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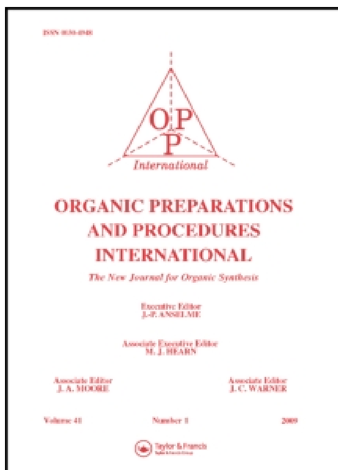

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SIMPLE METHODOLOGY FOR THE PURIFICATION OF AMINO ACIDS

Antonio José Mota[a]; Elena Castellanos[b]; Eusebio Juaristi[b]
[a] Departamento de Química Orgánica, Facultad de Farmacia, Universidad de Granada, Granada, SPAIN [b] Departamento de Química, Centro de Investigación y de Estudios Avanzados del IPN, México, D.F., MEXICO

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SIMPLE METHODOLOGY FOR THE PURIFICATION OF AMINO ACIDS

Submitted by (09/23/02) Antonio José Mota,[†*] Elena Castellanos[††] and Eusebio Juaristi.[††*]

[†] *Departamento de Química Orgánica, Facultad de Farmacia Universidad de Granada, Campus Cartuja 18071-Granada, SPAIN*
[††] *Departamento de Química, Centro de Investigación y de Estudios Avanzados del IPN, Apartado Postal 14-740, 07000 México, D.F., MEXICO*

Amino acids are among the most important substances for life and are present in all living organisms. They are the basic constituents of proteins, which participate in a large number of biochemical processes. Indeed, with the completion of the human genome sequence, the next major scientific goal is the determination of the structure and function of all proteins of biological relevance.[1] This effort requires, among others things, the specific preparation of both natural and unnatural amino acids, as well as their coupling in the synthesis of model peptides and proteins and remarkable activity is presently under way in this area.[2-4] In this context, the synthesis and purification of amino acids continues to present a significant challenge to synthetic chemists. While ionic exchange resins are still one of the most reliable techniques especially for large-scale preparations,[5] on occasion polar contaminants in reaction mixtures may cause interference that complicates the detection and purification (impurities may go unnoticed). Indeed,

adsorption of the amino acid to the resin may be quite strong, leading to low yields. Because of these drawbacks, alternative processes are being developed.[6] For the analytical detection of amino acids, silica gel thin-layer chromatography with propanol-aqueous ammonia as eluent has been described.[7] We now report that adaptation of this technique turns out to be equally efficient for the isolation and separation of amino acids on a preparative scale. Furthermore, the implementation of this simple methodology allows not only the isolation, but also the separation of amino acids mixtures in high purity and good recovery. These results compare favorably with the use of ion-exchange resins and represent practical procedures for the work-up of these substances.

EXPERIMENTAL SECTION

The applied methodology consists in the adsorption of the crude material in a chromatographic column packed with normal silica gel (70-230 mesh). The eluent employed consists of a mixture of isopropanol-methanol-NH_3 in ratios that vary from 0:1:1 (for very polar substances) to 9:1:0.5 (for samples of lower polarity). It is advisable to select the appropriate eluent polarity in thin layer assays. The indicated highly polar solvent mixture prevents retention of the amino acid on the silica. The employment of NH_3 (ammonium hydroxide) is essential since it forms an ammonium salt with the carboxylic group, while keeping the amino group(s) in a neutral state. Under these conditions, amino acids move easily through the column, to be then isolated by removal of solvents at moderate temperature and reduced pressure, in order to eliminate ammonia. In this way, the isolation and characterization of several amino acids in pure form has been possible. Thin layer chromatographic detection is carried out by means of a ninhydrin spray solution (where amino acids appear in colors from rose to violet). This solution was prepared as follows: to 95 mL of absolute ethanol, 5 mL of concentrated H_2SO_4 was added. The resulting solution was treated with 100 mg of ninhydrin and the mixture was stirred until complete solution. The resulting solution must be, preferably, kept away from the light.

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Determination of Adsorption Coefficients for Representative Amino Acids.- In order to establish the degree of "irreversible" retention of typical amino acids on the silica gel under the conditions studied, 50 mg of commercial amino acids was applied to a chromatographic column (1.0 cm diameter) containing 5.0 g of 70-230 mesh silica gel. Elution with isopropanol-methanol-ammonia (3:2:1) afforded the recovered amino acid, whose adsorption coefficient was calculated as shown below.

$$C_{ads} = \left(1 - \frac{\text{recovered sample}}{\text{initial sample}}\right) * 100$$

In this fashion, C_{ads} = 0 corresponds to negligible adsorption (zero loss) of amino acid, whereas C_{ads} = 100 represents complete retention (total loss) of the amino acid. *Table 1* collects the observed C_{ads} values for a series of 10 commercial amino acids. It can be seen that all samples were recovered with no appreciable loss.

Table 1. Adsorption Coefficients (C_{ads}) for Ten Commercial Amino Acids[a]

Amino Acid (50 mg)	R_f [b]	Recovered sample (mg)	C_{ads}
DL-Alanine	0.28	49.6	0.8
DL-3-Aminobutyric Acid	0.22	49.5	1.0
L-Asparagine	0.40	49.3	1.0
Glycine	0.23	49.5	1.0
DL-Phenylalanine	0.62	49.5	1.0
DL-a-Phenylglycine	0.63	49.8	0.4
L-Proline	0.24	49.2	1.6
L-Tyrosine	0.53	48.9	2.2
L-Threonine	0.24	49.3	1.4
L-Valine	0.35	48.1	3.8

a) Adsorbed on silica gel (70-230 mesh) and eluted with *i*-PrOH/CH_3OH/NH_3 (3:2:1). b) These R_f values were determined from silica gel (TLC aluminium sheets, silica gel 60 F_{254}) plates. These R_f values are in accordance with the retention time from column elution.

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Separation of Amino Acid Mixtures.- Several mixtures containing both α- and β-amino acids (1:1 by weight) were prepared and adsorbed on silica gel (70-230 mesh). Twenty five miligrams of each of the two amino acids was used, and adsorbed on a chromatographic column (1.0 cm of diameter, 20 cm of length), packed with 8.0 g of silica. The samples were eluted with isopropanol-methanol-ammonia to afford complete separation of the amino acids, in nearly quantitative recovery (*Table 2*).

Table 2. Separation of Three Amino Acid Mixtures Adsorbed on Silica Gel (70-230 mesh)[a]

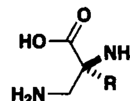
Mixture of Amino Acids A and B	Eluent	Recovered A (mg)	Recovered B (mg)
DL-3-Aminobutyric acid and L-Proline	5:2:1	24.3	24.8
DL-Phenylalanine and L-Threonine	5:2:1	24.9	24.5
L-Asparagine and L-Tyrosine	3:2:1	24.4	24.8

a) Eluted with *i*-PrOH/CH_3OH/NH_3.

Separation and Purification of α-β-Diamino Acids.- Several α-β-diamino acids prepared in our research group were purified by means of the procedure described in this paper [ca. 60-80 mg of amino acid, 10 g of 70-230 mesh silica, 1.0 cm diameter and 30 cm long chromatography column, *i*-PrOH/CH_3OH/NH_3 (3:2:0.5) eluent]. *Table 3* summarizes the experimental details.

Table 3. Purification of Three α,β-Diamino Acids by Adsortion on Silica Gel (70-230 mesh)

R	Initial sample (mg)	Isolated diamino acid (mg)	Recovery (%)
Me	58.0	52.0	90
Et	76.5	57.4	75
Bn	86.2	25.4	30[b]



a) Elution with *i*-PrOH/CH_3OH/NH_3 (3:2:0.5). b) The low yield in this case may be due to the crude sample being contaminated with material that originates from side reactions under the conditions of the preparation procedure.

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