Method for Isolation and Determination of α-Methyl DOPA Ethyl Ester in Aqueous Solution

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The ethyl ester of α-methyl-3,4-dihydroxyphenylalanine can be quantitatively separated from the free acid by column partition chromatography. Best results have been achieved by using a pH 4.5, 0.1 *M* citrate buffer adsorbed on powdered cellulose as the stationary phase and *n*-butanol as the mobile phase. If the eluate is analyzed colorimetrically using a reagent specific for intact catechol nuclei, interference by any oxidized ester is avoided. The method has been found useful for accelerated stability studies and provides recoveries averaging 99 per cent of theory.

IN AQUEOUS solution the ethyl ester of α-methyl-3,4-dihydroxyphenylalanine (α-methyl DOPA ethyl ester) is subject to two modes of degradation. The ester function may hydrolyze to yield the free amino acid, and the catechol nucleus is, as would be expected, quite prone to oxidation. In view of the two degradative pathways, initial study of the stability of the ester in aqueous solution did not seem amenable to straightforward functional group analysis. Instead, it seemed most desirable to attempt to preferentially isolate the totally intact ester and to conveniently determine its concentration by simple ultraviolet spectrophotometry.

In the event that such complete separation was found too difficult or time consuming, it was felt that isolation of the oxidized and unoxidized ester *together* would be entirely satisfactory if followed by an analytical procedure specific for the intact catechol nucleus.

The main problem, then, concerned separation of the intact or oxidized ester from any hydrolysis products. Toward this end, partition chromatography using suitably buffered and stabilized columns seemed a desirable approach.

Preliminary experiments indicated that successful separation of the ester could be achieved by using columns buffered at a pH of about 5. It was quite evident that the stationary phase should preferably contain both sodium bisulfite and ethylenediamine tetraacetic acid to prevent oxidation of the ester during column residence. Separation of the ester was most easily achieved by elution with *n*-butanol saturated with buffer.

The choice of a holding material was somewhat vexing in that silicic acid columns gave a very slow rate of flow. While columns prepared with Celite 545[1] seemed quite suitable, it was soon apparent that the trace metal contaminants in the Celite promoted oxidation of the ester. Fortunately, columns prepared with powdered cellulose were found entirely acceptable if stored no longer than one week prior to use.

EXPERIMENTAL

Reagents.—pH 4.5, 0.1 *M* citrate buffer; chloroform, Merck reagent; *n*-butanol, Merck reagent; sodium bisulfite; ethylenediamine tetraacetic acid; and powdered cellulose, chromatography grade.

Apparatus.—Chromatographic columns, 40 cm. long × 2 cm. i.d., constricted at the end and provided with a delivery tube to fit into the neck of a 100-ml. volumetric flask.

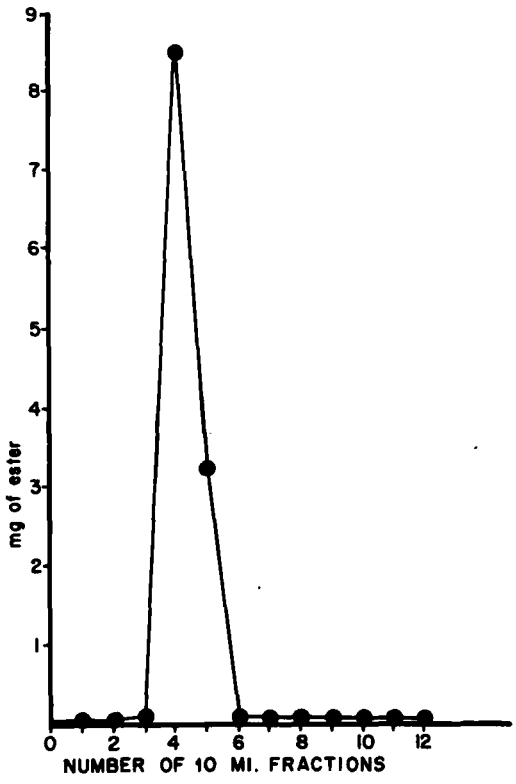

Fig. 1.—Chromatogram showing the elution of α-methyl DOPA ethyl ester from a cellulose column with *n*-butanol.

Procedure.—Sixteen grams of powdered cellulose was mixed mechanically with *ca.* 150 ml. chloroform. Twelve milliliters of buffer previously equilibrated against *n*-butanol and containing 0.35% sodium bisulfite and 0.05% ethylenediamine tetraacetic acid was then added to the cellulose-chloroform mixture with vigorous agitation. The

Received April 25, 1962, from the Pharmaceutical Research Department, Merck Sharp and Dohme Research Laboratories, West Point, Pa.

Accepted for publication August 29, 1962.

The authors wish to express their thanks to Mr. Donald Rodgers for making the details of the colorimetric method available and for supplying the reagent.

[1] Tradename for mineral filler marketed by Johns-Manville Sales Corp., New York, N. Y.

resulting slurry was packed into a column incrementwise using moderate pressure.

Prior to adding the sample, the column was washed with 50 ml. *n*-butanol previously equilibrated against buffer. When the last traces of butanol had entered the column, 1 ml. of a solution of α-methyl DOPA ethyl ester (5–10 mg./ml.) in water or buffer was added to the column and allowed to drain into the body of material. Five milliliters of *n*-butanol was then added to wash the walls of the column and followed by a second 5 ml. of *n*-butanol.

Twenty-five milliliters of *n*-butanol saturated with buffer was then added, and a constant head maintained on the column. At this point, collection of the effluent was started and either small fractions or gross volumes were collected.

Initially all assays were done spectrophotometrically by diluting the effluent with 0.1 *N* methanolic hydrochloride. Subsequently, when it appeared that small amounts of oxidation products were being eluted, all determinations were made by a modification of the procedure of Heinrich and Schuler (1). The procedure used was: the eluate, collected in a volumetric flask, was diluted with 5 *M* acetic acid to provide a concentration of 10 to 15 mcg. of ester per ml. A 5-ml. aliquot of this dilution was transferred to a 25-ml. volumetric flask along with 5 ml. of a 0.6% solution of 4-nitro-2-chloro-1-diazophenyl-β-naphthylsulfonic acid in 5 *M* acetic acid. The mixture was brought to volume with 5 *M* acetic acid and heated at 35° for 1 hour. The flasks were removed from the constant temperature bath, cooled rapidly, and read at 410 mμ *vs.* a suitable reagent blank. In the concentration range employed, all colors showed excellent adherence to Beer's law.

The reagent in 5 *M* acetic acid is stable for at least a week if kept cold. The partition columns should not be kept longer than 1 week.

DISCUSSION OF EXPERIMENTAL RESULTS

When 10 mg. each of α-methyl DOPA ethyl ester and the free amino acid were applied to separate cellulose columns and the effluent collected in 10-ml. fractions, no elution of the free acid could be detected after a total of 25 fractions had been assayed (ultraviolet). In contrast, elution of the ethyl ester was very easily accomplished in a reasonably narrow band as shown in Fig. 1. Although it has been found consistently that all of the ester is eluted in the third through the sixth fraction, we found it simpler to initiate collection of the effluent in a 100-ml. volumetric flask and to collect a total of 100-ml. of effluent. The slight dilution caused by collection of the larger volume is insignificant in view of the high sensitivity of the colorimetric method.

The recoveries of α-methyl DOPA ethyl ester obtained by the method outlined are given in Table I. It is evident from these data that recoveries are well within the limits desired for stability studies and that both the precision and accuracy are satisfactory.

TABLE I.—RECOVERY OF α-METHYL DOPA ETHYL ESTER AFTER PARTITION CHROMATOGRAPHY AND COLORIMETRIC ASSAY

Mg. Added	Mg. Found	% Theory Found[a]
2.56	2.60	102
5.05	4.95	98
9.84	9.84	100
10.00	9.95	99.5
10.67	10.13	95

[a] Average recovery = 98.9% of theory.

Subsequent experiments, in which known quantities (*ca.* 5 mg. each) of α-methyl DOPA ethyl ester and the free acid were chromatographed on single columns, showed the amino acid to have no effect on elution of the ester. Similarly, artificially degraded solutions of α-methyl DOPA ethyl ester (air oxidation at 60°) showed substantial loss after chromatography and colorimetric assay.

REFERENCES

(1) Heinrich, P., and Schuler, W., *Helv. Chim. Acta*, 31, 320(1948).