Preparation of 2-Isopropylthiamorpholin-3-one-5-carboxylic Acid

ISOVALTHINE is a new sulphur-containing amino-acid which is isolated from the urine of hypercholesterolaemic patients^{1,2}. A molecular model of isovalthine suggests a possibility of forming a thiamorpholinone derivative by the intramolecular cyclization. Such thiamorpholinone derivative may also be produced in the animal body and have some biological effects. From these considerations, 2-isopropylthiamorpholin-3-one-5-carboxylic acid was prepared from L-isovalthine and its biological effect was examined.

L-Isovalthine (30 mg, decomposition point 186°C)³ was heated in a small flask immersed in an oil-bath at about 190°C for 15 min. During this operation a vacuum was applied to the flask to remove water which was produced during cyclization. After cooling the flask to room temperature, the brown residue was dissolved in a small amount of 2 N ammonia and filtered. The filtrate was made weakly acidic with acetic acid, and transferred on a

column containing strong cation exchanger (*H*-form). The effluent and water washings were collected and evaporated to dryness in vacuum. The residue was taken up in ether and filtered. The ether solution was concentrated to a proper volume and left standing at room temperature. Needles obtained were recrystallized from ether, giving 18 mg, m.p. 140°C [α]_D²⁵ = +4.1° in ethanol. It was slightly soluble in water, soluble in ether, and easily soluble in methanol, ethanol, and alkali.

Anal. calc. for C₈H₁₃O₃NS: C, 47.27; H, 6.45; N, 6.89; found: C, 47.18; H, 6.44; N, 6.89. The molecular weight (calculated, 203) was found to be 186 and 217 by the Rast method. The ultra-violet absorption spectrum of the compound is shown in Fig. 1. $\lambda_{\max}$ = 202.8 m μ (ϵ = 13,000). Since another synthetic sample of thiamorpholin-3-one-5-carboxylic acid prepared from *S*-(carboxymethyl)-L-cysteine⁴ also showed a similar absorption, the $\lambda_{\max}$ = 202.8 m μ band is thought to be specific for the thiamorpholinone ring. The infra-red spectrum of the compound is shown in Fig. 2.

Although the thiamorpholinone derivative prepared as described here is similar in chemical structure with amytal, it has neither hypnotic effect on mice nor inhibitory effect on the electron transport system of isolated rat liver mitochondria.

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¹ Mizuhara, S., and Ohmori, S., *Arch. Biochem. Biophys.*, **92**, 53 (1961).

² Ohmori, S., and Mizuhara, S., *Arch. Biochem. Biophys.*, **96**, 179 (1962).

³ Mizuhara, S., *Tanabe Amino Acids Res. Foundation*, **2**, No. 4, 1 (1962).

⁴ Armstrong, M. D., and Levis, L. D., *J. Org. Chem.*, **16**, 749 (1951).

Fig. 1. Ultra-violet absorption spectrum of 2-isopropylthiamorpholin-3-one-5-carboxylic acid in methanol solution

Fig. 2. Infra-red spectrum of 2-isopropylthiamorpholin-3-one-5-carboxylic acid in potassium bromide

Configuration of Urinary Isovalthine

ISOVALTHINE crystals first isolated from urine were rhombic plates which gave DL-alanine after treatment with Raney nickel. Needle-shaped crystals were obtained by condensation of L-cysteine and (±)- α -bromoisovaleric acid. They were a mixture of L- and L-allo-isovalthine, and readily converted into the rhombic form by heating in hydrochloric acid^{1,2}.

Recently we have synthesized L- and L-allo-isovalthine separately by using (+) and (-)- α -bromoisovaleric acid. The resolution of (±)- α -bromoisovaleric acid into each of optical antipodes was achieved by using ephedrine. Optical rotation of the isomer synthesized from L-cysteine and (+)- α -bromoisovaleric acid was -52.4° ([α]_D²⁵ in 2 N hydrochloric acid) and that of the other isomer synthesized from (-)- α -bromo acid was +82° ([α]_D²⁵ in 2 N hydrochloric acid). The decomposition point of the isomer of [α]_D = -52.4° was 186°C and that of [α]_D = +82° was 185°C. The elemental analyses of these isomers were correct, and these single isomers gave L-alanine after treatment with Raney nickel.

On an amino-acid analyser (Beckman model 120 B), the needle-shaped crystals yield two