CHEMISTRY

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1957-01-01T00:00:00+00:00

SovietRxiv

Abstract

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ON CERTAIN PRODUCTS OF THE ELECTROREDUCTION OF GRAMICIDIN C

(Presented by Academician A. N. Nesmeyanov, May 7, 1957)

The idea that gramicidin C contains a diketopiperazine ring consisting of proline and phenylalanine was obtained ((⁀{1})) indirectly—from the decrease in amino nitrogen by 2 amino groups in the hydrolysate of reduced gramicidin C as compared with the hydrolysate of the unreduced compound. We considered it important to isolate from the reduction products 1,2-trimethylene-5-benzylpiperazine, derived from *d*-phenylalanyl-*l*-proline anhydride.

We reduced 1 g of gramicidin C; however, it was not possible to detect the desired piperazine. A base was isolated which proved to be *d*-phenylalaninol (α-benzyl-α-aminoethanol). N. I. Gavrilov and A. V. Koperina ((⁀{2})) observed the reducibility of linear dialkylamides of phenylsuccinic acid, but the reaction products were not studied by them in detail. For the present we refrain from interpreting the reasons for the formation of the amino alcohol during the electroreduction of gramicidin C; apparently, this is the principal direction of the reaction, since phenylalanine disappears completely, and one *d*-phenylalaninol is found among the reduction products.

Experimental Part

For electroreduction we used electrophoretically homogeneous gramicidin C, recrystallized twice from alcohol and once from a chloroform–petroleum ether mixture.

Electroreduction of gramicidin C. Gramicidin C was reduced by the method described previously ((⁀{1})), at a temperature not higher than 25°. After reduction, the catholyte was evaporated in vacuo at 30–35°; the residue was dissolved in a small amount of water and extracted with methylene chloride to remove unreduced gramicidin C. The aqueous solution was evaporated in vacuo; the residue was a hygroscopic substance, m.p. 200–210° (with decomposition). In portions, 1 g of gramicidin C was reduced. An electrochromatogram of the reduced gramicidin C was run in 30% acetic acid at a potential gradient of 6.8 cm for 5 hr. On development with benzidine and ninhydrin, one intense spot shifted 7 cm from the point of application toward the cathode was found, as well as several less intense spots shifted toward the cathode by 4, 5, and 10 cm.

Chromatographic study of the hydrolysate of reduced gramicidin C. 100 mg of reduced gramicidin C was hydrolyzed with 10 ml of 20% HCl for 42 hr; the hydrolysate was evaporated in vacuo, the residue was dissolved in 2 ml of water and chromatographed on Leningrad chromatographic paper No. 2 in the solvent system butanol—water—acetic acid in the ratio 4 : 5 : 1. A hydrolysate of gramicidin C was used as a reference. Four chromatograms

developed with benzidine, isatin, and a 0.4% solution of ninhydrin in methanol at room temperature and at 105°.

a. On development with ninhydrin at room temperature, the following amino acids were detected: ornithine ($R_f = 0.06$), proline ($R_f = 0.28$), valine ($R_f = 0.47$), leucine ($R_f = 0.64$). Proline appeared as an intense yellow spot. Phenylalanine is absent.

b. On development with ninhydrin at 105°, the amino acids ornithine, proline, valine, leucine, and one more new substance with $R_f = 0.75$ were detected. Phenylalanine is absent.

c. On development with isatin ([3]), in the solution under study and in the hydrolysate of gramicidin C, proline appears as intense blue spots.

d. On development with benzidine ([4]), spots of approximately equal color intensity were detected, corresponding to ornithine, proline, valine, leucine, and a spot with $R_f = 0.76$. Phenylalanine is absent.

Isolation of an organic base from the hydrolysate of reduced gramicidin C. 1 g of reduced gramicidin C was hydrolyzed with 50 ml of 20% HCl for 42 hours. The hydrolysate was evaporated, the residue was dissolved in a small amount of water, made alkaline, and extracted 5 times with distilled methylene chloride (10 ml each). The methylene chloride was washed with 3 ml of water, dried over fused KOH, and the solvent was distilled off in vacuo. A white crystalline substance with a faint amine odor remained, weight 100 mg. It was recrystallized from dry methylene chloride with addition of low-boiling petroleum ether. Weight 60 mg, m.p. 90-91°. The m.p. of *d*-phenylalaninol, according to the literature, is 91.5° ([5]).

$C_9H_{13}NO$.	Found, %:	N 9.25
	Calculated, %:	N 9.25

The acid oxalate was obtained by precipitation from alcohol with an ethereal solution of oxalic acid, m.p. 161-162°. According to the literature, for the acid oxalate of *l*-phenylalaninol the m.p. is 161-163° ([6]).

$C_{11}H_{15}NO_5$.	Found, %:	C 54.98; H 6.41; N 6.12
	Calculated, %:	C 54.77; H 6.26; N 5.87

The isolated *d*-phenylalaninol is soluble in alcohol, chloroform, and ether, insoluble in petroleum ether; its acid oxalate is insoluble in alcohol, soluble in water; on treatment with periodic acid they liberate ammonia, which was detected with Nessler's reagent, this being a qualitative reaction for α-aminospirits ([7]). The isolated *d*-phenylalaninol is chromatographically homogeneous and has $R_f = 0.75$. On the electrochromatogram in 30% acetic acid at a potential gradient of 6.8 V/cm it moves toward the cathode at a rate of 2 cm/hour.

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Received
4 V 1957

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Note: Figure translations are in progress. See original paper for figures.