Kopparbergs Bergslags AB, Svenska Cellulosa AB and Uddeholms AB. This support is gratefully acknowledged.

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Received September 3rd, 1970.

J. Chromatog., 53 (1970) 572–575

CHROM. 5015

The isolation, separation and determination of isopropylnoradrenaline and its O-methyl derivative in blood serum and tissue homogenate

The assay procedure was essentially that of COHEN AND GOLDENBERG[1] commonly applied for the estimation of epinephrine and norepinephrine using the final evaluation of the isoproterenol and O-methylisoproterenol in the form of corresponding adrenolutines. For the estimation in serum the procedure of KAHANE AND VESTERGAARD[2] was modified, and for the determination of the isoproterenol level in tissue homogenates the original procedure of BERTLER *et al.*[3] was applied. The final part of the analytical procedure was the chromatographic separation of isoproterenol and its O-methyl derivative from adrenalin and noradrenalin. For this purpose tandem chromatography on cellulosophosphate and carboxymethylcellulose was used.

Experimental

Isolation of isoproterenol and the corresponding O-methylderivative from plasma. A 15-ml plasma sample was incubated with 0.5 g of aluminium oxide under vigorous shaking for 3 min. The suspension of aluminium oxide was spun off at 700 × *g*, and the sediment was resuspended in an equal volume of double-distilled water and spun off again. The whole procedure was repeated twice. In the last phase the resuspended mixture of aluminium oxide (containing adsorbed catecholamines including isoproterenol and O-methylisoproterenol) were poured into a small column (0.8 × 15 cm) and eluted with distilled water; the eluate was discarded. The catecholamine mixture was eluted with 5 ml of 0.4 *M* acetic acid. The eluate was taken to dryness and repurified by chromatography on a Dowex 50 X2 column. The ion-exchange column was prepared as follows. A column (1.2 × 4 cm) was filled with swollen ion exchanger

(minus 400 mesh) and introduced into the ammonia cycle by subsequent washing with the following series of solvents:

(1) 10 ml of 2 *N* hydrochloric acid;
(2) 5 ml deionized water;
(3) 10 ml of 1 *M* ammonium acetate, pH adjusted to 6.0 by dropwise addition of glacial acetic acid;
(4) 5 ml of deionized water.

The dissolved sample from the first purification step was applied to the Dowex 50 column and the column was eluted twice with 8 ml of 0.4 *N* acetic acid. Before the subsequent run, the column was regenerated by washing in the same series of solvents as indicated above. The eluate from the Dowex column was evaporated to dryness, dried in a desiccator overnight, dissolved in 0.5 ml of 0.05 *M* ammonium acetate, the pH value of which had been adjusted before to 6.1 by addition of glacial acetic acid. This sample was subjected to the final chromatographic separation as described below.

Isolation of isoproterenol and the corresponding O-methylderivative in a heart tissue homogenate. Pooled hearts of five albino rats were homogenized in 30 ml of 0.4 *M* perchloric acid. The homogenate was spun off and the sediment reextracted under identical conditions. Pooled extracts were stored overnight at —20°. The next morning, the pH of this sample was adjusted by 5 *N* potassium carbonate to 4.0 and the precipitate of potassium perchlorate was spun off at a temperature not exceeding 0°. The precipitate was quickly washed on a Buechner funnel and both the supernatant and the washings were used for the further assay. An aliquot containing 150–500 μg of catecholamines each was applied to the Dowex 50 column (see previous paragraph), and catecholamines were eluted by 8 ml of 1 *N* hydrochloric acid. The flow rate in the Dowex column did not exceed 0.25 ml/min. The eluate was, as in the case of plasma samples, evaporated to dryness and prepared for further analysis by dissolving it in 0.5 ml 0.05 *M* ammonium acetate (pH 6.1).

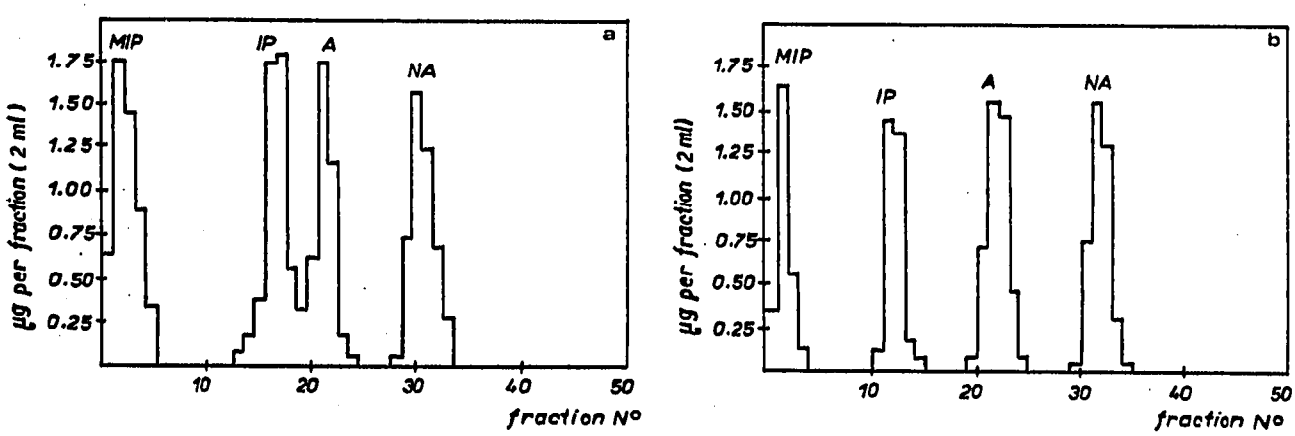

Fig. 1. The chromatographic profile of the mixture of O-methylisopropylnoradrenaline (MIP), isopropylnoradrenaline (IP), adrenaline (A) and noradrenaline (NA) on cellulosophosphate (a) and on the tandem system of cellulosophosphate and carboxymethylcellulose (b). For experimental details see text.

Separation of isopropylnoradrenaline and the corresponding O-methyl derivative using a tandem chromatographic procedure on cellulosophosphate and carboxymethylcellulose. For the separation of both catecholamines from epinephrine and norepinephrine two columns 0.6 × 40 cm combined in series were used. These columns were filled up to 35 cm with cellulosophosphate Whatman CP 11 (Column No I) and carboxymethyl cellulose Whatman CM 32 (Column No II). Columns were eluted by a linear gradient of ammonium acetate buffer with a concentration change from 0.05 to 0.25 *M*. The pH of the buffer was adjusted previously to 6.1; flow rate on both columns was less than 0.8 ml/min. Fractions of 2 ml were collected. Columns were loaded with 0.5 ml of a sample obtained after either purification procedure. The elution of the column was stopped after 100 ml of the eluant had passed through the system. Under these conditions *O*-methylisopropylnoradrenaline is eluted in fractions No. 3–7, isopropylnoradrenaline in fractions No. 10–15 (see Fig. 1).

Oxidation of isopropylnoradrenaline and its O-methyl derivative to the corresponding lutines. Combined fractions 3–7 and 10–15 were evaporated to a final volume of 0.2 ml, diluted to 1 ml with borate buffer (0.66 *M*, pH 7) and the following reagents were added subsequently: 0.05 ml of 0.02 % $CuCl_2 \cdot 2H_2O$ and 0.05 ml of 0.25 % potassium ferricyanide. The reaction was stopped after 3 min by adding 0.05 ml of 10 % BAL in 25 % formaldehyde. After 10–20 sec 0.2 ml of 10 *N* NaOH was added, and after another 5 min 0.15 ml of glacial acetic acid was pipetted into the sample. The reaction mixture was vigorously mixed and subjected to fluorimetric evaluation.

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Received August 31st, 1970

CHROM. 5027

Separation and determination of cobalamins on an SP-Sephadex column

In a previous paper[1] we have described the separation of a mixture of methylcobalamin, cobamamide, cyanocobalamin and hydroxocobalamin by means of successive chromatograms carried out on CM-cellulose and Dowex 50 W-X2 columns. The present note describes a simple method of separating the above derivatives of vitamin