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Separation of aminoketones in biological fluids by thin-layer chromatography

There are two physiologically important aminoketones, δ-aminolaevulinic acid (ALA) which is formed enzymically by the condensation of glycine and succinyl coenzyme A[1,2], and aminoacetone (AA) which is produced by the condensation of glycine and acetyl coenzyme A[3-6] or by the oxidation of threonine[5-11]. The enzymes which catalyse the above reactions are δ-aminolaevulinic acid (ALA) synthetase and aminoacetone (AA) synthetase. There is compelling evidence to show that ALA synthetase is the rate-limiting enzyme of the porphyrin biosynthetic pathway in animal, bacterial and plant cells[1,12-20].

The activity of this enzyme is increased in the livers of patients with acute intermittent porphyria[21] and in the livers of animals rendered porphyric by administration of a variety of drugs[22]. ALA-synthetase activity is usually measured by converting the ALA produced by the enzyme during incubation to a pyrrole by condensation with either ethyl acetoacetate or acetylacetone[23]. The pyrrole formed is reacted with modified Ehrlich reagent and the absorbency measured at 552 mμ. Aminoacetone which is present in the liver also reacts with ethyl acetoacetate or acetylacetone to give a pyrrole. Using the method available to separate ALA and AA, the activity of ALA-synthetase and AA-synthetase can be estimated[24]. In the present communication a method is described for the separation and identification of AA and ALA pyrroles by thin-layer chromatography (TLC). This method has been used to separate and identify aminoketones in the biological fluids.

Materials and methods

Table I lists the standard pyrroles[23,25] used and the compounds used to prepare these pyrroles.

A suspension of Silica Gel G (E. Merck, Darmstadt, 30 g in 60 ml of water) was spread on glass plates 20 × 20 cm to a thickness of 250 μ with a Desaga applicator. The plates were dried at 105–110° for 30 min and stored in a desiccator. A 0.1 % solution of the pyrroles in methanol was prepared and 1 mg of each pyrrole was spotted 2 cm from the edge of the plate. The plates were developed with 150 ml of a mixture of 50 % diethyl ether and 50 % *n*-hexane containing 2 % glacial acetic acid[26]. After the solvent had travelled a distance of 12–15 cm (usually 45 min), the plates were taken out, dried and sprayed with Ehrlich reagent[27]. After heating

TABLE I

ALA AND AA PYRROLES USED FOR TLC

Pyrrole	*Prepared from*	*m.p.* (°C)
2-Methyl-3-carbethoxy-4-propionic acid pyrrole	δ-Aminolaevulinic acid and ethyl acetoacetate	163–165°
2-Methyl-3-acetyl-4-propionic acid pyrrole	δ-Aminolaevulinic acid and acetylacetone	193–195°
2,4-Dimethyl-3-carbethoxypyrrole	Aminoacetone and ethyl acetoacetate	75–76°
2,4-Dimethyl-3-acetylpyrrole	Aminoacetone and acetylacetone	135–137°

to 100° for 5 min, the pyrroles appeared as red or violet spots. Results of the above separations are shown in Table II. AA and ALA pyrroles derived from ethyl acetoacetate are separated by this system. Similar separation of AA and ALA pyrroles derived from acetylacetone is also possible.

This procedure has been used for the isolation and identification of aminoketones generated by 17-days old chick embryo livers. Chicks were rendered porphyric by administration of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (DDC) by the RACZ AND MARKS method[28]. DDC (4 mg) was dissolved in dimethylsulfoxide (0.1 mg) and was injected into two 17-day old chick embryos with a sterile 1-in., 21-gauge disposable needle attached to the tip of a graduated 0.2-ml pipette. A small hole was then made in the egg shell above the air sac and the drug injected through the chorioallantois into the fluid surrounding the embryo. The opening of the shell was then covered with cellophane tape and the chick embryo incubated at 38°. At the end of the incubation period (12 h) the embryo was sacrificed and the liver removed, blotted, and weighed. The aminoketones were generated by the MARVER *et al.*[29] method by homogenizing the weighed liver in 3 volumes of 0.9% sodium chloride solution containing 0.5 m*M* EDTA and 10 m*M* Tris, pH 7.4. Aliquots (0.5 ml) of the homogenate were added to two Erlenmeyer flasks (25 ml) containing 1.5 ml of a solution at pH 7.2 of the following constitution: 200 μmoles of glycine, 20 μmoles EDTA and 150 μmoles of Tris·HCl. The two flasks were incubated at 37° for 1 h in a metabolic shaker and 1 ml of 25% trichloroacetic acid added. The precipitates were removed by centrifugation and 2 ml of supernatant added to 1 ml of 1 *M* phosphate buffer pH 6.8 in test tubes (two in number). After adding six drops of 5 *N* NaOH, 0.1 ml of ethyl acetoacetate was added to one tube and 0.1 ml of acetylacetone to the other, the tubes were stoppered and heated at 100° for 7 min. The tubes were cooled and the pH was adjusted to 7. Each one was extracted three times with 3 ml of diethyl ether. The ether layer was washed with water and dried over Na_2SO_4. The diethyl ether was removed under reduced pressure and the residue dissolved in 0.1 ml of methanol. The sample was applied to the plate and developed as described above. The plate was dried and sprayed with Ehrlich reagent[27] and heated to 100° for 5 min. The results are shown in Table III.

From the result it can be seen that the intense spot was due to ALA pyrrole in both cases and the faint spot was due to AA pyrroles.

Recently, ALA-synthetase activity has been measured in liver biopsies[30]

TABLE II

SEPARATION OF AA AND ALA PYRROLES BY TLC

Solvent system used: diethyl ether–*n*-hexane containing 2% glacial acetic acid (1:1).

Pyrrole	$R_F \times 100$	*Colour with Ehrlich*
2-Methyl-3-carbethoxy-4-propionic acid pyrrole	32	Violet
2-Methyl-3-acetyl-4-propionic acid pyrrole	7	Violet
2,4-Dimethyl-3-carbethoxypyrrole	80	Red
2,4-Dimethyl-3-acetylpyrrole	41	Red

TABLE III

SEPARATION OF AA AND ALA PYRROLES DERIVED FROM THE PORPHYRIC CHICK EMBRYO LIVER

Condensation reagent	*No. of spots*	$R_F \times 100$	
Ethyl acetoacetate	2	32 (intense)	80 (faint)
Acetylacetone	2	7 (intense)	41 (faint)

and in cell cultures[31]. In these procedures only a small amount of aminoketones are generated making their separation by solvent extraction or column chromatography impractical. The separation of small quantities of aminoketone is possible using this procedure and therefore may have application in estimation of ALA-synthetase activity of liver biopsies[30] or in cell culture[31].

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1 D. SHEMIN AND C. S. RUSSEL, *J. Amer. Chem. Soc.*, 75 (1953) 4873.
2 J. G. WRISTON, L. LACK, JR. AND D. SHEMIN, *J. Biol. Chem.*, 215 (1955) 603.
3 K. D. GIBSON, W. G. LAVER AND A. NEUBERGER, *Biochem. J.*, 70 (1958) 71.
4 G. KIKUCHI, A. KUMAR AND D. SHEMIN, *Fed. Proc. Fed. Amer. Soc. Exp. Biol.*, 18 (1959) 259.
5 G. URATA AND S. GRANICK, *Biochem. Biophys. Res. Commun.*, 4 (1961) 96.
6 G. URATA AND S. GRANICK, *J. Biol. Chem.*, 238 (1963) 811.
7 W. H. ELLIOT, *Nature*, 183 (1959) 1051.
8 A. NEUBERGER AND G. H. TAIT, *Biochim. Biophys. Acta.*, 41 (1960) 164.
9 W. H. ELLIOT, *Biochem. J.*, 74 (1960) 478.
10 K. D. GIBSON, A. NEUBERGER AND G. H. TAIT, *Biochem. J.*, 83 (1962) 539.
11 D. P. TSCHUDY, F. H. WELLAND, A. COLLINS AND M. RECHCIGL, JR., *Cancer Res.*, 24 (1964) 2033.
12 A. NEUBERGER AND J. J. SCOTT, *Nature*, 172 (1953) 1093.
13 E. I. B. DRESEL AND J. E. FALK, *Nature*, 172 (1953) 1185.
14 D. SHEMIN, T. ABRAMSKY AND C. S. RUSSEL, *J. Amer. Chem. Soc.*, 76 (1954) 1204.
15 D. SHEMIN, C. S. RUSSEL AND T. ABRAMSKY, *J. Biol. Chem.*, 215 (1955) 613.
16 E. I. B. DRESEL AND J. E. FALK, *Biochem. J.*, 63 (1956) 80.
17 J. E. FALK, E. I. B. DRESEL, A. BENSON AND B. C. KNIGHT, *Biochem. J.*, 63 (1956) 87.
18 S. GRANICK, *Science*, 120 (1954) 1105.
19 D. SHEMIN, *Methods Enzymol.*, 5 (1962) 883.
20 B. F. BURNHAM AND J. LASCELLES, *Biochem. J.*, 87 (1963) 462.
21 D. P. TSCHUDY, M. G. PERLOTH, H. S. MARVER, A. COLLINS, G. HUNTER AND M. RECHEIGL, *Proc. Nat. Acad. Sci., U.S.A.*, 53 (1965) 841.
22 S. GRANICK AND G. URATA, *J. Biol. Chem.*, 238 (1963) 821.
23 D. MAUZERALL AND S. GRANICK, *J. Biol. Chem.*, 219 (1956) 435.
24 H. S. MARVER, D. P. TSCHUDY, M. G. PERLOTH, A. COLLINS AND G. HUNTER, JR., *Anal. Biochem.*, 14 (1966) 53.
25 H. FISCHER AND H. ORTH, *Die Chemie des Pyrroles*, Vol. 1, Akad. Verlag. Leipzig, 1934, pp. 185 and 247.
26 M. W. ROOMI, *J. Chromatogr.*, 65 (1972) 580.
27 J. E. FALK, *Porphyrins and Metalloporphyrins*, Elsevier, Amsterdam, 1964, p. 160.
28 W. J. RACZ AND G. S. MARKS, *Biochem. Pharmacol.*, 18 (1969) 2009.
29 H. S. MARVER, D. P. TSCHUDY, M. G. PERLOTH AND A. COLLINS, *J. Biol. Chem.*, 241 (1966) 2803.
30 R. D. LEVERE, E. L. GITEN AND S. SASSA, *J. Lab. Clin. Med.*, 75 (1970) 137.
31 D. L. TYRELL AND G. S. MARKS, unpublished.

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