- A. Streitwiesser Jr., T. D. Walsh, and J. K. Wolfe, Jr., J. Am. Chem. Soc., <u>87</u>, 3682 (1965).
- 5. M. Tamura and J. Kochi, Synthesis, 303 (1971).
- 6. K. Abe, T. Yamasaki, N. Nacamura, and T. Sakan, Bull. Chem. Soc. Jpn., 50, 2792 (1977).
- 7. G. Foequet and M. Schlosser, Angew. Chem., 86, 50 (1974).
- 8. R. L. Cargill and M. Rosenblum, J. Org. Chem., 37, 3971 (1972).

ACYLATION OF STEROID ALCOHOLS WITH 3-CARBOXYPROPANAMIDO ACIDS

L. N. Volovel'skii, I. A. Rastrepina, N. V. Popova, V. N. Koryukina, and S. P. Kustova

UDC: 615.857.06

Using N-(3-carboxypropanoy1)- β -phenyl- α -alanine as a model it has been established that when steroid alcohols are esterified with 3-carboxypropanamido acids, the carboxy group of the succinic acid residue takes part in the esterification reaction. In order to obtain a steroid ester of a 3-carboxypropanamido acid in which the carboxyl of the amino acid is the esterifying group, the carboxy group of the succinic acid residue must first be esterified.

Androstane esters of 3-carboxypropanamido acids are biologically active substances. The esterification of a steroid hydroxy ketone with the chloride of a 3-carboxypropanamido acid leads to the formation of a monosteroid ester. It was of interest to determine which of the carboxy groups of the amino acid takes part in the esterification reaction.

Potentiometric titration showed that in their acidities the carboxy groups were close to one another and did not titrate separately. To demonstrate the structure of a dihydrotestosterone ester of a 3-carboxypropanamido acid we obtained the desired ester by three methods. The carboxy group participating in the acylation reaction was determined from the coincidence of the physicochemical properties of the desired product in two of the schemes. As the model acid we chose β -phenyl- α -alanine, with 17 β -hydroxyandrostan-3-one (dihydrotestosterone).

The synthesis of the dihydrotestosterone ester of 3-carboxypropanoyl- β -phenyl- α -alanine was carried out by the schemes given below.



Khar'kov Scientific-Research Institute of Endocrinology and Hormone Chemistry. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 700-704, September-October, 1985. Original article submitted October 29, 1984.



To synthesize substance (I) by a method described in the literature [1] we obtained the acid chloride of the monomethyl ester of succinic acid. In order to synthesize the acid chloride of N-(3-methoxycarbonylpropanoyl)- β -phenyl- α -alanine, a solution of the product of the reaction of β -phenyl- α -alanine with the acid chloride of monomethyl succinate in chloro-form was treated with thionyl chloride. The reaction of the acid chloride so synthesized with dihydrotestosterone followed by partial saponification gave the dihydrotesterone ester of α -benzyl-N-(3-carboxypropanoyl)glycine (I) in which the carboxy group of the β -phenyl- α -alanine had been esterified.

In the second scheme of synthesis, the hemisuccinate of dihydrotestosterone was obtained, and from it the acid chloride, which after reaction with β -phenyl- α -alanine was converted into substance (II) — the dihydrotestosterone ester of N-(3-carboxypropanoyl)- β -phenyl- α -alanine with mp 158-159°C.

By the third method N-(3-carboxypropanoyl)- β -phenyl- α -alanine was first isolated by the reaction of β -phenyl- α -alanine with succinic anhydride, and this was then converted by the action of thionyl chloride into the acid chloride of N-(3-carboxypropanoyl)- β -phenyl- α -alanine with which dihydrotesterone was esterified with the formation of dihydrotestosterone ester of N-(3-carboxypropanoyl)- β -phenyl- α -alanine with mp 157-159°C. The properties of the ester obtained corresponded to those of compound (II).

Thus the syntheses performed with β -phenyl- α -alanine as a model have shown that in the reaction with N-(3-carboxypropanol)- β -phenyl- α -alanine dihydrotestosterone is esterified by the carboxy group of the succinic acid residue with the formation of a monoester. The carboxy group of the amino acid does not take part in the reaction under these conditions. In order to obtain steroid esters in which a steroid alcohol is esterified by the carboxy group of the succinic residue of the amino acid moiety the preliminary protection of the carboxy group of the succinic residue is required.

EXPERIMENTAL

The elementary analyses of the compounds described corresponded to the calculated figures. Monomethyl succinate and its acid halide were obtained by Cason's method [1] and their constants corresponded to those given in the literature.

Acid Chloride of α -Benzyl-N-(3-methoxycarbonylpropanoyl)glycine. With stirring and cooling to -(3-5)°C 3.2 g of the acid chloride of monomethyl succinate was slowly added to a suspension of 3.3 g of β -phenyl- α -alanine in 10 ml of dry pyridine. After the addition of the whole of the acid chloride, the mixture was stirred at the same temperature for another 2 h and was poured into a solution of 3 ml of concentrated sulfuric acid in 50 ml of water cooled to 0°C. The resulting mixture was extracted three times with chloroform (50, 25, and 25 ml) and the chloroform extract was washed with water and dried over anhydrous sodium sulfate; after the latter had been filtered off the solution was concentrated to 20 ml. It was cooled to room temperature, and 10 ml of freshly distilled thionyl chloride was added to

it and the mixture was left at the same temperature for 3 h. The excess of thionyl chloride and the solvent were distilled off in vacuum. The residue was diluted with 25 ml of dry benzene and was mixed with 100 ml of petroleum ether. The yellow-brown precipitate that then deposited was filtered off, washed with petroleum ether, and dried. Yield 1.1 g (18.5% of theoretical) of the acid chloride of α -benzyl-N-(3-methoxycarbonylpropanoyl)glycine with mp 104-105°C.

Dihydrotestosterone Ester of 3-Carboxypropanoyl- β -phenyl- α -alanine. With stirring and cooling to 0-5°C, a solution of 2 g of the acid chloride of α -benzyl-N-(3-methoxycarbonyl-propanoyl)glycine in 10 ml of anhydrous pyridine was slowly added to a solution of 2 g of dihydrotestosterone in 6 ml of anhydrous pyridine, and stirring at the same temperature was continued for another 3 h. The mixture was poured into a solution of 6 ml of concentrated sulfuric acid in 100 ml of water cooled to 0°C, and the resulting precipitate was separated off, washed with water, and dried. After crystallization from methanol, 2.4 g (63.3% of theoretical) of the dihydrotestosterone ester of α -benzyl-N-(3-methoxycarbonylpropanoyl)gly-cine was obtained with mp 127-128°C.

IR spectrum: v_{max}^{KBr} , cm⁻¹: 1710 (CO of a carboxy group), 3440 (NH-CO); 1240 (C-O-C).

Dihydrotestosterone Ester of α -Benxyl-N-(3-carboxypropanoyl)glycine (I). A solution of 2 g of the dihydrotestosterone ester of α -benzyl-N-(3-methoxycarbonyl-propanoyl)glycine in a mixture of 16 ml of dichloroethane and 40 ml of methanol was added to a solution of 0.17 g of caustic potash in 10 ml of methanol, and the mixture was stirred at room temperature for 5 h. Then it was acidified with acetic acid, evaporated in vacuum to half volume, and diluted with 100 ml of water. The precipitate that deposited was separated off and dried. This gave 0.5 g (25% of theoretical) of the dihydrotestosterone ester of α -benzyl-N-(3-carboxypropanoyl) glycine (I) with mp 131-132°C (from methanol).

IR spectrum, v_{max}^{KBr} , cm⁻¹: 1720 (CO of a carboxy group); 1705 (CO at C-3); 3450 (NH-CO), 2870 (COOH).

Dihydrotestosterone Hemisuccinate. A mixture of 1.7 g of dihydrotestosterone, 2 g of succinic anhydride, and 15 ml of anhydrous freshly distilled dioxane was boiled under reflux for 3 h. Two thirds of the solvent was driven off and the residue was diluted with 100 ml of water. To free it from the excess of succinic anhydride, the precipitate that had deposited was twice heated with water, filtered off from the hot mixture, and dried. This gave 1.8 g (78.9% of theoretical) of dihydrotestosterone hemisuccinate with mp 125-127°C (from aqueous methanol).

IR spectrum, v_{max}^{KBr} , cm⁻¹: 1720 (CO of a carboxy group); 2860 (COOH).

Acid Chloride of Dihydrotestosterone Hemisuccinate. A solution of 1.8 g of dihydrotestosterone hemisuccinate in 5 ml of thionyl chloride was kept at room temperature for 4 h. The excess of thionyl chloride was distilled off in vacuum and the residue was dissolved in 20 ml of anhydrous benzene. This solution was diluted with 200 ml of petroleum ether, and the resulting precipitate was separated off, washed with petroleum ether, and dried. The yield of the acid chloride of dihydrotestosterone hemisuccinate with mp 189-190°C (from benzene) was 1.7 g (90.4% of theoretical).

Dihydrotestosterone Ester of N-(3-Carboxypropanoyl)- β -phenyl- α -alanine (II). With stirring and cooling to 0-5°C, a solution of 1.7 g of the acid chloride derivative of dihydrotestosterone hemisuccinate in 5 ml of anhydrous pyridine was added to a suspension of 0.7 g of β -phenyl- α -alanine in 3 ml of anhydrous pyridine, and stirring at the same temperature was continued for 2 h, after which the mixture was poured into a solution of sulfuric acid (3 ml of concentrated acid in 50 ml of water) cooled to 0°C. The resulting precipitate was separated off, washed with water, and dried. Yield 1.5 g (67.2% of theoretical) of the dihydrotestosterone ester of N-(3-hydroxypropanoyl)- β -phenyl- α -alanine (II) with mp 158-159°C (from methanol).

IR spectrum, v_{max}^{KBr} , cm⁻¹: 1710 (CO of a carboxy group); 1640 (carbonyl CO); 2850 (COOH); 3420 (CO-NH).

<u>N-(3-Hydroxypropanoy1)-\beta-phenyl- α -alanine.</u> A mixture of 5 g of β -phenyl- α -alanine, 3.3 g of succinic anhydride, and 20 ml of anhydrous freshly distilled dioxane was boiled under reflux until the ingredients had dissolved completely. Then 2/3 of the volume of the solvent was distilled off in vacuum and the residue was diluted with 150 ml of chloroform. The

precipitate that deposited was separated off, washed with chloroform, and dried. The yield of N-(3-hydroxypropanoyl)- β -phenyl- α -alanine with mp 143-144°C (from methanol) was 6.7 g (83.5% of theoretical).

IR spectrum, $v_{\text{max}}^{\text{KBr}}$, cm⁻¹: 1720 (CO of a carboxy group); 3400 (CO-NH); 2690 (COOH); 1680 (CO of an amide group).

<u>Acid Chloride Derivative of N-(3-Carboxypropanoyl)- β -phenyl- α -alanine. A solution of 5.3 g of N-(3-carboxylpropanoyl)- β -phenyl- α -alanine in 20 ml of freshly distilled thionyl chloride was boiled under reflux for 5 h. The excess of thionyl chloride was separated off, the residue was dissolved in 30 ml of ether, and the resulting solution was poured into 150 ml of petroleum ether. The precipitate that deposited was separated off, washed with petroleum ether, and dried. This gave 2.7 g (47.4% of theoretical) of the acid chloride derivative of N-(3-hydroxypropanoyl)- β -phenyl- α alanine with mp 65-66°C (from ether).</u>

<u>The Dihydrotestosterone Ester of N-(3-Hydroxypropanoyl)- β -phenyl- α -alanine. With stirring, a solution of 3.5 g of the acid chloride derivative of N-(3-hydropropanoyl)- β -phenyl- α -analine in 10 ml of anhydrous pyridine was slowly added to a solution of 3 g of dihydrotestosterone in 5 ml of anhydrous pyridine cooled to -5° C. Stirring at the same temperature was continued for 2 h and the mass was poured into a solution of 6 ml of sulfuric acid in 100 ml of water cooled to 0°C. The precipitate was separated off and dried. Yield 1.8 g (32.4% of theoretical) of the dihydrotestosterone ester of N-(3-hydroxypropanoyl)- β -phenyl- α -alanine with mp 157-159°C (from methanol).</u>

A mixture with the ester (II) obtained from dihydrotestosterone hemisuccinate gave no depression of the melting point. The IR spectra of the compound obtained and of compound (II) were completely identical.

SUMMARY

1. Using N-(3-hydroxypropanoy1)- β -pheny1- α -alanine as a model it has been established that in the esterification of sterol alcohols with 3-hydroxypropanamido acids it is the carboxy group of the succinic acid residue that takes part in the esterification reaction.

2. To obtain a steroid ester of a 3-hydroxypropanamido acid in which the carboxy group of the amino acid is esterified, the carboxy group of the succinic acid residue must first be esterified.

LITERATURE CITED

1. J. Cason, Org. Synth., Coll. Vol. III, 169-171 (1955).