Cystathionine has been reported to undergo enzymatic changes leading to the formation of seven members of cyclic products (Ricci, G., Santoro, L., Achilli, M., Matarese, R. M., Nardini, M., and Cavallini, D. (1983) J. Biol. Chem. 258, 10511-10517; Cavallini, D., Costa, M., Pensa, B., and Coccia, R. (1985) Biochem. Int. 10, 641-646). Gas-chromatographic and mass-spectrometric evidence reported in this paper indicates that the cyclic derivative of cystathionine, 1,4-hexahydrothiazepine-3,5-dicarboxylic acid, here simply named cyclothionine, is a normal component of bovine brain. This finding together with the detection of the same compound in the urine of cystathioninuric patients (Kodama, H., Sasaki, K., Mikasa, H., Cavallini, D., and Ricci, G. (1984) J. Chromatogr. 311, 183-188) supports the conclusion that cystathionine, apart from its role in trans-sulfuration, is converted also into cyclic compounds whose biochemical significance is as yet unknown.

Recent investigations in this laboratory have been directed toward the search of possible novel nontrans-sulfuriferous enzymatic changes of L-cystathionine. One sided oxidative deamination by L-amino acid oxidase was found to yield the cyclic compound cystathionine ketimine (1-3). Rat liver (4) and a number of bovine tissues (5), including brain, were also found to produce cystathionine ketimine through nonoxidative deamination. Nonenzymatic (6) and enzymatic (4) transaminations also yield the cyclic ketimine derivative.

1,4-Hexahydrothiazepine-3,5-dicarboxylic acid, here named cyclothionine for brevity, has been detected in the urine of cystathioninuric patients (7, 8) deficient in cystathionine γ-lyase (9, 10, and literature therein). It is likely that the excess of cystathionine occurring in these patients is channeled through minor metabolic routes whose existence is revealed by the lack of cystathionase. One of these routes could be responsible for the deamination, cyclization, and reduction of part of cystathionine to cyclothionine (Fig. 1). If so, cyclothionine should be a normal mammalian product coming from cystathionine via a minor metabolic path running alongside the trans-sulfurative route. In the present note we provide the first evidence for the occurrence of cyclothionine in a mammalian brain which supports this conclusion.

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for \( H_2 \) and 110 ml/min for air in the flame photometric detector.

Gas-chromatographic mass-spectrometric analyses were performed on a GCD Pye Unicam gas chromatograph connected to a low resolution mass spectrometer (LKB 2091) equipped with a digital PDP 11 computer. Chromographic separations were carried out on a 25 m × 0.2 mm inner diameter fused-silica capillary column with OV-101 as stationary phase. The flow rate of helium carrier gas was 0.6 ml/min. The column temperature was programmed from 120 to 250 °C at a rate of 8 °C/min; injector temperature was 250 °C; the molecular separator temperature was 260 °C. Mass spectrometer experimental conditions were: ionization mode, electron impact; electron energy, 70 eV; ion source, 250 °C; ion source vacuum, \( 0.5 \times 10^{-6} \) mm Hg.

RESULTS

The gas-liquid chromatography of the bovine brain extract as revealed by the flame ionization detector is illustrated in Fig. 2. One large and one minor peak having the same retention times of two similar peaks exhibited by the authentic sample of cyclothionine are seen in the shaded area of the chromatograms. As reported earlier (11) the large peak is the dimethyl ester of cyclothionine; the minor one is the trimethyl derivative produced by the partial methylation of the imino nitrogen by the diazomethane reagent. Fig. 3 reports the chromatograms of the brain extract revealed by the flame photometric detector for sulfur-containing compounds. It appears that at least five sulfur-containing products have been extracted from the brain by the procedure adopted, two of which (shaded area) exhibit the same properties of authentic cyclothionine (B) as regards retention time, the presence of sulfur, and the production of two unequal peaks. Co-chromatography with authentic cyclothionine (C) indicates the identical behavior of these two compounds. An approximate estimation of the amount of cyclothionine contained in the bovine brain was obtained from the peak areas and from the recovery of added cyclothionine used in the pilot assay. This value is of the order of 0.5 \( \mu g \) of cyclothionine/100 g of brain, wet weight.

The identification of cyclothionine has been confirmed by mass spectrometry of the large peak separated by gas chromatography. Fig. 4 shows the same fragmentation pattern of synthetic and endogenous cyclothionine, thus confirming the identity of the two compounds.

![Cyclic derivatives of cystathionine.](image)

**Fig. 1.** Cyclic derivatives of cystathionine. II and III, products of monodeamination followed by cyclization (Ref. 1-3). IV, products of reduction of II and III (Ref. 3).

![Gas-liquid chromatography of methylated extract](image)

**Fig. 2.** Gas-liquid chromatography of methylated extract from about 300 g of bovine brain (A) and of 1 \( \mu g \) of methylated authentic cyclothionine (B), as revealed by flame ionization detector. For analysis conditions see text.

![Gas-liquid chromatography of methylated extract](image)

**Fig. 3.** Gas-liquid chromatography of methylated extract from about 100 g of bovine brain (A); of 1 \( \mu g \) of methylated authentic cyclothionine (B); of A plus B (C), as revealed by flame photometric detector for sulfur-containing compounds. For analysis conditions see text.
FIG. 4. Mass spectra of endogenous (A) and synthetic (B) methylated cyclothionine. The main peak of the compound seen in Fig. 2 has been analyzed. Fragments with relative intensity higher than 1% are reported. m/z = 233, M⁺, the molecular ion; m/z = 201, M⁺ – H₂O, m/z = 174, M⁺ – CO₂CH₃, the monocarboxymethyl fragment; m/z = 114, M⁺ – H – 2(CO₂CH₃), m/z = 100 is m/z 114 – 14N and ring contraction.

DISCUSSION

The detection of cyclothionine in bovine brain, together with the reported occurrence in the same tissue (5) of enzymes converting cystathionine into the cyclic ketimine form, suggests cystathionine as the most likely precursor of cyclothionine. A plausible route for the production of cyclothionine through the ketimine form is shown in Fig. 1. The excretion of cyclothionine in cystathionuric patients (7, 8) is an indication of the occurrence of this enzymatic route, at least in man. Cyclothionine and similar compounds, to our knowledge, have never been reported in plants, and a dietary source of the compound seems unlikely although not ruled out. A supply to the brain from other tissues of the same animal is of course another possibility. The cystathionine content is unusually high in mammalian brain (13) and in certain tumoral forms of the nervous system (14). This is in accord with the higher cystathionine synthase activity compared with the lower cystathionine γ-lyase activity of the brain of most mammals and in particular in man (15). The high level of cystathionine cannot be explained by its being only a cysteine precursor, and the presence of cyclothionine in brain suggests a role of cystathionine other than that of trans-sulfuration. Apart from proline other cyclic amino acid, i.e. piperolic acid, has been detected in brain (16). Cyclothionine is a cyclic imino acid with three unusual properties: it is a dicarboxylic imino acid, it contains sulfur, and it contains an uncommon seven-membered heterocyclic ring. The last property appears of relevance if one considers the presence of similar seven-membered rings in the structure of benzodiazepine so extensively used in the therapy of some central nervous system disturbances. The relationship between cyclothionine and benzodiazepine at a functional level in the brain is therefore worthy of investigation. Cyclothionine is not reactive to ninhydrin, making difficult the study of its distribution in tissues and of the elucidation of its biochemical role. A simple and sensitive analytical method is necessary for the determination of cyclothionine, and work in this direction is now in progress in this laboratory.

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REFERENCES