Isolation of *S*-Adenosyl-3-thiopropylamine from the Eye of the Sea Catfish (*Arius felis*)

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A method is reported for the separation of *S*-adenosyl-3-methylthiopropylamine and other basic compounds in the eye of the sea catfish (*Arius felis*) by ion-exchange chromatography on CM-Sephadex. One of the basic compounds was isolated in crystalline form and was shown to be *S*-adenosyl-3-thiopropylamine by chemical and spectroscopic characterizations and by comparison with a synthetic sample.

We have shown that the reflecting material of the eye of the sea catfish (*Arius felis* L.) consists of acidic and basic components. The former are oligomers of 5,6-dihydroxyindole-2-carboxylic acid (Ito & Nicol, 1974). The latter consists of *S*-adenosyl-3-methylthiopropylamine (decarboxylated *S*-adenosyl-methionine), and several minor ones, including thiamin and the compounds 'B' and 'C' (Ito & Nicol, 1975). We have suggested that the compound B may be *S*-adenosyl-3-thiopropylamine (decarboxylated *S*-adenosylhomocysteine) on the basis of the colour reactions and chromatographic behaviour. We now describe the isolation and identification of *S*-adenosyl-3-thiopropylamine from the eye of the catfish.

Materials and Methods

Animal

Sea catfish were collected from channels and bays near Port Aransas, Texas, U.S.A.

Chemicals

S-Adenosyl-L-methionine (chloride hydrochloride; 88% purity) and *S*-adenosyl-L-homocysteine (98% purity) were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A. A natural sample of *S*-adenosyl-3-methylthiopropylamine was isolated from eyes of the catfish as reported by Ito & Nicol (1975). A synthetic sample of *S*-adenosyl-3-thiopropylamine was prepared according to the method of Jamieson (1963) from 2',3'-isopropylidene-*S*-adenosyl-3-thiopropylamine, which was kindly supplied by Dr. G. A. Jamieson, National Red Cross, Bethesda, Md., U.S.A.

Instruments

U.v.-absorption spectra were taken on a Beckman model 24 spectrophotometer, and i.r. spectra on a Perkin-Elmer grating i.r. spectrophotometer, model 237B.

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For this, 40 eyes (wet wt. 14.8 g) were homogenized and extracted in 4 vol. (v/w) of 0.5M-H₂SO₄ and centrifuged at 10000g for 10 min. The residue was again extracted in 2 vol. of 0.5M-H₂SO₄, and the combined extracts were adjusted to pH 4 with freshly prepared 0.5M-BaCO₃ suspension. The resulting BaSO₄ was filtered off and washed with water. The filtrate was then adjusted to pH 3 with 0.5M-H₂SO₄, kept at 2°C overnight, and filtered. The filtrate was passed through a column (1.9 cm × 3.0 cm) of Dowex-1 (X8, acetate form) to remove residual sulphate ions, and concentrated to about 20 ml under decreased pressure at 40°C. The insoluble material was removed by centrifugation at 10000g for 10 min and the supernatant was evaporated to dryness; the residue was taken up in 3 ml of 0.5M-ammonium formate, kept overnight at 2°C, and centrifuged at 10000g for 10 min. The clear colourless supernatant was chromatographed on a column of CM-Sephadex C-25 as shown in Fig. 1. The fractions 16–21 were evaporated to a small volume, freeze-dried, and then subjected to sublimation under decreased pressure to remove ammonium formate. The residue was dissolved in 2 ml of 1M-formic acid and applied to a column (1.0 cm × 27 cm) of CM-Sephadex C-25, equilibrated with 1M-formic acid, and eluted with the same solvent, 5.5 ml fractions being collected. Fractions 19–24, which contained *S*-adenosyl-3-thiopropylamine, were evaporated to near-dryness, transferred to a small centrifuge tube, and evaporated with a stream of N₂. The residue was crystallized from 0.1 ml of 0.5M-H₂SO₄ and 2 ml of ethanol to give 1.6 mg (3.6 μmol) of *S*-adenosyl-3-thiopropylamine bisulphate as needles.

To isolate *S*-adenosyl-3-methylthiopropylamine, the fractions 44–55 (see Fig. 1) were freeze-dried, the residue was chromatographed on the CM-Sephadex column and eluted with 1M-formic acid as described above. The yield of *S*-adenosyl-3-methylthiopropyl-

Fig. 1. Elution pattern of an extract of eyes of the catfish on a column of CM-Sephadex C-25

The extract (3 ml) was applied to a column (1.05 cm $\times$ 40 cm), which had been equilibrated with 0.5 M-ammonium formate, pH 6.5, and was eluted with the same solvent at a flow rate of 24 ml/h. Fractions (3.8 ml) were collected and monitored by their E_{260} . [The fractions 12–14, 16–21, 25–30 and 44–55 were found to contain thiamin, *S*-adenosyl-3-thiopropylamine, compound C (Ito & Nicol, 1975) and *S*-adenosyl-3-methylthiopropylamine respectively.]

Table 1. *T.l.c. and paper electrophoresis of S-adenosylhomocysteine, S-adenosylmethionine and related compounds isolated from catfish eyes*

Authentic samples of *S*-adenosylhomocysteine and *S*-adenosylmethionine were compared with compounds isolated from catfish eye extracts (see Fig. 1). Thin-layer chromatograms (cellulose; E. Merck, Darmstadt, West Germany) were developed in propan-1-ol/1 M-HCl (3:2, v/v), or 95% ethanol/1 M-ammonium acetate, pH 7.0 (7:3, v/v). Paper electrophoresis was carried out on Whatman no. 3MM paper with a voltage gradient of 10 V/cm. The solvents used were: 90% (w/w) formic acid/acetic acid/water (1:3:16, by vol.), pH about 1.6; acetic acid/pyridine/water (10:3:500, by vol.), pH 4.0. The compounds were located with a u.v. (254 nm) lamp, ninhydrin reagent and iodoplatinate reagent (Toennies & Kolb, 1951).

	T.l.c. (R_F values)		Mobility towards cathode on paper electrophoresis (cm/h)	
	Propanol/ HCl	Ethanol/ ammonium acetate	pH 1.6	pH 4.0
Fractions 16–21 (catfish eye)	0.32	0.58	3.6	2.1
<i>S</i> -Adenosylhomocysteine	0.31	0.26	2.7	0.6
Fractions 44–55 (catfish eye); <i>S</i> -adenosyl-3-methylthiopropylamine	0.18	0.42	4.5	3.3
<i>S</i> -Adenosylmethionine	0.17	0.20	3.5	1.9

amine was spectrophotometrically determined to be 9.6 μ mol.

Results

The chromatography on CM-Sephadex effectively separated the basic components of the eye extract (Fig. 1). The fractions 44–55 contained *S*-adenosyl-3-methylthiopropylamine, which had been previously isolated from the catfish by another method (Ito & Nicol, 1975). The fractions 25–30 contained the compound C (Ito & Nicol, 1975), which was identified by means of t.l.c. Another basic compound was isolated from the fractions 16–21 and crystallized as the sulphate. The compound gave single spots

on thin-layer chromatograms which could be detected under a u.v. lamp and with ninhydrin and iodoplatinate reagents.

The purified compound exhibited u.v.-absorption spectra in 0.1 M-HCl and 0.1 M-NaOH almost identical with those of adenosine. Acid hydrolysis of the compound in 1 M-HCl at 100°C for 10 min yielded adenine and a compound which reacted with ninhydrin and iodoplatinate sprays, but did not absorb u.v. light. These features were characteristics of *S*-adenosyl-L-homocysteine (Duerre, 1962); however, t.l.c. and paper electrophoresis clearly distinguished the compound from *S*-adenosyl-L-homocysteine (Table 1).

Fig. 2. I.r.-absorption spectra of natural and synthetic *S*-adenosyl-3-thiopropylamine

—, Natural *S*-adenosyl-3-thiopropylamine bisulphate (0.70mg) isolated from catfish eyes; ···, synthetic *S*-adenosyl-3-thiopropylamine bisulphate (1.15 mg); both spectra were taken in KBr pellets.

Fig. 3. Structure of *S*-adenosyl-3-thiopropylamine

The faster migration toward cathode and the co-existence of *S*-adenosyl-3-methylthiopropylamine seemed to indicate that the compound might be *S*-adenosyl-3-thiopropylamine. In fact, t.l.c. and paper electrophoresis in the solvents described in Table 1 showed that the compound and synthetic *S*-adenosyl-3-thiopropylamine migrated at the same rates. Further, the i.r.-absorption spectra of the compound and of synthetic *S*-adenosyl-3-thiopropylamine were identical with each other (Fig. 2). The mixed melting point was 174–176°C (decomp.), and samples of the synthetic *S*-adenosyl-3-thiopropylamine and the compound had m.p. values of 175–176°C (decomp.) and 173–175°C (decomp.) respectively. Accordingly, the basic compound was identified as *S*-adenosyl-3-thiopropylamine. The structure is shown in Fig. 3.

Discussion

The method used previously for the isolation from the catfish of *S*-adenosyl-3-methylthiopropylamine (Ito & Nicol, 1975) was not suitable for the isolation of *S*-adenosyl-3-thiopropylamine, as the latter is not stable in strong acid. Although the method described in the present paper is somewhat

tedious, it allows the isolation of both *S*-adenosyl-3-methylthiopropylamine and *S*-adenosyl-3-thiopropylamine as well as the compound C (Fig. 1). Further, since the method is mild, it is most unlikely that the isolated *S*-adenosyl-3-thiopropylamine is produced as an artifact of the isolation procedure.

This is the first report of the isolation of *S*-adenosyl-3-thiopropylamine from a biological source, although a chemical synthesis has been described by Jamieson (1963). The fact that the compound had not been detected hitherto in any animal tissue seems to indicate that it is present in extremely low quantities or is metabolized very rapidly. The isolated yield of *S*-adenosyl-3-thiopropylamine is 0.24 μmol/g wet wt. of eye; this is considerably higher than the contents of *S*-adenosyl-homocysteine in various tissues (approx. 0.05 μmol/g wet wt. of tissue; Salvatore *et al.*, 1971). It may be that being a strongly acidic compound, the tapetal pigment, which consists of oligomers of 5,6-dihydroxyindole-2-carboxylic acid (Ito & Nicol, 1974), forms *in vivo* a complex with *S*-adenosyl-3-methylthiopropylamine and *S*-adenosyl-3-thiopropylamine and other basic compounds including thiamin and compound C (Ito & Nicol, 1975); this might explain the extremely high content of *S*-adenosyl-3-methylthiopropylamine and *S*-adenosyl-3-thiopropylamine in the eye of the catfish.

Three mechanisms may be considered for the synthesis *in vivo* of *S*-adenosyl-3-thiopropylamine; decarboxylation of *S*-adenosylhomocysteine, demethylation (transmethylation) of *S*-adenosyl-3-methylthiopropylamine, and condensation of adenosine and 3-mercaptopropylamine. It is noteworthy that, *in vitro*, homocysteine methyltransferase uses *S*-adenosyl-3-methylthiopropylamine as a methyl donor (Zappia *et al.*, 1969).

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