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SYNTHESIS AND PROPERTIES OF DERIVATIVES OF DIAMINOPROPIONIC AND DIAMINOBUTYRIC ACIDS

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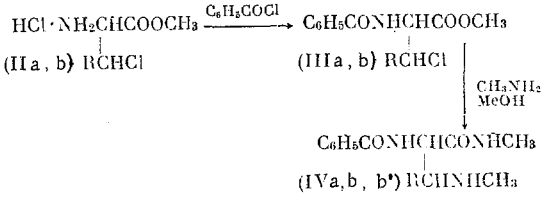
In an earlier publication we have proposed a method for the selective cleavage of alkali-labile carbohydrate-protein bonds in glycopeptides and glycoproteins based on the substitution by methylamine of the glycosyloxy radicals bound to serine or threonine [1]. Conversion of the serine and threonine residues into derivatives of β-N-methyldiaminopropionic (Ia) and β-N-methyldiaminobutyric (IIb) acids by the action of alkali and methylamine, in the presence or absence of $NaBH_4$, permits the position of the carbohydrate chains on the polypeptide core to be marked. Development of the proposed method and its effective utilization in the chemistry of the glycoproteins together with other known methods of structural analysis of these biopolymers necessitates a detailed study of the properties of the indicated diamino acids. Compounds (Ia) and (Ib) formed during acid hydrolysis are stable under the standard conditions of hydrolytic cleavage of polypeptides and can be determined quantitatively by means of an automatic amino acid analyzer. By this (Ia) is detected as an individual compound [2] and (Ib) as a mixture of apparently the erythro and threo isomers [1].

The present work has been concerned with the synthesis of derivatives of (Ia) and (Ib) and a study of their transformations under conditions used in current methods of structural analysis of polypeptides. Synthesis of the methylamides of α-N-benzoyl-β-N-methyldiaminopropionic (IVa) and the isomers of α-N-benzoyl-β-N-methyldiaminobutyric acids (IVb) and (IVb') was accomplished by the scheme shown:



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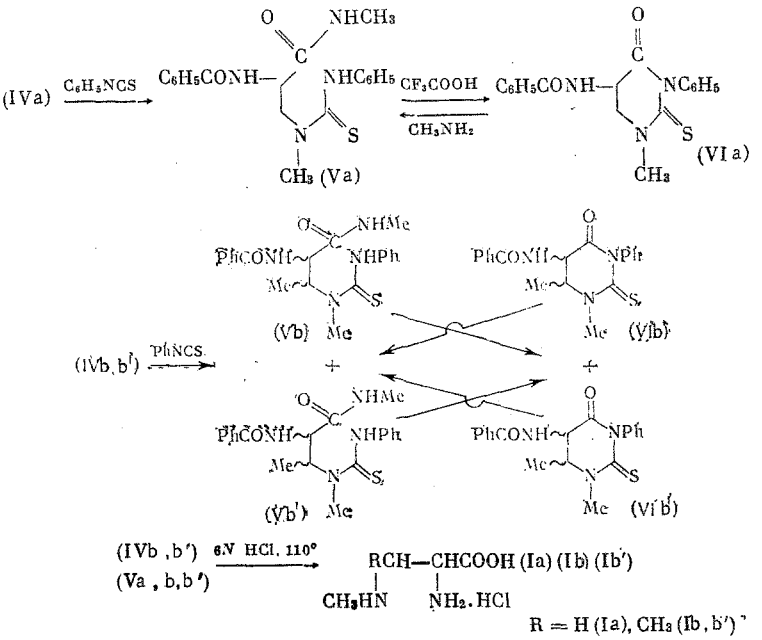
The hydrochlorides of the methyl esters of α-amino-β-chloropropionic (IIa) and butyric (IIb) [3, 4] acids were treated with benzoyl chloride in dioxane [5]. The methyl esters of α-N-benzoyl-β-chloropropionic (IIIa) and butyric (IIIb) acids which were obtained were isolated by chromatography on silica gel and were further purified by crystallization from an ether–hexane mixture. The yield of the analytically pure substances (IIIa, b) was 80-82%, their structure was confirmed by elemental analysis, a negative ninhydrin test, and the presence in their IR spectra of absorption bands corresponding to aromatic (1600 and 1490 cm^{-1}), amide (1645 and 1520 cm^{-1}) and ester (1740 and 1240 cm^{-1}) groupings. Treatment of (IIIa) and (IIIb) with 10% methylamine in absolute methanol yielded derivatives of (Ia) and (Ib), the methylamides (IVa) [6] and (IVb, b'). By this the methylamides (IVb, b') were obtained as a mixture of two substances, consisting, apparently, of the methylamides of D, L-erythro- and D, L-threo-α-N-benzoyl-β-N-methyldiaminobutyric acids. Separation of the mixtures was realized by TLC on silica gel. Hydrolysis of the isomers (IVb, b') which were obtained with 6 N HCl and subsequent analysis by the amino acid analyzer showed the presence of the two peaks of (Ib) and (Ib'), identical to the peaks which we had observed previously in the hydrolysis of the methylamides of α-N-acetyl-β-N-methyldiaminobutyric acids formed by treatment with alkali and methylamine of the methylamide of O-(2-acetamido-2-desoxy-β-D-glucopyranosyl)-N-acetyl-D, L-threonine [1]. In view of the difficulty in preparing large quantities of the methylamides (IVa, b, b') in an analytically pure state, the compounds were characterized as their N-phenylthiocarbamates. Synthesis of the latter derivatives from (IVa, b, b') was also made due to the necessity to study the behavior of these diamino acid residues under the Edman method conditions of dehydration of peptide chains, which is commonly used for determination of the amino acid sequence. The methylamides of α-N-benzoyl-β-N-methyldiaminopropionic (IVa) and butyric (IVb, b') acids were reacted with phenylisothiocyanate without additional purification immediately after removal of methylamine from the reaction mixture.



The methylamide of α-N-benzoyl-β-(N'-methyl-N''-phenylthiocarbamyl)propionic acid (Va) was obtained in a yield of 78% in the crystalline state. Its structure was confirmed by elemental analysis and IR spectrum: bands at 1640, 1660, and 1535 cm^{-1} (amide groupings), 1350 cm^{-1} (thiourethane grouping), 1600 and 1490 cm^{-1} (aromatic system). In the PMR spectrum of the methylamide (Va) signals were observed for the aromatic protons of two monosubstituted phenyl rings (δ, ppm, 7.2-8), the N-methyl group at the β-C atom (3.03) and the N-methyl group of the methylamide group (2.86). Hydrolysis of (Va) in 6 N HCl for 24 h at 110° gave β-N-methyldiaminopropionic acid (Ia) which was detected as a single peak when run on the amino acid analyzer.

From the mixture of methylamides (IVb) + (IVb'), as a result of reaction with phenylisothiocyanate two compounds (Vb), (Vb') were obtained, differing in their chromatographic behavior, and which could be separated by crystallization from ethanol. The individual methylamides (Vb) and (Vb') had the same composition, corresponding to the formula $C_{20}H_{24}N_4O_2S$, closely similar PMR spectra (see Experimental) and IR spectra in which absorption bands were present corresponding to the two amide (1650, 1670, and 1530 cm^{-1}) and the thiourethane (1340 cm^{-1}) groups, as well as the aromatic system (1600 and 1460 cm^{-1}). Compound (Vb) had a mp 199-200°, its isomer (Vb') 190-193°; the mixed-melting point of both compounds gave a depressed value (174-176°). The structure of the methylamides (Vb) and (Vb') was confirmed additionally by the results of acid

hydrolysis in 6 N HCl at 110°, which for each individual compound led to the formation of a mixture of the erythro and threo isomers (Ib, b'). The ratio of these isomers depended on the time of hydrolysis. Hydrolysis of (Vb) and (Vb') was completely finished after 12 h. The isomer (Ib) was formed as the main hydrolysis product, the content of which considerably increased on heating with acid for 14 h, and after 48 h was predominant. When (Vb) was subjected to acid hydrolysis in all cases the (Ib') isomer predominated, the content of which gradually decreased and was practically the same after 24 h and 48 h treatment with acid. These results suggest that the isomers (Vb') and (Ib) on the one hand, and (Vb) and (Ib') on the other, have the same (erythro or threo) configurations and that the isomer (Ib') is the more stable with respect to the isomerizing action of acid. It may be assumed that (Ib') has the threo configuration. However, this assumption requires rigorous proof.

In the next stage of our work we studied the transformation of the methylamides (Va, b, b') under the action of trifluoroacetic acid (TFA). Under the dehydration conditions of the Edman method such treatment leads to a cleavage of the amide bond and a splitting off of the N-terminal amino acid residue. In our case treatment of (Va) with 99% TFA led to a derivative of hexahydropyramidine (VIa) (yield 79.5%). Its structure was confirmed by elemental analysis and the PMR spectrum in which were observed signals due to the aromatic protons of two monosubstituted phenyl rings (δ, 7-8 ppm) and one N-methyl grouping (3.58); a signal due to a N-methylamide grouping (2.86) was not present in the spectrum. And lastly the structure of the hexahydropyrimidine derivative (VIa) was confirmed by the fact that when it was treated with methylamine in absolute methanol, the original methylamide (Va) was obtained as a sole product, identical with the methylamide of α-N-benzoyl-β-(N'-methyl-N"-phenylthiocarbamyl)propionic acid (from the chromatographic behavior and constants).

The methylamides derived from diaminobutyric acid (Vb, b') under the same conditions form the same mixture of substances in which the principal components are substances (VIb) and (VIb'); the mixture was separated from the reaction products by crystallization. The yield of the mixture (VIb) + (VIb') obtained from the acyclic derivative (Vb) was 88%, and from (Vb') was 86%. Separation of these substances was achieved by means of preparative chromatography on silica gel and crystallization from ethanol. The chromatographically pure substances (VIb) and (VIb') which were obtained had the same composition by elemental analysis, corresponding to the formula $C_{18}H_{17}N_3O_2S$, closely similar IR spectra and differed in mp: (VIb) 251-253°, (VIb') 159-161°. It should be noted that in both cases the isomer (VIb) predominated. From cyclization of (Vb) the ratio of isomers (VIb) and (VIb') was 1.95 : 1, and from cyclization of (Vb') 1.45 : 1. The structure of compounds (VIb) and (VIb') as cis/trans isomers of 1-phenyl-2-thio-3,4-dimethyl-5-benzamido-6-oxohexahydropyrimidine was deduced from the PMR spectra and the reactions of compounds with methylamine. In the PMR spectrum of (VIb) signals are displayed due to the aromatic protons of two monosubstituted phenyl rings (δ, 7-8 ppm), as well as signals corresponding to a single N-methyl group (3.58), a single C-methyl group (1.28) and two vicinal protons of ring methine groups (4-C, 4.58; 5-C, 5.20; $J_{4,4} = 6$ Hz). A signal corresponding to the methylamide grouping (2.85-2.9) was not present in the spectrum. In the PMR spectrum of (VIb') there were also observed signals of aromatic protons (7-7.9), N-methyl group protons (3.52), C-methyl group protons (1.52) and two vicinal protons of ring methine groups (4-C, 4.04; $_5$-C, 4.98; $J_{4,5} = 5.2$ Hz). A signal corresponding to the methylamide group (2.85-2.9 ppm) was also absent from the spectrum of (VIb'). These data are insufficient to permit assignment of cis or trans configurations to the isomers which were obtained. By the action of methylamine on the individual compounds (VIb) or (VIb') the same mixture of substances was obtained, in which the methylamides of (Vb) and (Vb') were identified as the principal components, which also confirms the cyclic structure of (VIb) and (VIb').

Thus on the basis of the data obtained it is possible to conclude that both the acyclic and cyclic derivatives of D, L-erythro- and D, L-threo-diaminobutyric acids are readily and reversibly converted into one another, by both the action of acids and of bases, which in all probability is a result of inversion of the configuration at the α-C atom.

In conclusion we note that the present study of the conversion of the methylamides of the diamino acid derivatives (Va, b, b'), and notably their cyclization, which occurs with cleavage of the amide bond, can serve as a basis for the development of a new method of selective splitting from the polypeptide chain of glycoproteins, serine or threonine residues which are linked by an O-glycoside bond to the carbohydrate chains. By the action of methylamine these residues are modified to derivatives of the β-N-methyldiamino acids and consequently after treatment with phenylisothiocyanate and TFA can be converted, similarly to the above described methylamides (IV), into cyclic products with cleavage of the peptide bond.

EXPERIMENTAL

Thin-layer chromatography was carried out on Silufol plates (Czechoslovakia) with $CHCl_3$–methanol systems: 9 : 1 (system A) and 8 : 2 (system B). The substances were detected with starch–chlor–iodide, ninhydrin,

and iodine. Preparative chromatography was carried out on columns of silica gel L 40/100 (Czechoslovakia). Melting points (corrected) were determined on a "Boetius" microscope stage (GDR). IR spectra were recorded on a "Perkin–Elmer 257" spectrophotometer using KBr tablets. PMR spectra were recorded on a "Varian XL-100" instrument. Determination of amino acids was carried out on a modified amino acid analyzer of the BC 201 type (LKB Bio Cal). Citrate buffer (0.7 N), pH 5.28, a 35 cm × 0.9 cm column, rate of elution 60 ml/h, and the resin Aminex A-5 were employed.

Methyl Ester of α-N-Benzoyl-β-chloropropionic Acid (IIIa). Substance (IIIa) was prepared from 4.6 g of the methyl ester of α-amino-β-chloropropionic acid (IIa) as in [5]; yield 5.1 g (80%), mp 113-114°.

Methyl Ester of α-N-Benzoyl-β-chlorobutyric Acid (IIIb). One gram of the methyl ester of α-amino-β-chlorobutyric acid (IIb) was suspended in 13 ml of dioxane, then 0.4 ml water and 1.85 g K_2CO_3 were added and stirred 15 min. With cooling with ice, 15 ml of benzoyl chloride was added and the reaction mixture stirred for 15 h at ~20°. The precipitated KCl was filtered off, and the filtrate evaporated to dryness. The residue was dissolved in $CHCl_3$, washed with 5% K_2CO_3, concentrated by evaporation and chromatographed on silica gel. The product was eluted with system A. The fraction containing the substance with R_f 0.45 (system B) was evaporated, and the residue crystallized from ether with hexane. The yield of (IIIb) was 1.1 g (82%), mp 77-78°, IR spectrum (ν, cm^{-1}): 1600 and 1490 (aromatic system), 1645 and 1520 (amide group), 1740 and 1240 (ester group). Found: C 56.55; H 5.12; N 5.38; Cl 13.53%. $C_{12}H_{14}NO_3Cl$. Calculated: C 56.50; H 5.52; N 5.47; Cl 13.9%.

Methylamide of α-N-Benzoyl-β-(N'-methyl-N"-phenylthiocarbamyl)propionic Acid (Va). To 0.28 g of (IIIa) was added 15 ml of 10% methylamine in absolute methanol. This was kept for 4 h at ~20° and evaporated to dryness repeatedly by adding methanol in order to remove methylamine. The dry residue was dissolved in 5 ml ethanol; 0.42 ml of phenylisothiocyanate was added and then it was heated 5 min at the boiling point, cooled, left for 2 h at ~20°, and then concentrated and chromatographed on silica gel, eluting with system A. The fraction containing one substance (R_f 0.31, system A) was concentrated and crystallized from ethanol. Yield of (Va) 0.33 g (78%), mp 185-187°. IR spectrum (ν, cm^{-1}): 1660, 1640, and 1535 (amide groups), 1600 and 1490 (aromatic system), 1350 (thiourethane group). PMR spectrum (δ, ppm, $CDCl_3$): 7.2-8 m (10 H, 2 Ph); 3.03 s (3 H, NMe); 2.86 d (3 H, NHMe). Found: C 61.31; H 6.09; N 14.97; S 8.83%. $C_{18}H_{22}N_4O_2S$. Calculated: C 61.59; H 5.98; N 15.12; S 8.65%.

Methylamide of α-N-Benzoyl-β-(N'-methyl-N"-phenylthiocarbamyl)butyric Acid (Vb and b'). Ester (IIIb) (2.55 g) was dissolved in 100 ml of 10% methylamine in abs. methanol, then kept 4 h at ~20° and evaporated repeatedly by adding methanol. The residue was crystallized from ethanol. The methylamides of (IVb) and (IVb') were obtained as a mixture of two substances with R_f values of 0.21 and 0.38 (system B); yield 2.32 g (93%). Found: C 54.28; H 7.32; N 14.56; Cl 12.49%. $C_{13}H_{20}N_3O_2Cl$. Calculated: C 54.70; H 7.03; N 14.56; Cl 12.49%. To the mixture obtained, was added 3.6 ml of phenylisothiocyanate, dissolved in 10 ml ethanol, it was then boiled 5 min, cooled, kept for 2 h at ~20° and evaporated to initiate crystallization. The crystals were filtered off, washed with ethanol, and dried in vacuum. Yield of (Vb) 1.4 g (36.4%), mp 199-200° (from ethanol), R_f 0.18 (system A). The mother liquor was evaporated to small volume and ether added. Crystallization of compound (Vb') resulted: yield 0.85 g (22.1%), mp 190-193° (from ethanol), R_f 0.32 (system A). The mixed-melting point of (Vb) + (Vb') was 174-176°. The mother liquor from the crystallization of (Vb') was evaporated to dryness and chromatographed on silica gel in system A. There was obtained an additional 0.5 g of (Vb) and 0.75 g of (Vb'). Total yield of (Vb) 49.5% and of (Vb') 41.5%. IR spectrum of (Vb) (ν, cm^{-1}): 1650, 1670, and 1530 (amide groups), 1600 and 1480 (aromatic system), 1340 (thiourethane group). PMR spectrum (Vb) (δ, ppm, $CDCl_3$): 7.2-8 m (10 H, 2 Ph), 3.03 s (3 H, NMe), 2.85 d (3 H, NHMe), 1.41 d (3 H, CMe). Found: C 62.76; H 6.37; N 14.91; S 8.17%. $C_{20}H_{24}N_4O_2S$. Calculated: C 62.48; H 6.29; N 14.56; S 8.32%. IR spectrum of (Vb') (ν, cm^{-1}): 1640, 1660, and 1530 (amide groups), 1600 and 1490 (aromatic system), 1340 (thiourethane group). PMR spectrum (Vb') (δ, ppm, $CDCl_3$): 7.2-8 m (10 H, 2Ph), 3.08 s (3 H, NMe), 2.9 d (3 H, NHMe), 1.34 d (3 H, CMe). Found: C 62.78; H 6.38; N 14.79; S 8.23%.

1-Phenyl-2-thio-3-methyl-5-benzamido-6-oxohexahydropyrimidine (VIa). Methylamide (Va) (0.22 g) was dissolved in 1.1 ml of 99% TFA, boiled 20 min, cooled and evaporated in vacuum repeatedly with addition of ethanol. The residue was crystallized from ethanol. Yield of (VIa) 0.16 g (79.5%), mp 176-177°, R_f 0.68 (system A). PMR spectrum of (VIa) (δ, ppm, $CDCl_3$): 7.8 m (10 H, 2Ph), 3.58 s (3 H, NMe), 4.3 q (1 H, 4-CH_{eq}), 3.58 (1 H, 4-CH_{ax}), 5.04 m (1 H, C_5H). Found: C 63.37; H 4.82; N 12.01; S 9.28%. $C_{18}H_{17}N_3O_2S$. Calculated: C 63.39; H 5.05; N 12.38; S 9.45%.

1-Phenyl-2-thio-3,4-dimethyl-5-benzamido-6-oxohexahydropyrimidine (VIb, b'). Substance (Vb) (0.75 g) was treated with 3.75 ml of 99% TFA analogously to (Va). According to TLC in system A the reaction mixture

consisted of two main substances with R_f 0.59 and 0.8 and an insignificant amount of a substance with R_f 0.47 which was not further investigated. After removal of TFA and crystallization from ethanol, 0.619 g (68%) of a mixture of the two substances (VIb) + (Vb') was obtained, which was further chromatographed on silica gel, eluting with system A. Yield of (VIb) was 0.39 g (56.5%), R_f 0.59, mp 251-253° (from ethanol). PMR spectrum (δ, ppm, $CDCl_3$): 7.8 m (10 H, 2Ph), 3.58 s (3 H, NMe), 1.28 d (3 H, CMe), 4.58 m (1 H, 4-CH), 5.2 t (1 H, 5-CH), $J_{4,5}$ = 6 Hz. Found: C 64.97; H 5.42; N 11.80; S 8.83%. $C_{19}H_{19}N_3O_2S$. Calculated: C 64.57; H 5.42; N 11.89; S 9.07%.

Yield of (VIb') 0.2 g (29%), R_f 0.8, mp 159-161° (from ethanol). PMR spectrum (δ, ppm, $CDCl_3$): 7-7.9 m (10 H, 2Ph), 3.52 s (3 H, NMe), 1.52 d (3 H, CMe), 4.04 m (1 H, 4-CH), 4.98 m (1 H, 5-CH), $J_{4,5}$ = 5.2 Hz. Found: C 64.72; H 5.38; N 11.71; S 8.94%.

An identical mixture of the two substances was obtained from 0.38 g of the compound (Vb') by treating it with 2 ml of TFA; yield of (VIb) + (VIb') 0.3 g (86%). Chromatography on silica gel gave 0.16 g (45.7%) of (VIb), mp 251-253° [admixture with a sample of (VIb) obtained from (Vb) gave no depression in mp], and 0.11 g (31.4%) of (VIb'), mp 159-161° [admixture with (VIb) from (Vb) gave no depression in mp].

Reaction of the Hexahydropyrimidine Derivatives (VIa, b, and b') with Methylamine. Substance (VIa) (50 mg) was dissolved in 2 ml of 10% methylamine in abs. methanol and kept for 3 h at ~20°. The solution was evaporated to dryness under vacuum and the residue crystallized from ethanol. The yield of methylamide (Va) was 37 mg (67.7%), mp 185-187° [admixture with (Va) from (IVa) gave no depression in mp], R_f 0.31 (system A).

Analogous treatment of compound (VIb) or (VIb') with a solution of methylamine in methanol in each case resulted in the same mixture of two main substances with R_f 0.18 and 0.32 (system A). Preparative separation of this mixture on silica gel gave compounds (Vb) with mp 199-200° and (Vb') with mp 190-193°.

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CONCLUSIONS

1. The synthesis has been accomplished of the methylamides of α-N-benzoyl-β-(N'-methyl-N"-phenylthiocarbamyl)propionic and butyric acids, which under the conditions used for dehydration of polypeptide chains by the Edman method through treatment with trifluoroacetic acid were converted into cyclic derivatives of hexahydropyrimidine.

2. Derivatives of D, L-erythro- and D, L-threo-diaminobutyric acid were readily and reversibly converted into one another under the action of acidic and basic reagents.

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