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LETTERS TO THE EDITORS

A Synthesis of L-Arginyl-L-Arginine

The dipeptide arginylarginine is one of the last remaining peptide sequences as yet not synthesized. As the first step in this direction, L-arginine was converted to N^{α},N^{ω},N^{ω}-tricarbobenzoxy-L-arginine (1), and this compound then coupled in chloroform solution in the presence of ethyl chloroformate and anhydrous triethylamine with N^{ω}-carbobenzoxy-L-arginine benzyl ester (2) to yield N^{α},N^{ω},N^{ω}-tricarbobenzoxy-L-arginyl-N^{ω}-carbobenzoxyarginine benzyl ester (2). After three crystallizations from ethyl acetate, the latter compound has been found to melt at 160° (instead of the lower value previously reported (2)). Calculated for $C_{51}H_{57}N_8O_{11}$: C, 64.0; H, 6.0; N, 11.7; found: C, 63.9; H, 5.9; N, 11.6; $[\alpha]_D = +3.0°$ (c = 2.5, chloroform) at 25°.

In methanol–acetic acid solution and in the presence of freshly prepared palladium black, the coupling product was hydrogenated to completion of the reaction. Evaporation *in vacuo* yielded a noncrystallizable oil, which gave a single ninhydrin-positive spot on paper in two solvent systems. The first was formic acid–water–*tert.* butanol (15:15:70) in which the R_f was 0.11, as compared with a value of 0.20 for L-arginine under the same conditions. The second solvent was methanol–water–pyridine (40:10:1) in which the R_f was 0.09, as compared with the value of 0.19 for L-arginine. On hydrolysis in 5 *N* HCl at 121° and 15 lb. pressure for 2.5 hr., the hydrogenation product yielded a single spot in the two solvent systems which was indistinguishable from that obtained with an authentic sample of L-arginine treated under the same conditions of heating and acidity as the hydrogenation product.

Treatment of the aforesaid sirupy residue with flavianic acid produced a crystalline diflavianate dihydrate with m.p. 230–234°. Calculated for $C_{12}H_{26}O_3N_8 \cdot 2[C_{10}H_4OH(NO_2)_2SO_3H] \cdot 2H_2O$: C, 38.6; H, 4.2; N, 16.9; found: C, 38.3; H, 4.4; N, 17.0. The melting point of L-arginine monoflavianate is 260°. A crystalline salt of the preparation was also obtained with picrolonic acid, the analysis of which was consistent with the formulation of a tripicrolonate; m.p. 290–295° (the monopicrolonate of L-arginine melts at 238°). Calculated for $C_{42}H_{50}N_{20}O_{18}$: C, 44.9; H, 4.5; N, 24.9; found: C, 44.4; H, 4.7; N, 24.5 (Dumas, and Kjeldahl after reduction); $[\alpha]^{24}_{546} = +32.4°$ (c = 1, dimethylformamide).

On the basis of the chromatographic and analytic evidence, it is concluded that the compound prepared by the synthetic procedure described above, is arginylarginine.[1]

[1] Our former associate, Dr. Nobuo Izumiya of Kyushu University, Japan, has notified us that he has succeeded in preparing chromatographically pure arginylarginine by the coupling of carbobenzoxynitroarginine with nitroarginine ethyl ester followed by saponification and catalytic hydrogenation.

REFERENCES

1. ZERVAS, L., WINITZ, M., AND GREENSTEIN, J. P., *Arch. Biochem. Biophys.* **65,** 573 (1956).
2. ZERVAS, L., WINITZ, M., AND GREENSTEIN, J. P., *J. Org. Chem.* **22,** 1515 (1957).

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Formation of α-Amino-γ-Butyrolactone from *S*-Adenosylmethionine[1]

S-Adenosylmethionine has been recognized as a donor and precursor of several important biochemical groups (1). Homoserine and 5′-methylthioadenosine are derived from it on hydrolysis (2). Recently it has been observed in this laboratory that cell-free preparations of *Aerobacter* metabolize *S*-adenosylmethionine and accumulate a product which after paper chromatography gives a brown color with the ninhydrin spray (3). This compound is also formed by chemical splitting of *S*-adenosylmethionine in slightly acid solution. Attempts to identify it seemed warranted for a better understanding of the mechanism of degradation of the adenosyl sulfonium compound.

A sample of *S*-adenosylmethionine containing 15 μmoles/ml. was adjusted to pH 4. The solution was heated at 100° for 20 min. Aliquots were chromatographed on Whatman No. 1 filter paper using butanol, acetic acid, water (60:15:25, v/v) as the developer. The papers were examined for ultraviolet quenching reaction and sprayed with ninhydrin. Duplicates were treated with the chloroplatinate reagent. Sulfur and adenine were accountable only as 5′-methylthioadenosine. A small amount of homoserine was present, and a brown ninhydrin product which contained no adenine or sulfur was evident at R_f 0.34.

The main portion of the hydrolyzate was chromatographed in bands on Whatman No. 1 paper and the unknown material eluted. Since the compound appeared to be related to homoserine, the eluate was heated at 100° and aliquots were removed at intervals and chromatographed. Homoserine was found to accumulate as the other material disappeared in the course of the heat treatment.

The homoserine precursor was found also on hydrolysis of *S*-adenosylethionine (4), *S*-ribosylmethionine, and to a lesser degree from *S*-methylmethionine. Methionine and dimethyladenosylthetin do not yield this compound when treated similarly. Thus it became clear that the unknown homoserine precursor was derived from the amino acid moiety of the *S*-adenosylmethionine. Under the conditions of

[1] This work was performed under the auspices of the U. S. Atomic Energy Commission.