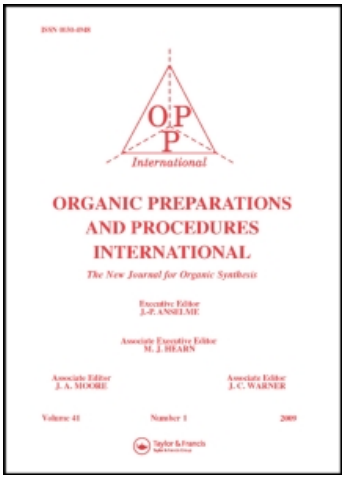

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THE STRUCTURE OF "ITCA", A URINARY METABOLITE OF CYANIDE

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(10/24/03)

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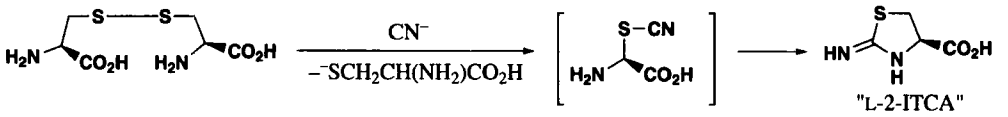
Mammals have several enzyme systems for the detoxification of cyanide, a military/civilian threat agent of contemporary concern, *viz.*, rhodanase (thiosulfate: cyanide sulfurtransferase; EC 2.8.1.1)[1] and β-mercaptopyruvate sulfurtransferase (3-mercaptopyruvate: cyanide sulfurtransferase, EC 2.8.1.2),[1,2] both enzymes catalyzing the conversion of cyanide to the non-toxic thiocyanate, the latter being excreted in the urine. Cyanide also reacts *in vivo* with L-cystine, the oxidized disulfide form of the endogenous sulfur amino acid, L-cysteine, to produce L-2-iminothiazolidine-4-carboxylic acid ("L-2-ITCA"; *Scheme 1*).[3]

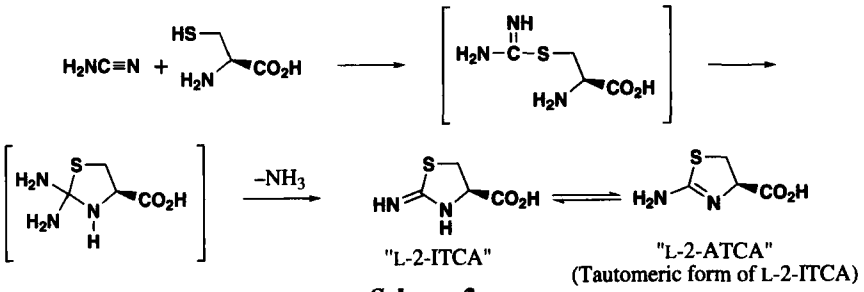


Scheme 1

Whether this reaction is enzyme catalyzed has not been unequivocally established, but the reaction can readily be reproduced in the test tube without any enzymes.[4] Since L-2-ITCA is, likewise, excreted in the urine,[3] it was also assumed to be a detoxification product of cyanide. However, Bittner *et al.*[5] have shown recently that L-2-ITCA, when administered intracerebroventrically (icv) to mice, elicited wild–running seizures, and when infused icv to rats, resulted in selective loss of neurons in the hippocampus. This implied that L-2-ITCA was an excitotoxin that may possibly be responsible for the neurotoxicity observed in humans on chronic exposure, such as by ingestion of improperly processed cassava, a cyanide-containing staple of certain populations in South Africa.[6]

A number of synthetic methods for the preparation of ITCA have been described in the literature[3,4,7,8] but many of these are multi-steps leading to racemic ITCA, which is not useful for biological studies. Moreover, the chiral integrity of ITCA prepared by other methods is uncertain, based on the wide range of optical rotations reported.[3,4,9]

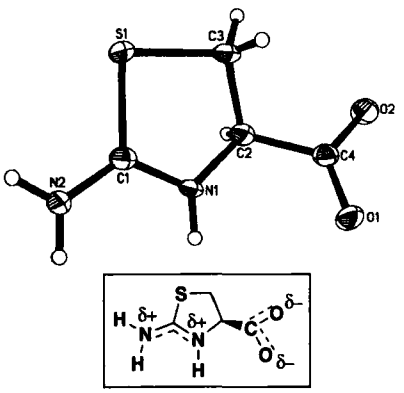
We have now developed a simple, one-step, one-pot synthesis of L-2-ATCA, the tautomeric form of L-2-ITCA, by condensation -- under nitrogen -- of L-cysteine with cyanamide in aqueous solution at reflux temperatures (*Scheme 2*). The course of the reaction can readily be followed by monitoring the ammonia formed as well as the formation of the product by tlc.



Scheme 2

By this means, chiral L-2-ITCA ($[\alpha]_D^{23}$ -96.9°C) was prepared in reproducible yields of 45-50%. When D-cysteine was used as reactant, D-2-ITCA ($[\alpha]_D^{23}$ +93.0°C) was obtained. Also, L-5,5-dideutero-2-ITCA, for use in biological studies was prepared with deuterium-labeled L-cysteine. This condensation reaction can also be conducted using microwave techniques on smaller scale[10,11], which reduced the reaction time from 4-5 hours to 5 minutes. However, tlc indicated that other by-products reflecting the presence of NH_3 were formed, suggesting that the purification process would be more tedious; hence, no attempts were made to isolate the products. We believe the mechanism of this reaction to proceed by initial condensation of cyanamide on the cysteine thiol, followed by ring closure (*Scheme 2*); since, under identical conditions, L-serine was unreactive and did not give the corresponding oxazoline derivative.

Because the literature is ambiguous about the structure of "L-2-ITCA", and its tautomeric form, *viz.*, 2-amino-2-thiazoline-4-carboxylic acid (2-amino-4,4-dihydrothiazole-4-carboxylic acid, *Scheme 2*), appears to be used interchangeably, the "L-2-ITCA" prepared above was subjected to x-ray crystallographic analysis. The data (*Figure 1*) indicated that (a) N-2 is an amino (not an imino) group, (b) the carboxyl group is ionized, and (c) the N-1 ring nitrogen is protonated. Moreover, the N1-C1 (1.315 Å) and C1-N2 (1.303 Å) bond lengths are nearly identical and shorter than the N1-C2 bond (1.459 Å) (*Table 1*; compare ref 12 for the copper complex), suggesting a partial double-bond character for the former. This is depicted in *Fig. 1*. These data suggest that the ring structure of "ITCA", at least in the crystalline state, *is not a thiazolidine*, but a thiazoline, *i.e.*, the structure of "L-2-ITCA" is 2-amino-2-thiazoline-4*R*-carboxylic acid (suggested acronym, L-2-ATCA), and the compound exists as a zwitterion.



Indeed, these x-ray data now confirm Schoeberl's assignment[4] of the structure of the product from the reaction of cyanide with cystine as a thiazoline. Later, its zwitterionic character was postulated by Gawron *et al.*[7] based on (a) its high decomposition temperature, (b) ir and nmr spectra, (c) titration studies, and (d) studies on the optical rotation changes as a function of pH.

The toxicological aspects of L-ITCA are presently under investigation.

EXPERIMENTAL SECTION

Cyanamide was purchased from Fluka Chemical Co., L-cysteine and its hydrochloride from Sigma Chemical Co., and L-cysteine-3,3-d_2 (98%) from Cambridge Isotope Laboratories, Inc. Melting points were taken on a Fischer-Johns hot stage melting point apparatus and are uncorrected. Thin-layer chromatography was performed using Analtech silica gel GF Uniplates with n-butyl alcohol/acetic acid/water (4:1:1) as solvent. The reaction products were visualized under 254 nm UV light by fluorescence quenching, and by exposure to iodine vapors in an iodine chamber. Microanalyses were provided by M-H-W Laboratories, Phoenix, AZ.

2-Amino-2-thiazoline-4-carboxylic Acid. a) From L-Cysteine Hydrochloride. 2-Amino-2-thiazoline-4*R*-carboxylic Acid (L-2-ATCA).- To L-cysteine hydrochloride (5.2 g, 33 mmol) dissolved in 50 mL deionized H_2O was added $NaHCO_3$ (2.8 g, 33 mmol) and cyanamide (1.4 g, 33 mmol) with stirring. The mixture was heated under reflux and a continuous stream of nitrogen gas was maintained over the reaction. H_2O was occasionally added to replace evaporative loss, and the pH of entrained gases monitored at the condenser outlet with Instachek 0-13 pH papers. The pH remained consistent at 10-11 (apparently reflecting evolution of gaseous NH_3) until the reaction was nearly complete, at which time it decreased to 8-9, when reflux was discontinued. The reaction mixture was concentrated on a rotary evaporator to approximately 25% of the original volume when crystals spontaneously precipitated. Following overnight refrigeration, the precipitate was collected (0.98 g) and the filtrate evaporated to a dry, white residue. The residue was dissolved in a few ml of hot H_2O and upon cooling produced 1.38 g of crystals. The solids were combined, recrystalized from H_2O/ethanol (1:5), and dried over P_4O_{10} to yield 1.89 g (39%) of white crystals, mp 225-235°C (dec.), *lit.*[13] 212°C. TLC (n-butanol/acetic acid/H_2O, 4:1:1) of product vs authentic sample was identical, R_f = 0.30. $[a]_D^{23}$ -96.9 (c = 1.04, H_2O), *lit.*[13] $[a]_D^{23}$ –99, (c = 1.0, H_2O); $[a]_D^{23}$ -93.4 (c = 1.15, 2% Aq. HCl), *lit.*[(14)] $[a]_D^{23}$ -88.8, (c = 3, 2% aq. HCl).

Anal. Calcd for $C_4H_6N_2O_2S$: C, 32.87; H. 4.14; N, 19.17. Found: C, 32.95; H, 4.07; N. 19.02

b) From L-Cysteine.- To L-cysteine (5.00 g, 41.3 mmol, free base, anhydrous) dissolved in 100 mL H_2O was added cyanamide (1.73 g, 41.2mmol) with stirring. The resulting clear, colorless solution was heated under reflux under a continuous stream of N_2 gas for 7 hours. The pH of the entrained gas remained about 11 as the reaction proceeded, then dropped to about 8 at which time reflux was discontinued. The reaction mixture was stirred overnight at room temperature, and then concentrated on a rotary evaporator until white crystals spontaneously formed in substantial quantity. Following overnight refrigeration, the solids were collected, recrystalized from EtOH/H_2O (5:1), and the product dried in a vacuum desiccator over P_4O_{10} to yield 3.47 g of L-2-ATCA (58% yield), mp 220-240°C dec., $[\alpha]_D^{23}$ -94.4 (c = 1.18, H_2O).

c) From D-Cysteine (free base). 2-Amino-2-thiazoline-4*S*-carboxylic Acid (D-2-ATCA).- This enantiomer, obtained as white crystals in 39% yield (mp 220-240°C dec.), was prepared

from D-cysteine (free base) using the above procedure (b); $[\alpha]_D^{23}$ +93.0 (c = 1.02, H_2O), $[\alpha]_D^{23}$ +89.5 (c = 1.05, 2% Aq. HCl).

Anal. Calcd. for $C_4H_6N_2O_2S$: C, 32.87; H. 4.14; N, 19.17. Found: C, 33.09; H, 4.53; N, 19.50

d) 2-Amino-5,5-dideutero-2-thiazoline-4*R*-carboxylic Acid.- L-Cysteine-3,3-d_2 (0.106 g, 0.864 mmol, free base, anhydrous), cyanamide (0.041 g, 0.864 mmol) and 20 mL of H_2O were reacted as above to give the dideteurated product, albeit in diminished yield after recrystalization (31 mg). TLC of the crude reaction mixture showed the formation of many by-products not seen in the prototype reactions above, perhaps due to impurities in the deuterated L-cysteine.

Table 1. Pertinent Bond Lengths [Å] and Angles [°] for L-2-ATCA

N1-C1	1.315(3)	O2-C4	1.239(3)	N1-C2-C4	114.2(2)
C1-N2	1.303(3)	C1-N1-H1A	120.6(17)	C1-N2-H2A	121.2(18)
N1-C2	1.459(3)	C2-N1-H1A	118.9(17)	C1-N2-H2B	120.4(17)
O1-C4	1.252(3)	N2-C1-N1	124.6(2)	H2A-N2-H2B	118(2)

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A PRACTICAL SYNTHESIS OF ETHYL L-GLUTAMINE

(L-Theanine)

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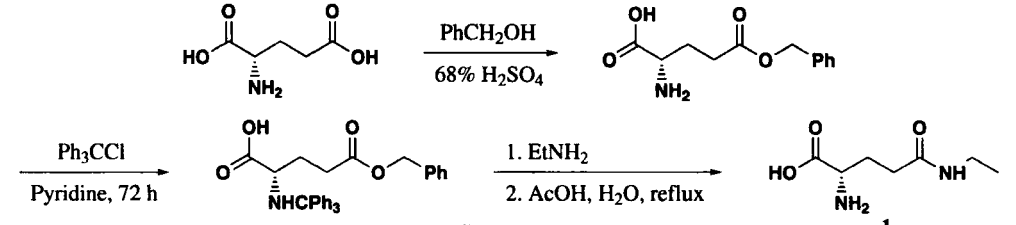
L-Theanine (**1**) is a unique free form amino acid found in the tea plant and in the mushrooms Xerocomus badius and certain species of genus *Camellia*, *C. japonica* and *C. sasanqua*. It increases α-waves-producing mental and physical relaxation and decreases stress and anxiety without drowsiness.[1] Studies suggest that L-theanine may also find other applications such as controlling hypertension,[2] improving learning performance,[3] heightening mental acuity, promoting concentration, acting antagonistically against the paralysis induced by caffeine,[4] supporting the immune system, lowering blood pressure, and increasing brain dopamine levels; there are no known side-effects.[5-7]

As part of our research program, we required an efficient method to prepare L-theanine. Surprisingly however, a review of the literature, including patents, indicated the absence a practical synthesis. A summary of the known procedures[8-12] is shown in *Schemes 1*,[13] *2*[14] and *3*.[15]



Scheme 1