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SYNTHESIS AND SOME PHARMACOLOGICAL PROPERTIES OF N-BENZOYL-α,β-DEHYDRODIPEPTIDES

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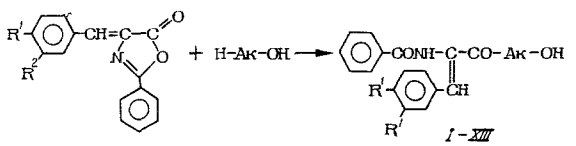
Some derivatives of α,β-dehydroaminoacids possess antitumor activity or are inhibitors of the CNS [12]. In an attempt to explore new physiologically-active materials we synthesized the N-benzoyl-α,β-dehydrodipeptides I-XV, containing phenylalanine or tyrosine residue and studied their pharmacological properties.

The synthesis of dehydropeptides I-XIII was accomplished by the azlactone method.



R^1=H (I—IV), OCH_3 (V—VIII), OC_3H_7-*i* (XI), CH_3COO (XII, XIII); R^2=H (I—VIII, XI—XIII), $R^1+R^2=OCH_2O$ (IX—X); Ak* = β-Ala (I, V, IX, XI, XII), β-Ph-β-Ala (II), γ-Abu (III, VII), ε-Aca (IV, VIII), β-Me-β-Ala (VI), DL-Met (X), D-Ala (XIII)

The derivatives of N-benzoyl-α,β-dehydrotyrosine (XIV and XV) were synthesized by removal of the acetyl group from the corresponding O-acetyl derivatives XII and XIII in 2 N sodium hydroxide solution.

The yields and physicochemical data for compounds I-XV are presented in Table 1.

To test the influence of the N-terminal amide group on the biological activity of derivatives of α,β-dehydro-O-alkyltyrosine, p-methoxy- and p-isopropoxy-cinnamoyl-β-alanine (XVI and XVII, respectively) were synthesized by the Schotten-Baumann method.

The structures of the compounds obtained were confirmed by IR and ^{1}H NMR data. In the IR spectra of dipeptides I-XV were maximum absorptions at 1625-1640 cm^{-1} and 1710-1730 cm^{-1} for the amide and acid carbonyl groups, respectively. The frequency of the NH valence oscillations of the amide groups occurred in the 3240-3310 cm^{-1} region. In the case of the O-acetyl derivatives of tyrosine XII and XIII, the IR spectra also showed a band at 1750 cm^{-1} for the ester carbonyl of the acetyl group.

The ^{1}H NMR spectra of all the synthesized α,β-dehydrodipeptides I-XV showed singlet signals for the CH group of the dehydroaminoacid residue in the 7.18-7.25 ppm range which indicates the Z-configuration for the synthesized compounds [11].

The results of the pharmacological studies (Table 1) show that the greater part of the dipeptides I-XV, as well as the cinnamoyl-β-alanines XVI and XVII provide morphine activity. Analysis of the data shows that the introduction of the alkoxy groups into the benzene ring of the phenylalanine residue of the dipeptides leads to the appearance of opiate antagonistic activity (compare compound I with peptides V, IX, XI, and also IV with VIII). However,

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TABLE 1. Physicochemical and Biological Characteristics for Dehydropeptides I-XV*

Compound	% Yield	mp, °C	Rf (A)	Rf(B)	Empirical formula	Antagonist activity, %*
I	80,6	218—220	0,59	0,89	$C_{19}H_{18}N_2O_4$	0
II	58,8	183—185	0,65	0,88	$C_{25}H_{22}N_2O_4$	18,6
III	65,4	214—216	0,53	0,65	$C_{20}H_{20}N_2O_4$	24,2
IV	73,6	132—134	0,68	0,66	$C_{22}H_{24}N_2O_4$	0
V	97,1	241—243	0,61	0,64	$C_{20}H_{20}N_2O_5$	70,0
VI	81,1	202—203	0,58	0,63	$C_{21}H_{22}N_2O_5$	46,6
VII	87,7	222—224	0,55	0,68	$C_{21}H_{22}N_2O_5$	18,9
VIII	93,9	154—156	0,62	0,65	$C_{23}H_{26}N_2O_5$	23,4
IX	96,1	238—239	0,43	0,83	$C_{20}H_{18}N_2O_6$	37,4
X	41,6	159—160	0,64	0,86	$C_{22}H_{22}N_2O_6$	39,0
XI	85,8	189—191	0,66	0,82	$C_{22}H_{24}N_2O_5$	25,7
XII	97,1	202—204	0,35	0,87	$C_{21}H_{20}N_2O_6$	0
XIII	61,23	208—209	0,41	0,85	$C_{21}H_{20}N_2O_6$	0
XIV	95,6	215—216	0,26	0,78	$C_{19}H_{18}N_2O_5$	0
XV	94,9	136—137	0,30	0,80	$C_{19}H_{18}N_2O_5$	0

* The antagonist activities of compounds XVI and XVII were 38.7 and 28.4%, respectively, while the standard preparation (Naloxone) was 95%.

derivatives of tyrosine-containing peptides with O-acetyl or free hydroxyl groups do not show morphine activity (compounds XII-XV). In the case of phenylalanine-containing dipeptides, transition from the C-terminal β-alanine to γ-aminobutyric acid leads to the appearance of the desired activity (compounds I and III), but further elongation of the carbon chain of the C-terminal amino acid residue to ε-aminocarboxylic acid gives loss of activity (compound IV). Comparison of compounds V and XI with XVI and XVII shows that removal of the N-terminal benzamide group does not lead to the disappearance of the antagonistic properties. Even in the case of N-benzoyl-α,β-dehydro-O-methyltyrosyl-β-alanine (V), this structural change leads to a perceptible decrease in activity (compounds V and XVI).

By the example of N-benzoyl-α,β-dehydro-3,4-dioxymethylenephenylalanine dipeptides it was established that the exchange of the C-terminal β-alanine for DL-methionine does not give a perceptible change in antagonistic properties (compounds IX and X).

Data on the anticonvulsant and antibacterial properties of dipeptides I-XV are also presented in the Experimental Part.

EXPERIMENTAL (CHEMICAL)

The chemical purity of the compounds obtained was monitored by TLC on Silufol UV-254 plates in the solvent systems A) chloroform—acetone (1:1) or B) propanol—water (7:3) with visualization by UV-light or iodine.

The IR spectra were recorded on an UR-20 spectrometer, and the ^1H NMR spectra on a Varian T-60 instrument. Application of the azlactone method was carried out analogously to [9]. Elemental analysis data corresponded with the calculated values.

N-Benzoyl-α,β-dehydrodipeptides (I-XIII). To a solution of 15 mmoles of sodium hydroxide in 60 ml of 1:1 aqueous acetone was added 15 mmoles of the corresponding azlactone and the mixture was stirred at room temperature for 48 h. The reaction mixture was diluted with water to 150 ml, the unreacted azlactone was filtered off and the filtrate was acidified with dilute hydrochloric acid to pH 1.0. The resulting precipitate was filtered off, washed with water, dried, and recrystallized from ethanol or ethyl acetate.

The yields and physicochemical properties of dipeptides I-XIII are presented in Table 1.

N-Benzoyl-α,β-dehydrotyrosylalanines (XIV and XV). A mixture of 10 mmoles of O-acetylated dehydrodipeptide XII or XIII and 30 mmoles of sodium hydroxide in 15 ml of water was stirred at room temperature for 24 h, diluted with water to 100 ml, and acidified with dilute hydrochloric acid to pH 1.0. The resulting precipitate was filtered off, washed with water, dried, and recrystallized from ethanol.

The physicochemical data for dipeptides XIV and XV are presented in Table 1.

N-p-Alkoxycinnamoyl-β-alanines (XVI and XVII) were synthesized by an analogous method [2] in 1:1 dioxane—water.

N-p-Methoxycinnamoyl-β-alanine (XVI). Yield 47.8%, mp 136-137°C (from a mixture of ethyl acetate–hexane). R_f(A) 0.48, (B) 0.82. IR spectrum, ν, cm^{-1}: 1630, 1660, 1710, 3280. ^1H NMR spectrum (DMSO d_6), δ, ppm: 2.51 t (2H, α-CH_2, β-Ala); 3.40 q (2H, β-CH_2, β-Ala); 3.81 s (3H, OCH_3); 6.43 d (1H, CH); 7.16 m (4H, aromatic protons); 7.33 d (1H, CH); 8.00 t (1H, NH). $C_{13}H_{15}NO_4$.

N-p-iso-Propoxycinnamoyl-β-alanine (XVII). Yield 65.8%, mp 127-129°C (from a mixture of tetrahydrofuran–hexane), R_f(A) 0.51, (B) 0.86. IR spectrum, ν, cm^{-1}: 1650, 1670, 1700, 3265. ^1H NMR spectra (acetone–d_6), δ, ppm: 1.06 d (6H, 2·CH_3); 2.43 + (2H, α-CH_2, β-Ala); 3.43 q (2H, β-CH_2, β-Ala); 4.53 m (1H, CHO); 6.48 d (1H, 6H); 7.15 m (4H, aromatic protons); 7.40 t (1H, NH); 7.50 d (1H, CH). $C_{15}H_{19}NO_4$.

EXPERIMENTAL (BIOLOGICAL)

Local anesthetic activity was studied on anesthesia brought about by concentrations of 0.25% on isolated frog nerves [1]. Novocaine (procaine hydrochloride) was used as control.

The topical anesthetic activity of the compounds in the form of 1% solution was determined on rabbit eye corneas by the method of Ren [3]; the results obtained were compared with dicain (tetracaine hydrochloride).

Central anesthetic action of the compounds was studied by means of the "hot plate" method in doses of 30 mg/kg [6]; the control was morphine.

The opiate antagonism activity (suppression of the analgetic activity of morphine in doses of 7 mg/kg ED_{99}) was conducted on the basis of the mechanical stimulation of rats' tails in doses of 10 mg/kg [8], using naloxone as control.

The dehydrodipeptides I-XV and the cinnamoyl-β-alanines XVI and XVII did not show local anesthetic, topical anesthetic, or central anesthetic activity. Data on the morphine antagonist activity of these compounds is summarized in Table 1.

The antispasmodic activity was studied on non-hybrid white mice weighing 18-22 g. The spasms were induced by maximal electroshock and convulsions were induced by the introduction of korazol (pentylenetetrazole), nicotine, and arecoline [4, 7, 13, 14]. The compounds were introduced over 30 min before injection of the convulsive agent and applying electric stimulation. The average effectiveness and the toxic dose according to [10] were determined for the most active compounds. Zarontin (ethosuximide) was used as the control preparation. It was established that dipeptides I-XV were devoid of central N- and M-cholinolytic properties, i.e., did not influence the nicotinic spasms and the arecholinic tremors. Anti-electroshock properties also were absent from these compounds. However, this series showed antagonism to korazol. With respect to the korazol clonic convulsions, compounds III, X, and XII were 40% effective in doses of 200 mg/kg. Compounds I, XIII, and XIV prevented the korazol clonic spasm in 60% of the animals at the same dose. The most interesting among the studied materials was dipeptide I, for which the 50% effective dose for antagonism to korazol (ED_{50}) was 220 (163-297) mg/kg mass of the animal; the ED_{50} of zarontin was 150 mg/kg at 50% of the lethal dose LD_{50} 2200 (1692-2860) mg/kg, with $p = 0.05$. The therapeutic index of compound I was sufficiently high: $TI = LD_{50}/ED_{50} = 10$.

The antibacterial action of the compounds was studied on the basis of generalized staphylococcal and dysenteric infections of white mice [5] stimulated by intraperitoneal injection of the test cultures, leading to 100% death of untreated control animals. We used the Smith strain of staphylococcus and the Flexner strain 6858 of dysentery bacteria. The compounds were introduced in a single injection of 1500 pg/kg. The activity was evaluated as the overall prolongation of the life of the animals. Compounds increasing the lifetime of the mice under conditions adequate to distinguish from the control according to the χ^2 criterion were considered to have antibacterial activity. In experiments on healthy mice we determined the transportability of the test compounds by one-time oral administration. The experiments were carried out on 290 white non-hybrid mice weighing 17-19 g. Introduction of the subject compounds in a dose of 2000-3000 mg/kg did not produce a change in the behavior or the condition of the animals. The chemotherapeutic action of compound I appeared as an increase in longevity of the infected animals with staphylococcal as well as with dysentery infection by 30-40% ($p < 0.001$). Introduction of an alkoxy group in the benzene ring of the phenylalanine residue of dipeptide I produced selective activity of the compounds. Thus, for dipeptide V, activity was shown only for staphylococcal infection (40%, $p < 0.001$), and compound XI, for dysentery infection (40%, $p < 0.001$). However, the analog of peptide I

with the dioxymethylene group (IX) was completely devoid of antibacterial properties. The same was observed for dipeptides II, III, IV, and VIII.

The tyrosine-containing dipeptides XII-XV somewhat increased the lifetimes of the mice by 10-30% ($p < 0.01$-0.001), depending upon compound and infecting strain. Compound VII increased the life of the infected animals by 30% ($p < 0.001$) for the case of dysentery infection.

This series of dehydrodipeptides therefore shows antagonism with respect to narcotic analgesics as well as antispasmodic and antibacterial properties.

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ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF 1,3,5-TRIFERROCENYL-t-(1-FERROCENYLETHENYL)CYCLOHEXENE

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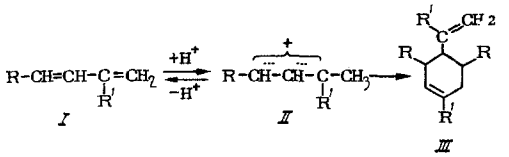
Organic compounds containing the ferrocenyl substituent in the molecule are often biologically active substances [1]. In recent years, we have developed a reaction for proton-catalyzed cyclodimerization of 1,3-diferrocenyl- and 1,3-arylferrocenyl-1,3-butadienes, which results in the production of 1,3,4,5-tetrasubstituted derivatives of cyclohexane (III) [2-4, 13].



I, II, III: a — R=R¹=Fc; b — R=Fc, R¹=Ph; c — R=Ph, R¹=Fc; d — R=Fc, R¹=p-$MeOC_6H_4$; e — R=p-$MeOC_6H_4$, R¹=Fc; Fc=$C_5H_5FeC_5H_4$

The starting ferrocenyl-1,3-butadienes (Ia-d) have low stability in most cases, and are difficult to obtain in pure form. We have found [13] that in place of dienes Ia-e, the corresponding ferrocene-containing allylic alcohols are suitable for use in the synthesis of compounds IIIa-e.

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