PREPARATION OF ARGININOL*

by

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In the course of a study of the terminal amino acid of salmine by reduction of the free carboxyl group with $LiAlH_4$ and chromatographic identification of the amino alcohol formed thereby, according to the method of FROMAGEOT, JUTISZ, MEYER, AND PENASSE[1], it was necessary to know the behaviour of argininol. This compound has not yet been reported in the literature and when its preparation was attempted by direct reduction of the esterified amino acid as has been done with the neutral amino acids[2], difficulties were encountered, owing to the instability of the guanido group. It was decided therefore to protect the guanido group by benzoylation prior to ester formation and reduction. Dibenzoyl-arginine ethyl ester hydrochloride was prepared according to the indications of FELIX AND DIRR[3].

EXPERIMENTAL

Benzoylation. 4.5 g L(+)-Arginine was dissolved in water and treated with 15 ml benzoyl chloride. 10% NaOH was added gradually to keep the solution about pH 11 during the 3 h reaction period. The mixture was stirred constantly and maintained at 0°. Dibenzoyl-arginine precipitated when the solution was adjusted to pH 4–5. It was recrystallized as fine needles by dissolving in dilute NaOH and readjusting to pH 7. The crystals were washed with water and ether. 4.2 g was obtained. The product was then recrystallized as the hydrochloride by heating briefly in 15% HCl. M.P. 215° C.

Esterification. 1.8 g of the dibenzoyl-arginine hydrochloride was suspended in 150 ml absolute alcohol and treated with dry HCl. Evaporation of the solvent *in vacuo* resulted in a syrupy mass. After the second esterification and concentration, a crystalline product formed. It was recrystallized from alcohol and ether. The yield was 1.5 g. M.P. 142° C.

Reduction. The ester (1.5 g) was dissolved in 150 ml anhydrous tetrahydrofuran by warming to 40°. 1.05 g $LiAlH_4$ was added and the reduction allowed to proceed in a nitrogen atmosphere at room temperature with stirring for 2 h. The excess reductor was decomposed with water and the solution adjusted to pH 5 with H_2SO_4. The solvent was removed by concentration *in vacuo*. The residue, which consisted of a white precipitate and a yellow oil, was evaporated to dryness in a dessiccator over P_2O_5, and then made slightly basic with anhydrous $Ba(OH)_2$. Dibenzoyl-argininol was extracted with 3

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portions of absolute alcohol at 40°. To remove any mineral salts which had dissolved in the alcohol, the solution was treated with 3 volumes of absolute ether, left in the cold room overnight, and then filtered. The filtrate was concentrated *in vacuo* to a syrup.

Hydrolysis of benzoyl groups. The dibenzoyl-argininol was not isolated from this fraction but was hydrolyzed directly by refluxing with 20% H_2SO_4 for 22 h. The aqueous solution was treated with $Ba(OH)_2$ to precipitate quantitatively SO_4^{-2}. The clear supernatant which resulted after centrifugation was concentrated *in vacuo*.

Isolation of argininol dipicrate. Attempts to crystallize argininol as the hydrochloride and oxalate were unsuccessful so it was decided to isolate it as the picrate. A portion of the concentrated solution was treated with an alcoholic solution of picric acid. An orange oil separated immediately but after the solution had been left in the refrigerator for several hours clusters of yellow crystals formed. When these crystals were dissolved in warm water there was again the formation of a small amount of oily substance. This was not observed, however, in the two subsequent crystallizations. The 4 times recrystallized picrate was washed with cold water and extracted several times with ether to remove any free picric acid. The crystals were dried *in vacuo* over P_2O_5 at 56° for 7 hours. The melting point taken by means of an electrically heated block was 168° C uncorrected.

Analysis.

$C_{18}H_{22}O_{15}N_{10} = 618.4$

Calc.	C 34.96%	H 3.59%	N 22.65%
Found	C 35.08%	H 3.72%	N 22.68%*

Analyses of argininol liberated from the picrate. 140 mg 3 times recrystallized picrate was hydrolyzed in 2 ml 6 *N* HCl for 1 h in a boiling water bath. Picric acid was removed by 5 ether extractions. The last 2 extractions were colourless. The picric-acid-free, aqueous solution of argininol was analysed for total N (Kjeldahl), NH_3 liberated by action of periodic acid, and total argininol (Table I): The action of periodic acid (20 min. at room temperature) on argininol should give 1 molecule of NH_3 per molecule of amino alcohol, resulting from the amino group adjacent to a C-atom bearing an alcoholic group oxidizable by the periodic acid[1,4,5]. As no NH_3 is formed from arginine under these conditions, this determination served to exclude the possibility of unreduced arginine. Total argininol was determined colorimetrically by the method of SAKAGUCHI[6]. It was assumed that the coloration given by 1 mole of argininol would be equal to that given by 1 mole of arginine (used as standard) since the reaction involves the guanido group.

TABLE I

mg ARGININOL FROM 140 mg PICRATE, AS RESULTING FROM DIFFERENT DETERMINATIONS

Determination	*Mg Argininol*
N total	26.3
N periodic	25.4
Colorimetry	25.2
Mean:	25.6
Theoretical value for argininol derived from 140 mg dipicrate:	36.2

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The results show only 71% recovery when referred to the original quantity of dipicrate hydrolysed. This loss was probably due to solubility of argininol in the ether extractions of picric acid. However, the ratios between the values obtained by the 3 methods are very close to those calculated for argininol.

Chromatography. A portion of the solution was subjected to paper chromatography to determine if argininol could be separated from arginine by this method. Whatman No. 1 paper was used and the solvent system was pyridine + collidine + water in proportion by volume 60:20:20. The experiment was run at room temperature for 26 h in which time the solvent front advanced 40 cm. The chromatograms were run in duplicate, one being revealed with ninhydrin and the other by the SAKAGUCHI reaction adapted to paper. The same spots were revealed by these two methods as both substances involved were ninhydrin- and Sakaguchi-positive.

Results.

1. arginine R_F 0.12
2. argininol R_F 0.35
3. arginine + argininol: 2 spots observed; positions corresponding to those found when each compound was run separately.

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SUMMARY

Argininol was prepared by reducing dibenzoylarginine ethyl ester with $LiAlH_4$ in tetrahydrofuran. Crystalline argininol dipicrate was obtained. An aqueous solution of free argininol (base) prepared by decomposition of the dipicrate was analysed for total N, periodic N, and SAKAGUCHI N. The ratios between the values obtained by the three methods are very close to those calculated for argininol. Argininol was separated by paper chromatography from arginine and its R_F was determined in the pyridine + collidine + water solvent system.

RÉSUMÉ

L'argininol a été préparé par réduction de l'ester éthylique de la dibenzoyl-arginine par $LiAlH_4$ dans le tétrahydrofurane. Le dipicrate d'argininol a été obtenu à l'état cristallisé. La base libre a été obtenue en solution aqueuse. Elle a été caractérisée par les dosages suivants: N total, N periodique, N SAKAGUCHI. L'argininol a été séparé de l'arginine par chromatographie sur papier et son R_F a été déterminé dans un solvant pyridine + collidine + eau.

ZUSAMMENFASSUNG

Das Argininol wurde durch Reduktion des Dibenzoylargininethylesters mit $LiAlH_4$ in Tetrahydrofuran hergestellt. Es wurde kristallisiertes Argininoldipikrat erhalten. Eine wässrige Lösung von freier Argininol-Base wurde durch Zersetzung des Dipikrats hergestellt. Die freie Argininol-Base wurde analysiert auf Total-N, Stickstoff, der durch Oxydation mit Periodsäure als NH_3 freigemacht wird und SAKAGUCHI-N. Die drei N-Werte, die nach den angegebenen Methoden der Stickstoffbestimmungen erhalten wurden, stimmten sehr gut mit den für Argininol berechneten überein. Argininol wurde vom Arginin durch Papierchromatographie getrennt und sein R_F im Lösungssystem Pyridin + Collidin + Wasser bestimmt.

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