SYNTHESIS OF 3-HYDROXY-AND 3-CHLORO-GLUTAMIC ACIDS

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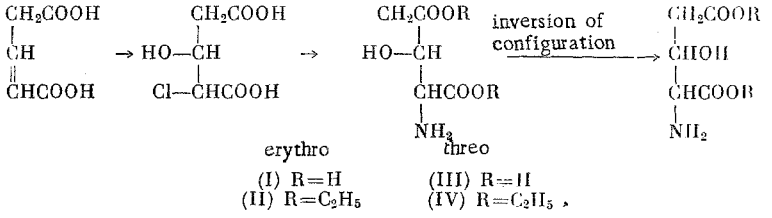
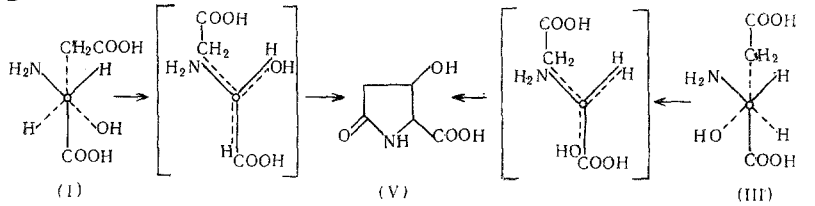
A promising line of work in the chemistry of enzymes is the use of analogs of substrates in the study of the mechanism of enzymic reactions. Thus, in the investigation of enzymic transformations of amino acids α-substituted amino acids, sulfonic acids, β-mercapto and β-hydroxy α-amino acids, etc. are often used as pseudosubstrates or inhibitors, and this provides valuable supplementary information on the structures of active centers of enzymes and the mechanism of the reactions which occur. Considerable interest from this point of view is presented by the isomeric 3-hydroxy- [1] and 3-chloro-glutamic acids, whose synthesis is the subject of the present paper. In doing this we envisaged the further use of the substances obtained in investigations on synthesis and in the study of their reactions, as glutamic acid analogs, with a number of enzymes, including those containing pyridoxal 5-phosphate.

As the syntheses of 3-hydroxyglutamic acid which have been described are fairly complicated for preparative purposes and lead to a mixture of diastereoisomers, it was desirable to use known stereochemically specific methods of preparing β-hydroxy α-amino acids, of which one of the simplest and most convenient is the hydroxyhalogenation of α,β-unsaturated acids with subsequent amination [2]. We used this method for the synthesis of 3-hydroxyglutamic acid, in accordance with the scheme:



trans-Glutaconic acid reacts rapidly and quantitatively with aqueous hypochlorous acid with formation of 2-chloro-3-hydroxyglutaric acid. When treated with concentrated aqueous ammonia, the latter is converted into erythro-3-hydroxyglutamic acid (I), which can be conveniently isolated from the reaction mixture in the form of the well cyrstallizing hydrochloride of the diethyl ester (II), with subsequent acid hydrolysis to (I). As in analogous synthesis of β-hydroxy α-amino acids, as a result of this reaction only the erythro isomer is formed; it can readily be converted into threo-3-hydroxyglutamic acid (III) by a described method via the oxazolin [1]. We must mention that previous attempts have been made to prepare (I) and (III) from glutaconic acid, but the only reaction product isolated was found to be 2,3-dihydroxyglutaramide [3].

In the study of the reaction of enzymes with compounds of the type (I) and (III) account must be taken of the preferred conformations of such structures. From general considerations it must be supposed that in the stable rotational isomers of (I) and (III) the large substituents (COOH and $HOCOCH_2$ groups) will occupy the most remote positions; in (I) the OH and NH_2 groups will then be remote, whereas in (III) they will be spatially close together.



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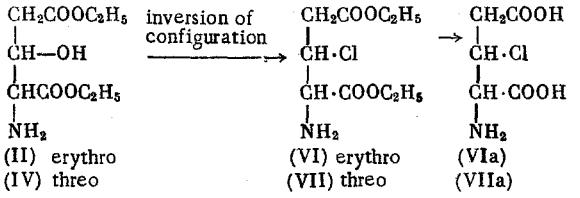
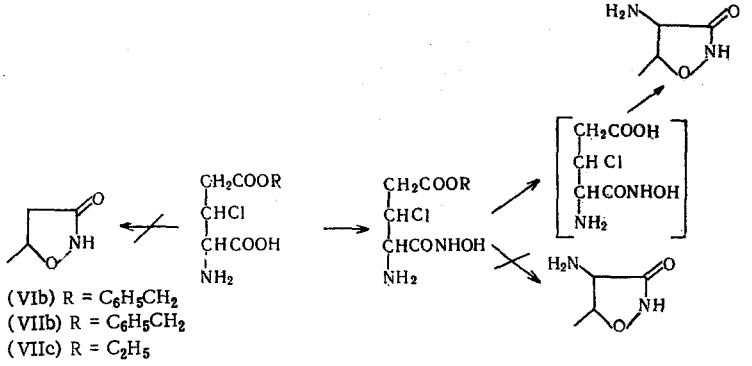
Like glutamic acid, (I) and (III) are able to cyclize to 3-hydroxy-5-oxo-2-pyrrolidinecarboxylic acids (V), but the reaction rates differ for (I) and (III). Whereas (I) is largely converted into trans-(V) in aqueous solution at 100° in the course of a few hours, (III) is scarcely changed under these conditions. This can be explained on the view that the cyclization reaction passes through the corresponding reaction conformations of (I) and (III), the stabilities of which determine the reaction rate. Since the formation of the screened conformation of (III) is, as will be seen, associated with high steric hindrance, the cyclization of (III) is slower.

For the synthesis of the 3-chloroglutamic acids (VIa) and (VIIa) having definite configurations we used the diethyl erythro- and threo-3-hydroxyglutamates (II) and (IV). The replacement of OH by halogen was effected by the action of phosphorus pentachloride, and this was accompanied by inversion of configuration at the 3-carbon atom. Decided difficulty was met at the stage of the hydrolysis of (VI) and (VII); this arose from the instability of 3-chloroglutamic acids [and, to a smaller extent, of (I) and (III)] when heated in acidic aqueous solutions. Hence, under optimum conditions the yields of (VIa) and (VIIa) were 25-40%, and they were accompanied by a considerable amount of 2-oxoglutaric acid.



We then investigated possible methods of converting (VIa) and (VIIa) into substituted 3-isoxazolidinones. One of the general methods for the synthesis of this class of compounds is the reaction of 3-halopropionic esters with an alkaline solution of hydroxylamine [4]. Under these conditions the ester (VII) gave a complex mixture of products, which contained only traces of isomeric 3-isoxazolidinones (paper chromatography and electrophoresis). An analogous picture was found for threo-3-chloroglutamic acid 5-ethyl ester (VIIc), for which the intermediate formation of the corresponding hydroxamic acid with subsequent cyclization did not occur, but processes predominated in which the elimination of the 3-chloro substituent occurred. Hence, the ester (VIIc) was converted into the corresponding hydroxamic acid — ethyl 4-amino-3-chloro-4-(hydroxycarbamoyl)butyrate — under mild conditions (yield 50-60%), and the cyclization of this was studied under various conditions. In this case, however, only traces of a substituted 3-isoxazolidinone were detected, which is associated with the formation of labile products. probably belonging to the pyridine or pyrrole system.



These difficulties were avoided by the cyclization of the hydroxamic acids containing a free carboxy group — 4-amino-3-chloro-4-(hydroxycarbamoyl)butyric acids — which were formed from the benzyl esters (VIb) and (VIIb). The known methods for the synthesis of the 5-benzyl esters of glutamic acid [5] proved to be inapplicable in this case, which was due either to the decomposition of (VI) and (VII) under the reaction conditions, or to the simultaneous formation of 1-benzyl esters. We prepared 5-benzyl esters of erythro- and threo-3-chloroglutamic acids free from 1-ester impurity (paper electrophoresis) by the esterification of (VIa) and (VIIa) with benzyl acetate in presence of perchloric acid. Under analogous conditions from (VIIa) and ethyl acetate we also synthesized (VIIc). Then (VIb) and (VIIb) were converted by the carboxy anhydride method into the corresponding hydroxamic acids, benzyl protection was removed by hydrogenolysis, and as a result of cyclization substituted 3-isoxazolidinones were obtained.*

*Date on the synthesis of these compounds and their properties are given in [6].

EXPERIMENTAL

Diethyl Erythro-3-hydroxyglutamate (II) and Its Hydrochloride. 6.5 g of glutaconic acid was added to 49.2 ml of 1 N hypochlorous acid solution at 0°, the mixture was kept at this temperature for 12 h and then lyophilized, and the residue was dried over KOH. The yield was quantitative. Found: Cl 19.63%. $C_5H_7O_5Cl$. Calculated: Cl 19.42%.

A solution of the oily 2-chloro-3-hydroxyglutaric acid in 10 ml of water was added with cooling and stirring to 50 ml of aqueous ammonia solution, saturated at 0°. After a few days the reaction mixture was evaporated, 250 ml of alcohol was added, and the mixture was saturated at the boil with dry hydrogen chloride. Ammonium chloride was separated, the filtrate was evaporated down to low bulk, ethyl acetate was added to the clear solution, and after 12 h at 0° 3.9 g (30%) of the hydrochloride of (II) was filtered off; m.p. 176° (alcohol).

[1] gives m.p. 173-174°.

50 ml of chloroform saturated with ammonia at 0° was added dropwise with stirring and cooling to 10° to a suspension of 3.5 g of the hydrochloride of (II) in 15 ml of chloroform. Ammonium chloride was filtered off, and the filtrate was vacuum-evaporated. The oily residue was dried over P_2O_5, and we obtained 2.6 g (88%) of (II), m.p. 57-58° (ether). Found: N 6.02%. $C_9H_{17}O_5N$. Calculated: N 6.35%.

Erythro-3-hydroxyglutamic Acid (I). A solution of 2.6 g of the hydrochloride of (II) in 26 ml of 20% HCl was heated for 2 h at 100° and then vacuum-evaporated. 50 ml of hot acetic acid was added, and the mixture was left for 12 h at 0°. The yield of the hydrochloride of (I) was 1.8 g (90%); m.p. 194° (decomp.) (2N HCl — CH_3COOH). [1] gives m.p. 194° (decomp.). (I) was prepared from its hydrochloride by described methods [1] in 80-90% yield; m.p. 208-209° (aqueous alcohol). [1] gives m.p. 209-210°.

trans-3-Hydroxy-5-oxo-2-pyrrolidinecarboxylic Acid (V). 1.6 g of (I) was boiled in 10 ml of water for 7 h, the mixture was evaporated, and the residue was extracted with alcohol. Unchanged (I) was separated, and the residue was treated with dichloroethane. The yield of (V) was 0.64 g (44%); m.p. 163-164° (decomp.). Found: N 9.10%. $C_5H_7O_4N$. Calculated: N 9.64%.

Ethyl trans-3-Hydroxy-5-oxo-2-pyrrolidinecarboxylate. An alcoholic solution of 4.4 g of (II) was heated for 8 h at 40° and then evaporated, the residue was dissolved in acetone, and petroleum ether was added until crystallization set in. The yield of the ester was 2.14 g (63%); m.p. 109°. Found: N 7.8%. $C_7H_{10}O_4N$. Calculated: N 8.14%.

Diethyl Threo-3-chloroglutamate (VII) as Its Hydrochloride. 5.1 g of (II) was added to a suspension of 4.6 g of PCl_5 in 30 ml of chloroform with stirring and cooling at such a rate that the temperature of the mixture did not rise above 8-10°. Stirring was continued further for 1.5-2 h at 5°, and the mixture was filtered and vacuum-evaporated at 25-30°. The residue was dissolved in 20 ml of absolute alcohol and decolorized with charcoal, and alcohol was vacuum-distilled off. The oily residue was dissolved in a little dichloroethane, dry ether was added, and after 12 h at 0° the hydrochloride of (VII) was filtered off. The yield of the hydrochloride of (VII) was 4.5 g (83%); m.p. 111-113° (decomp.) (alcohol — ether). Found: Cl 13.19%. $C_9H_{17}O_4NCl_2$. Calculated: Cl 13.12%.

Diethyl Erythro-3-chloroglutamate (VI) as Its Hydrochloride. In an analogous way from 6.5 g of the hydrochloride of (IV) and 5.8 g of PCl_5 in 40 ml of chloroform we obtained 5 g (74%) of the hydrochloride of (VI); m.p. 115-116° (decomp.) (alcohol — ether). Found: Cl 13.27%. $C_9H_{17}O_4NCl_2$. Calculated: Cl 13.12%.

Threo-3-chloroglutamic Acid (VIIa). A solution of 8.5 g of the hydrochloride of (VII) in 85 ml of 20% HCl was stirred for 1 h in a boiling water bath. Hydrochloric acid was then vacuum-distilled off, the residue was dissolved in 20 ml of water, and the solution was decolorized with charcoal and vacuum-evaporated. The residue was dissolved in a little glacial acetic acid, ammonium chloride was filtered off, acetic acid was removed in a vacuum, and the residue was dissolved in 3 ml of water. With cooling and stirring the calculated amount of 2 N LiOH was added cautiously, and the mixture was left overnight at 0°. Five times its volume of absolute alcohol was then added, and the mixture was kept for 12 h at 0°. The crystalline acid was filtered off and washed with absolute alcohol until the test for chloride was negative, and then with ether. The yield of (VIIa) was 2.4 g (43%); m.p. 145-146° (decomp.) (water — alcohol). Found: Cl 19.42; N 7.44%. $C_5H_8O_4NCl$. Calculated: Cl 19.52; N 7.16%.

Erythro-3-chloroglutamic Acid (VIa). The hydrolysis of 2.7 g of the hydrochloride of (VI) was conducted as in the preceding experiment. We isolated the hydrochloride of (VIa). It was dissolved in water, and 2 N LiOH was added; the solution was then treated with excess of absolute alcohol and isopropyl alcohol, and the amorphous amino acid was filtered off (35%) and crystallized from the least possible amount of hot water. The yield of (VIa) was 0.45 g (24%); m.p. 144-145° (decomp.). Found: Cl 19.71; N 7.25; 7.29%. $C_5H_8O_4NCl$. Calculated: Cl 19.52; N 7.16%.

Threo-3-chloroglutamic Acid 5-Benzyl Ester (VIIb). 1.25 ml of 73% perchloric acid was added with stirring to a suspension of 1.8 g of (VIIa) in 30 ml of benzyl acetate, and the mixture was left for a few days at 20°. It was diluted with ether to 100 ml, shaken with 50 ml of water, and after 2 h at 0° crystalline (VIIb) was separated. The yield of (VIIb) was 1.2 g (44%); m.p. 141-142° (decomp.). Found: N 5.20%. $C_{12}H_{15}O_4NCl$. Calculated: N 5.23%.

Erythro-3-chloroglutamic Acid 5-Benzyl Ester (VIb). 2.2 g of the hydrochloride of (VIa) was mixed with 1.25 ml of 73% perchloric acid, hydrogen chloride was removed in a vacuum, 30 ml of benzyl acetate was added, and the further procedure was as described above. The yield of (VIb) was 0.86 g (32%); m.p. 127° (decomp.). Found: N 5.48%. $C_{12}H_{14}O_4NCl$. Calculated: N 5.23%.

Threo-3-chloroglutamic Acid 5-Ethyl Ester (VIIc). 0.62 ml of 73% perchloric acid was added to a suspension of 0.9 g of (VIIa) in 10 ml of ethyl acetate, and the mixture was left for 7 days at 20°. Ethyl acetate was removed in a vacuum, the residue was dissolved in 20 ml of water, and the solution was passed through a column (1 × 95 cm) of Amberlite CG-45 with elution with water. Lyophilization of the fractions containing the 5-monoester gave 0.45 g of product (42%); m.p. 125-127°. Found: N 6.45%. $C_7H_{12}O_4NCl$. Calculated: N 6.68%.

Ethyl 4-Amino-3-chloro-4-(hydroxycarbamoyl)butyrate. Phosgene was passed for 30 min into a stirred suspension of 0.52 g of (VIIb) in 25 ml of dry dioxane at 40°. Excess of phosgene was removed with a stream of dry air, dioxane was vacuum-evaporated at 25-30°, and the residue was dried over P_2O_5 and KOH and dissolved in 10 ml of absolute alcohol cooled to − 5°. With vigorous stirring a solution of 0.2 g of hydroxylamine base in 5 ml of absolute alcohol was added rapidly, and after 1 h the precipitate was filtered off. The yield of the ester was 0.3 g (52%); m.p. 150° (decomp.). Found: N 12.43%. $C_7H_{13}O_4N_2Cl$. Calculated: N 12.47%. The substance was unstable to keeping.

CONCLUSIONS

1. The syntheses of erythro- and threo-3-hydroxyglutamic and erythro- and threo-3-chloroglutamic acids and some of their derivatives are described.

2. Some properties of the compounds obtained were studied.

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