treated simultaneously with captan and either cysteine or reduced glutathione[1] and also in urine of workers occupationally exposed to carbon disulfide[2].

The metabolism of captan as well as of carbon disulfide appears to involve a reaction with cysteine to yield TTCA[1,2].



(1)

Reaction of captan with cystein gives tetrahydrophtalimide and thiophosgene. The thiophosgene so generated ultimately condenses with another cysteine molecule to yield TTCA[3].

The pathway of TTCA formation after inhalation of CS_2 has not yet been elucidated. It seems that CS_2 reacts with glutathione to yield a dithiocarbamate derivative which after successive (non)enzymatic breakdowns results in a molecule that cyclizes to TTCA, which is excreted in the urine.

The detection of TTCA in urine of workers exposed to CS_2 opens new perspec-

tives for the evaluation of the exposure to CS_2[5]. To this purpose, it is necessary to have a pure standard of TTCA available. In the present work we wish to report the synthesis, purification and spectral properties of TTCA.

Experimental.

A slightly modified method of De Baun et al.[1] was used for the synthesis of TTCA.

To a stirred mixture of 34 g (0,193 mole) L-cysteine hydrochloride monohydrate in 300 ml of water and 31,2 g (0,78 mole) NaOH in 100 ml of water, a solution of 22,2 g (0,193 mole) thiophosgene in 200 ml of benzene was slowly added under nitrogen atmosphere at room temperature.

The two-phase reaction mixture was stirred at room temperature for 4 hours.

The aqueous layer was separated, acidified with HCl to pH 2 and extracted 5 times with 100 ml portions of ethyl acetate. The combined ethyl acetate layers were dried over anhydrous magnesium sulfate. After removal of ethyl acetate a crude yellow-orange product was obtained (yield 65%).

This product was purified on a Pye Unicam high-pressure liquid chromatograph equipped with a preparative column (length : 250 mm, O.D. : 1/2", I.D. : 10 mm) packed with RSil C18 HL particles of 10 micron diameter.

250 mg of the crude product was dissolved in methanol and injected by means of a 2,0 ml sample loop.

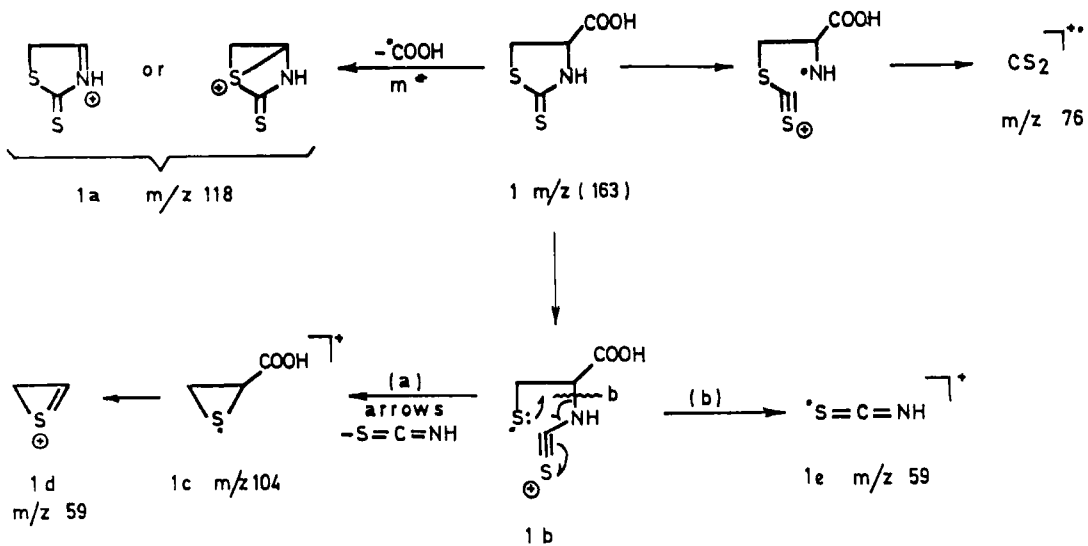
The eluent was a 10% HAc solution in water and the flow rate was set at 150 ml per hour.

This way TTCA was separated from its impurities in one run and after evaporation of the eluent, 75 mg of a white cristalline product was obtained (yield 38%).

The melting point was determined with a melting microscope (Reichert Thermovar).

The ^1H NMR spectrum was obtained using a Varian T-60A spectrometer.

The mass spectrum was obtained at 70 eV ionization potential by direct insertion into the ion source of a AFI-MS 50 instrument. The infrared spectrum was recorded on a Beckman IR-7 infrared spectrophotometer.


or
-•COOH
m•
COOH
NH
S
•NH
CS2
m/z 76
1a m/z 118
1 m/z (163)
(a)
arrows
-S=C=NH
(b)
b
•S=C=NH
1d
m/z 59
1c m/z 104
1b
1e m/z 59

SCHEME 1

Results and Discussion.

The melting point was established as 175-176°C, which is in accordance with the results of De Baun et al.[1]

The low-resolution mass spectrum of TTCA is shown in FIG.1 and the proposed fragmentation pattern in Scheme 1.

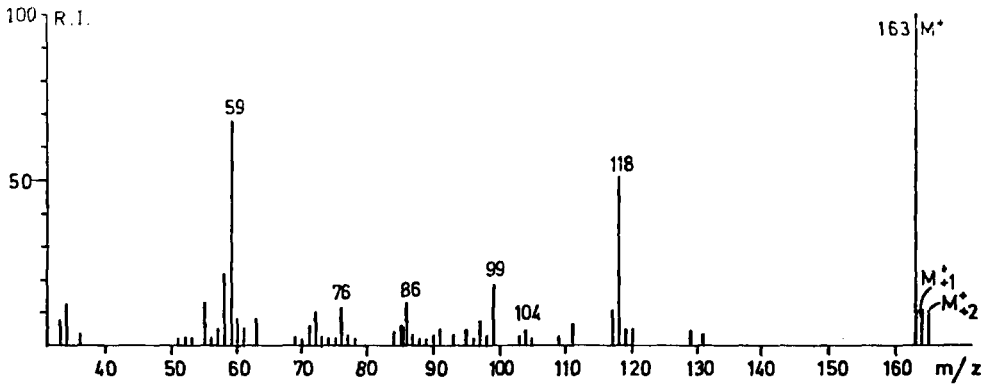


FIG. 1

The spectrum of TTCA shows an abundant molecular ion (1) at m/z 163, which is the base peak, and two major fragments at m/z 118 and 59. The fragment at m/z 118 is due to the loss of HOOC˙ from the molecular ion to form 1a.
This fragmentation is confirmed by the presence of a metastable ion at m/z 85,4.
The formation of the ion at m/z 59 can be explained by two possible pathways starting from ion 1b. In pathway (a) there is an elimination of S=C=NH from 1b which leads to the formation of 2-carboxythiirane (1c).
This ion loses the HOOC˙ group (alfa-cleavage) to form ion 1d.
On the other hand in pathway (b), ion 1b undergoes a beta-cleavage with respect to the C=S group to form $NH=C=S^+$ (1e).
Since there is no information from high resolution and deuterium labeling techniques, it is clear that some of the conclusions drawn are tentative.
The ^1H NMR data of TTCA in aceton-d^6 solution (TMS as internal standard) are gathered in Table 1.

TABLE 1 : ^{1}H NMR data of TTCA in aceton-d_6.

(TMS as internal standard)

	chemical shifts (in ppm)	coupling constants (in Hz)
H-5A	4,03	J (5A,4) : 9,0
H-5B	3,78	J (5B,4) : 5,0
H-4	5,01	J (5A,5B) : 11,3
NH	7,71	
COOH	9,12	

The spectrum is quite simple and shows a pattern peculiar to an ABX system.

The infrared spectrum is shown in FIG.2.

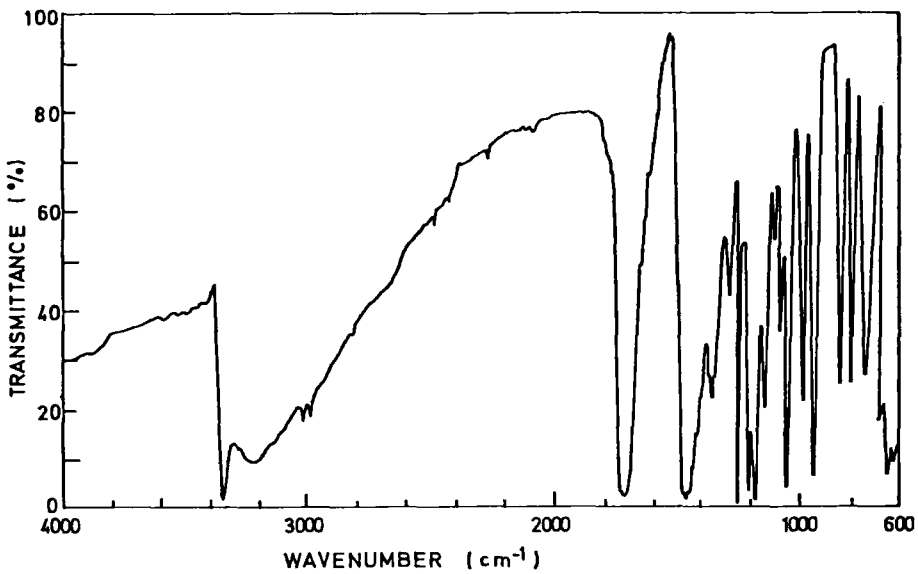


FIG. 2

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