SYNTHESIS AND STRUCTURE OF N-CARBOXYANHYDRIDE AND OTHER DERIVATIVES OF α,β-DEHYDROTRYPTOPHAN

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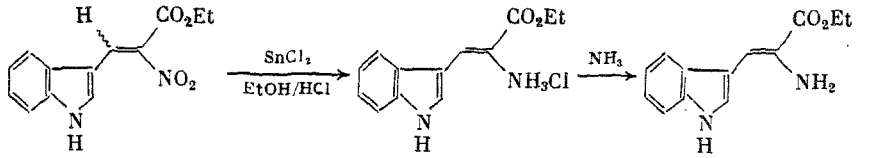
α,β-Dehydro-amino acids have been discovered in various peptide-like biogenic compounds: polypeptide-antibiotics, peptides of microbial origin, phytotoxic peptides, etc [1]. Recently, dehydroamino acids have been introduced into the composition of peptide hormones [2] to increase their activity. Great attention is being paid to the synthesis of α,β-dehydrotryptophan (1), which is responsible for the biological activity of telomycin antibiotics [3], A-128-00 [4], etc.

According to the general scheme of peptide synthesis, selectively removable (or transformable) protecting groups must be used for the temporary blocking of functional groups of amino acids. One of these paths, the classical Erlenmeyer azlactone method of synthesis, was found [5] to be ineffective for the preparation of Δ-tryptophan (I) derivatives because of the low yield of the corresponding Δ^2-oxazolin-5-ones. It should also be noted that in the preparation of dehydropeptides containing the residues of (I), very mild conditions should be used for removing the protective groups.

The present work deals with the preparation of certain derivatives of (I) and the study of their use for the synthesis of dehydropeptides.

It has already been shown [6, 7] that the catalytic reduction of the nitro group in the methyl ester of α-nitro-β-(3-indolyl)acrylic acid proceeds regioselectively over platinum group metals. The reduction of ethyl ester of α-nitro-β-(3-indolyl)acrylic acid (II) with $SnCl_2$ and HCl by the method in [7, 8] proceeds in a similar way



(II) (III)

The data of PMR and TLC on silica gel showed that the ethyl ester of Δ-tryptophan (III), obtained by the reduction of an equilibrium mixture [9] of Z- and E-isomers of (II), contains only one (Z) isomer. The singlet signal of the exocyclic CH= proton in the PMR spectrum of this compound is present in the same region (the chemical shift is equal to 6.81 ppm), as is the methyl ester of (I), the structure of which has been already established [7, 10] by ^1H and ^{13}C NMR spectroscopy and x-ray diffraction analysis.

Ester (III) reacts smoothly with various acylating agents (anhydrides of acetic and trifluoroacetic acids, 2-ethoxythiolan-4-one [11] and di-tert-butylpyrocarbonate) to form monoacyl derivatives (IVa-d). Alkaline hydrolysis of the ester group in (IVa) and (IVc) leads to acylated derivatives of Δ-tryptophan (Va, c). (See scheme on next page.)

When N-acetal-Δ-tryptophan (Va) is heated with Ac_2O, the azlactone ring is closed and simultaneously the NH group of the indole ring becomes acylated. The acetyl group is readily

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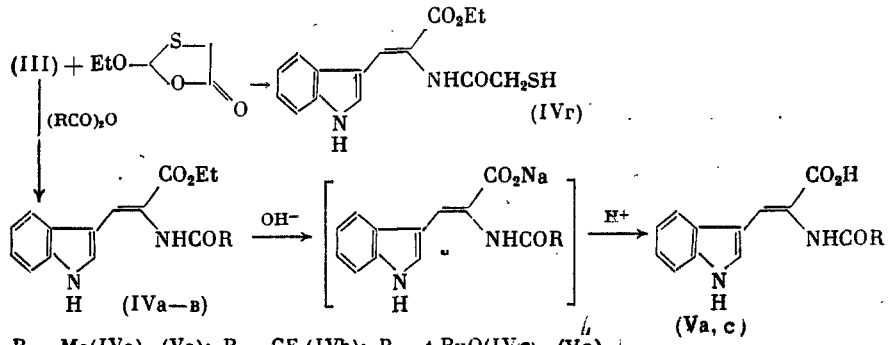
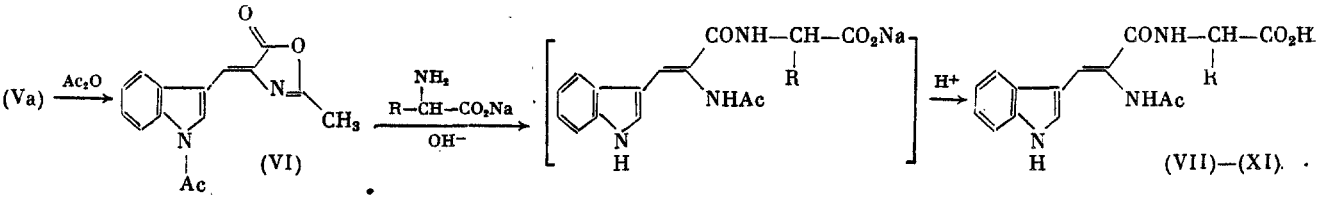


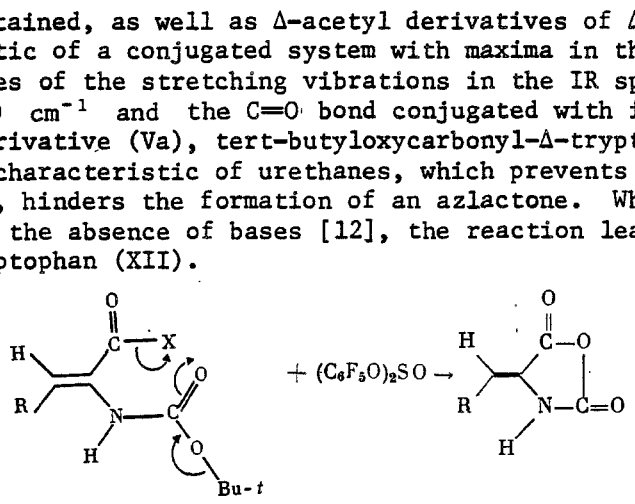
R = Me(IVa), (Va); R = CF_3(IVb); R = *t*-BuO(IVc), (Vc).

removed in an alkaline medium in the course of the synthesis of N-acetyldehydropeptides (VIIa-e)



Amino acids: S- and R-phenylalanine, S- and R-tryptophan, S-glutamic acid.

The dehydropeptides obtained, as well as Δ-acetyl derivatives of Δ-tryptophan (Va-d), have an absorption characteristic of a conjugated system with maxima in the region of 280 and 335 nm, which agrees with values of the stretching vibrations in the IR spectrum of these compounds: C=C bond (1615-1630 cm^{-1} and the C=O bond conjugated with it (1700-1730 cm^{-1}). In contrast to the N-acetyl derivative (Va), tert-butyloxycarbonyl-Δ-tryptophan (Vc) has an electron density distribution characteristic of urethanes, which prevents splitting of the acyl NH-proton. This, in turn, hinders the formation of an azlactone. When dipentafluorophenyl sulfite acts on (Vc) in the absence of bases [12], the reaction leads to the formation of N-carboxyanhydride of Δ-tryptophan (XII).



R= 3-Indolyl, X = OH.

Data of the ^{13}C NMR spectrum of carboxyanhydride (XII) in DMSO showed that the far spin–spin coupling constant (SSCC) in the $^{1}H-C=C-^{13}CO$ fragement does not exceed 1.5 Hz, which indicates a cis-orientation of the interacting nuclei, i.e., a Z-configuration of this compound. For the $^{13}CO-NH$ fragment, the SSCC value is equal to 6.5 Hz. The UV and IR spectra of (XII), which forms brightly yellow crystals, stable on prolonged storage, indicate a high degree of conjugation of the double bonds in this compound. It should also be noted that there are no known N-carboxyanhydrides of α,β-unsaturated amino acids. The N-carboxyanhydride of Δ^{Z}-tryptophan (XII) can be used for the synthesis of dehydropeptides.

EXPERIMENTAL

The PMR spectra were recorded on a "Bruker WP-200 SY" spectrometer with a working frequency of 200 MHz relative to TMS (δ = 0 ppm).

Ethyl Ester of Δ^{Z}-Tryptophan (III). By the method in [7], from 52 g of ethyl ester of α-nitro-β-(3-indolyl)acrylic acid and 157 g of $SnCl_2 \cdot H_2O$, 37.5 g (81.5%) of amino ester hydrochloride were obtained, and from it, 30.8 g (90%) of (III), mp 120-121°C (from CH_3CN). Found: N 12.15%. $C_{13}H_{14}N_2O_2$. Calculated: N 12.71%. IR spectrum (in $CHCl_3$, ν_{max}, cm^{-1}): 3440(NH_2), 1705 (CO), 1642 (C=C). UV spectrum (in EtOH), λ_{max}, nm 232 (log ε 4.32), 285 (3.84), 340 (4.35). PMR spectrum (in DMF-d_7, δ, ppm): 1.30 t and 4.23 q (3H and 2H, OC_2H_5), 6.81 s (1H, CH=), 11.38 s (1H, indole NH).

Ethyl ester of N-acetyl-Δ^Z-tryptophan (IVa) from amino ester (III) and Ac_2O, yield 90%, mp 171-172°C (cf. [13]).

Ethyl ester of N-trifluoroacetyl-Δ^Z-tryptophan (IVb) from (III) and $(CF_3CO)_2O$, yield 91%, mp 231-232°C (from CH_3CN). Found: F 17.27%. $C_{15}H_{13}N_2O_3F_3$. Calculated: 17.45%. IR spectrum (suspension in mineral oil, ν_{max}, cm^{-1}): 3200 (NH), 1735 and 1695 (CO), 1625 (C=C), 1150 (CF). UV spectrum (in DMSO, λ_{max}, nm): 293 (log ε 4.08), 330 (4.16). PMR spectrum (in DMF-d_7, δ, ppm): 1.17 t and 4.10 q (3H and 2H, OC_2H_5), 7.10 s (1H, CH=), 11.80 s (1H, indole NH).

Ethyl ester of N-tert-butyloxycarbonyl-Δ^Z-tryptophan (IVc) from (III) and di-tert-butylpyrocarbonate, yield quantitative, mp 156-157°C (from aq. DMF. Found: C 65.66; H 6.69%. $C_{18}H_{22}N_2O_4$. Calculated: C 65.43; H 6.71%. IR spectrum (suspension in mineral oil, ν_{max}, cm^{-1}): 3430, 3280 and 3230 (NH), 1695 and 1680 (CO), 1620 (C=C. UV spectrum (in EtOH), λ_{max}, nm): 227 (log ε 4.39), 278 (3.94), 338 (430).

Ethyl ester of N-mercaptoacetyl-Δ^Z-tryptophan (IVd) from (III) and 2-ethoxythiolan-4-one [11], yield 82%, mp 150-151°C. Found: C 59.41; H 5.26; N 8.90%. $C_{15}H_{16}N_2O_3S$. Calculated: C 59.20; H 5.30; N 9.20%. IR spectrum (suspension in mineral oil, ν_{max}, cm^{-1}): 3280 (NH), 1700 and 1680 (CO), 1620 (C=C). UV spectrum (in EtOH), λ_{max}, nm): 229.

N-Acetyl-Δ^Z-tryptophan (Va). A suspension of 2 g of (IVa) in 12 ml of aqueous (1:1) MeOH containing 1 g of NaOH was heated for 20 min at 70°C, the solution was poured onto ice and acidified by 5N HCl. The fine precipitate of the monohydrate of acid (Va) was separated, washed with water, and dried in vacuo. Yield, 1.85 g (96%), mp 232-233°C (from aq. DMF). Found: C 59.43; H 5.33; N 9.93%. $C_{13}H_{14}N_2O_4$. Calculated: C 59.53; H 5.38; N 10.68%. Anhydrous (Va) was obtained by heating the monohydrate over P_2O_5 in vacuo, mp 235-236°C. Found: C 63.88; H 5.00; N 11.37%. $C_{13}H_{12}N_2O_3$. Calculated: C 63.92; H 4.95; N 11.47%. IR spectrum (suspension in mineral oil, ν_{max}, cm^{-1}). 3340 and 3260 (NH), 1665 and 1615 (CO and C=C). UV spectrum (in i-PrOH, λ_{max}, nm): 227 (log ε 4.03), 277 (4.06), 336 (4.42). PMR spectrum (in DMF-d_6, δ, ppm): 2.15 s (3H, Ac) 7.80 s (1H, CH=), 9.25 s (1H, NHCO), 11.64 s (1H, indole NH).

4-(N)Acetylindol-3-yl-methylene)-2-methyl-Δ^Z-oxazolin-5-one (VI). A suspension of 6.3 g of (Va) monohydrate in 50 ml of Ac_2O was heated for 30 min at 80°C, and then evaporated in vacuo. Yield 5.8 g (92%), mp 203-204°C (from benzene); cf. [14].

A Typical Procedure for the Preparation of N-Acetyl-dehydropeptides. A 1.35 g portion (5 mmoles) of oxazolinone (V) was added, with stirring, to a solution of 5 mmoles of the α-amino acid in 50 ml of acetone, containing 5 ml of 1N NaOH. The mixture was heated to boiling, and left to stand overnight. After acetone has been removed in vacuo, the residue was treated by 20 ml of 2N NaOH, the mixture was acidified with 1N HCl, and the dehydropeptide precipitate was filtered and crystallized from aq. DMF.

N-Ac-Δ^Z-Trp-(S)-Phe-OH (VII). Yield 80%, mp 228-230°C, $[\alpha]_D^{25}$ +28.0° (c 1, Py). Found: C 67.59; H 5.58; N 10.62%. $C_{22}H_{21}N_3O_4$. Calculated: C 67.50; H 5.41; N 10.76%. IR spectrum (suspension in mineral oil, ν_{max}, cm^{-1}): 3350 and 3160 (NH), 1710 (COO), 1650, 1630 and 1605 (CO and C=C). UV spectrum (in EtOH, λ_{max}, nm): 228 (log ε 4.41), 278 (4.11), 336 (4.39). PMR spectrum (in DMF-d_7, δ, ppm): 2.13 s (3H, Ac), 3.17 d (2H, CH_2), 4.73 q (1H, CH), 7.81 s (1H, CH=), 9.19 s (1H, NHAc), 11.53 (1H, NH_{indole}).

N-Ac-Δ^Z-Trp-(R)-Phe-OH (VIII). Yield 81%, mp 230-232°C, $[\alpha]_D^{25}$ −28.5° (c 1, Py). Found: N 11.0%. $C_{22}H_{21}N_3O_4$. Calculated: N 10.76%. The spectral data are similar to those given above for (VII).

N-Ac-Δ^Z-Trp-(S)-Trp-OH (IX). Yield 75%, mp 246-247°C, $[\alpha]_D^{25}$ +42.0° (c 1, Py). Found: C 66.23; H 5.20; N 12.90%. $C_{24}H_{22}N_4O_4$. Calculated: C 66.92; H 5.15; N 13.01%. IR spectrum (suspension in mineral oil, ν_{max}, cm^{-1}); 3330 and 3160 (NH), 1728 (COO), 1650 and 1610 (CO and C=C). UV spectrum (in EtOH, λ_{max}, nm): 224 (log ε 4.78), 278 (4.16), 335 (4.32). PMR spectrum (in DMF-d_7, δ, ppm): 213 s (1H, Ac), 3.33 d (2H, CH_2), 4.80 q (1H, CH), 7.81 s (1H, CH=), 9.25 s, 10.89 s and 11.61 s (1H, NH).

N-Ac-Δ^Z-Trp-(R)-Trp-OH (X). Yield 72%, mp 250-252°C, $[\alpha]_D^{25}$ −41.5° (c 1, Py). Found: 13.07%. $C_{24}H_{22}N_4O_4$. Calculated: N 13.01%. The spectral data are similar to those given above for (IX).

N-Ac-Δ^Z-Trp-(S)-Glu-OH (XI). Yield 76%, mp 220-221°C, $[\alpha]_D^{25}$ +2.0° (c 1, Py). Found: C 57.93; H 5.10; N 11.01%. $C_{18}H_{19}N_2O_6$. Calculated: C 57.90; H 5.13; N 11.25%. IR spectrum (suspension in mineral oil, ν_{max}, cm^{-1}): 3340 and 3100 (NH), 1700 (COO), 1660 and 1610 (CO and

C=C). UV spectrum (in EtOH, λ_{max}, nm): 226 (log ε 4.29), 243 (4.26), 262 (4.25), 324 (4.21). PMR spectrum: in DMF-d_7, δ, ppm: 2.36 s (3H, Ac), 5.00 (1H, CH), 10.48 s (1H, NHAc), 11.60 s (1H, NH).

N-tert-Butyloxycarbonyl-Δ^z-tryptophan (Va) was obtained in the same way as the acetyl derivative, yield, 94%, mp 229-230°C (from aq. DMF). Found: C 63.36; H 6.34; N 9.15%. $C_{18}H_{22}N_2O_4$. Calculated: C 63.56; H 6.00; N 9.27%. IR spectrum (suspension in mineral oil, ν_{max}, cm^{-1}): 3400 and 3230 (NH), 1690 (COO), 1620 (C=C). UV spectrum (in EtOH, λ_{max}, nm): 227 (log ε 4.45), 278 (3.97), 335 (4.23). PMR spectrum (in DMF-d_7, δ, ppm): 1.45 s (9H, CH_3), 7.20 m (2H, CH=), 7.55 d (1H, CH=), 7.80 d (1H, CH=), 7.85 s (1H, CH=), 7.97 s (1H, CH=) 8.05 s (1H, NHCO), 11.65 s (1H, indole NH).

Reaction of N-tert-Butyloxycarbonyl-Δ^z-tryptophan (Vc) with Dipentafluorophenyl Sulfite. A 1.0 g portion of dipentafluorophenyl sulfite was added to a solution of 0.75 g of (Vc) in 2 ml of DMF. The mixture was evaporated to the beginning of crystallization, diluted with 10 ml of ethyl acetate, and the precipitate was filtered. The yield of the light yellow crystals of N-carboxyanhydride of Δ^z-tryptophan (XII) was 0.46 g (82%), mp 252°C (from DMF). Found: C 63.06; H 3.68; N 12.30%. $C_{12}H_8N_2O_3$. Calculated: C 63.10; H 3.53; N 12.28%. IR spectrum (suspension in mineral oil, ν_{max}, cm^{-1}): 3340 and 3200 (NH), 1815, 1805 and 1740 (CO), 1660 (C=C). UV spectrum (in DMSO), λ_{max}, nm): 282 (log ε 3.84), 376 (4.32). PMR spectrum (in DMF-d_7, δ, ppm): 7.20 s (1H, CH=), 7.30 m (2H, 2CH=), 7.55 d (1H, CH=), 7.95 d (1H, CH=) 8.35 s (1H, CH=), 12.10 s (1H, indole NH). ^{13}C NMR spectrum in DMSO-d_6, δ, ppm): 151.0 (COO), 162.2 (CONH), etc.

When N-tert-butyloxycarbonyl derivative (Vc) was boiled in Ac_2O, an unchanged product (Vc) was obtained.

CONCLUSIONS

1. N-acetyldehydrotryptophan peptides were obtained by the azlactone method from the Na-salts of the corresponding amino acids.

2. N-Carboxyanhydride of Δ^z-tryptophan is formed by the action of dipentafluorophenyl sulfite on tert-butyloxycarbonyl-Δ^z-tryptophan in the absence of bases.

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