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SYNTHESIS AND BIOLOGICAL ACTIVITY OF β-ACYLHYDRAZIDES OF AROYLPYRUVIC ACIDS

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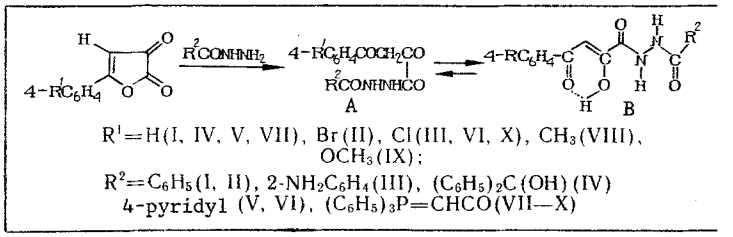
It was established earlier that amides of aroylpyruvic acids are characterized by a wide spectrum of biological actions, including antimicrobial [4], anti-inflammatory [1, 2], analgetic [1], and antispasmodic [5] activity. These compounds may also be used as intermediate materials for the synthesis of dyes, pesticides, and various other nitrogen-containing biologically-active compounds [3]. The structurally-related β-benzoylpyruvoylhydrazide of benzoylpyruvic acid possesses bacteriostatic effects with respect to strains of Staphylococcus aureus and Eschericia coli [6]. Physiologically active α-phenyl-β-benzoylhydrazides of aroylpyruvic acid are also known [7].

In an attempt to find new active compounds among the substituted hydrazides of aroylpyruvic acids, we prepared β-acylhydrazides of aroylpyruvic acids (I-X) by reaction of 5-aryl-2,3-dihydro-2,3-furandiones with various acylhydrazines.

The starting materials for the synthesis of compounds VII-X were the hydrazides of triphenyphosphoranylidinepyruvic acids (XI) obtained by hydrazinolysis of the ethyl esters of these acids (XII):



R^1=H(I, IV, V, VII), Br(II), Cl(III, VI, X), CH_3(VIII), OCH_3(IX);
R^2=C_6H_5(I, II), 2-$NH_2C_6H_4$(III), $(C_6H_5)_2C(OH)$(IV) 4-pyridyl (V, VI), $(C_6H_5)_3P{=}CHCO$(VII—X)

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TABLE 1. Physicochemical Characteristics and Antimicrobial Activity of Compounds I-X and XII

Compound	Yield, %	mp, °C (dec)	Empirical Formula	Minimal Inhibitory Concentration (MIC), μg/ml	
				E. coli M_{17}	S. aureus P-209
I	86	165—166*	$C_{17}H_{14}N_2O_4$	500	250
II	93	182—183	$C_{17}H_{13}BrN_2O_4$	250	125
III	92	201—202	$C_{17}H_{14}ClN_3O_4$	500	250
IV	78	178—179	$C_{24}H_{20}N_2O_5$	1000	1000
V	87	177—178	$C_{16}H_{13}N_3O_4$	250	250
VI	86	215—216	$C_{16}H_{12}ClN_3O_4$	500	500
VII	79	212—213	$C_{31}H_{25}N_2O_5P$	1000	1000
VIII	72	203—204	$C_{32}H_{27}N_2O_5P$	500	500
IX	84	218—219	$C_{32}H_{27}N_2O_6P$	500	500
X	77	205—206	$C_{31}H_{24}ClN_2O_5P$	500	500
XI	72	235—236†	$C_{21}H_{19}N_2O_2P$	500	500
XIII	75	264—265	$C_{31}H_{25}N_4O_3P$	Inact.	500
Mercuric Chloride	—	—	$HgCl_2$	1000	1000
Ethacridine Lactate	—	—	$C_{18}H_{21}N_3O_4$	2000	500

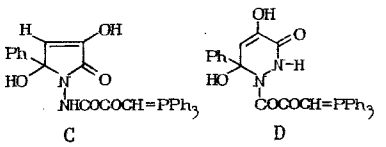
* Lit. mp 162-163°C (dec) [12].

† Melts without decomposition.

The physicochemical and spectral properties of some aroylhydrazides of aroylpyruvic acids, including I, are presented in the literature [12], and the constants for compounds III-X are given in Table 1. The structures of the compounds obtained were verified by data from IR and ^1H NMR spectroscopy, mass spectrometry, and elemental analysis.

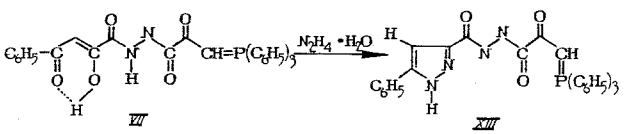
Compounds I-X exist in DMSO-d_6 solution mainly in the enol form B, as indicated by the ^1H NMR singlets for the methine proton C^3H at 7.05-7.27 ppm and a signal for the hydroxyl (C^2-OH) at 10.22-10.77 ppm. This coincides with literature data which claims that as a rule, aroylpyruvic acid derivatives, including β-aroylhydrazides, exist in the enol form with respect to the carbonyl group in position 2 by means of an intramolecular hydrogen bond [11, 12]. Moreover, the presence in the ^1H NMR spectra of compounds I-X of singlets for the two protons of the C^3H_2 group at 4.58-4.84 ppm requires the presence of the noncyclic 2,4-diketo-form A to the extent of 5-15%, judging by the intensity of the signals, which does not contradict the literature data [12].

The mass spectrum of hydrazide VII does not show a peak for the molecular ion, but has peak for ion fragments with the following significant mass numbers (relative intensity, %): 361(3) $(C_6H_5)_3P=CHCOCONHNH^+$, 303 (11) $(C_6H_5)_3P=CH-C\equiv O^+$, 278 (22) $(C_6H_5)_3PO^+$, 262 (4) $(C_6H_5)_3P^+$, 201 (6) $(C_6H_5)_2PO^+$, 174 (7) $C_6H_5CCH_2COC\equiv NH^+$, 146 (11) $C_6H_5CO-CH=C=O^+$, 119 (7) $C_6H_5COCH_2^+$, 105 (46) $C_6H_5CO^+$, 77 (35) $C_6H_5^+$, 58 (100) $H_2N-N=C=O^+$, 57 (63) $HN=N-C\equiv O^+$, 43 (72) $HNCO^+$. A peak with m/z of 103 ($C_6H_5CN^+$) was absent, and also the presence of a peak with mass number 146 allowed the elimination of the alternative structures 2,5-dihydro-2-pyrrolone (C) and 1,2,3,6-tetrahydro-3-pyridazinone (D), which agrees with literature data [12].



C D

The action of hydrazine hydrate on compound VII led to the formation of the heterocyclization product: the triphenylphosphoranylidinepyruvoyl hydrazide of 5-phenyl-3-pyrazole carboxylic acid (XIII), the constants and spectral characteristics of which are given in Experimental.



VII XIII

EXPERIMENTAL (CHEMICAL)

The IR spectra of the compounds obtained were determined in vaseline oil pastes with an UR-20 spectrometer. The ^1H NMR spectra were determined with a RY-2310 instrument (60 MHz)

TABLE 2. Antiviral Activity of Compounds I, IV, and V with Respect to Activation by Type A and B Groups in Experiments on Developing Chicken Embryos

Indicator	Type A Virus Group				Type B Virus Group			
	compound			remantadine	compound			adapromin
	I	IV	V		I	IV	V	
Ratio of Decrease in Number of Infected Embryos (Protection Ratio)	1,2	1,2	1,4	2,6	1,9	2,5	1,8	3,3
Index of Effectiveness, %	17	17	29	61	47	63	44	70
Comparative Evaluation of Compound and Standard Activities	±	±	±	+++	++	+++	++	+++

TABLE 3. Antiviral Activity of Compounds IV and V Relative to Activation by Type A and Type B Groups in Experiments on Mice

Indicator	Type A Virus Group		Type B Virus Group		
	compound V	remantadine	compound		adapromin
			IV	V	
Increase of Lifetime of Animals in Treated Group, Days	1,2	4,3	2,8	0,6	2,6
Ratio of Decrease in Number of Diseased Mice (Protection Ratio)	1,6	3,4	4,7	1,5	4,6
Index of Effectiveness	37	97	79	33	78
Comparative Evaluation of Compound and Standard Activities	+	++++	+++	+	+++

in DMSO-d_6, using HMDS as internal standard. The mass spectra were obtained with a Varian MAT-311 instrument using direct introduction, an emission current of 1000 mA, an ionization energy of 70 eV, and an injection temperature of 170°C. The homogeneity of the materials was determined on Silufol UV-254 plates with the following eluents: benzene-ether (3:2) and benzene-ether-acetone (10:9:1), with visualization with iodine or UV-light.

The characteristics of the new compounds are presented in Table 1. Elemental analyses data corresponded with the calculated values.

β-Acylhydrazides of Aroylpyruvic Acids (II-X). To a solution of 2 mmoles of 5-aryl-2,3-dihydro-2,3-furandione in 70 ml of benzene was added with stirring 2 mmoles of the corresponding carboxylic acid hydrazide, and the mixture was heated to boiling. After cooling the precipitate was filtered off and recrystallized from ethanol of toluene.

Hydrazide of Triphenylphosphoranylidinepyruvic Acid (XI). To a solution of 3.76 g (10 mmoles) of ethoxalylmethylenetriphenylphosphorane (XII) [9] in 80 ml of ethanol was added 1.0 ml of 70% aqueous hydrazine solution and the mixture was boiled for 2 h. The solvents were evaporated and the residue was recrystallized from aqueous ethanol to give 2.60 g (72%) of compound XI, mp 235-236°C, IR spectrum, ν, cm^{-1}, crystals: 3395, 3345-3360 (NH_2, NH), 1663 (CONH), 1615 (P – – – C – – – C – – – O), 1442, 1470-1480 (P – C_6H_5). ^1H NMR spectrum, δ, ppm (DMSO-d_6): 4.24 (2H, b, NH_2), 7.45-7.85 (16H, m, $3C_6H_5$, CH), 9.15 (1H, b, NH).

Triphenylphosphoranylidinepyruvoyl Hydrazide of 5-Phenyl-3-pyrazole Carboxylic Acid (XIII). To a suspension of 0.54 g (1 mmole) of β-triphenylphosphoranylidenepyruvoyl hydrazide of benzoylpyruvic acid VI in 30 ml of ethanol was added 0.2 ml of 70% aqueous hydrazine solution and the mixture was heated to boiling. After cooling the precipitate was filtered off and recrystallized from toluene to give 0.40 g (75%) of compound XIII, mp 264-265°C (dec). IR spectrum, ν, cm^{-1}, crystals 3280-3310 (amide); 1654 (CONH). ^1H NMR spectrum, δ, ppm (DMSO-d_6): 7.10-7.85 (23H, m, $4C_6H_5$, 2CH, NH), 9.85 (1H, b, NH), 13.60 (1H, b, ring NH).

EXPERIMENTAL (BIOLOGICAL)

The antimicrobial, antiviral, anti-inflammatory, and antispasmodic activity of the synthesized compounds was investigated.

The acute toxicity LD_{50} of the compounds was determined by the method of L. N. Pershin [14] by intraperitoneal and peroral introduction into white mice weighing 18.24 g in the form of 2% starch paste suspensions.

The antimicrobial activity compared with standard strains of E. coli M_{17} and S. aureus P-209 was determined by the standard 2-fold serial dilution method in beef bouillon [14] with a bacterial load of 250 thousand microbe units per ml of solution. The activity of the

dose was evaluated as the minimal inhibitory concentration (MIC) of the compound, i.e., the maximum dilution resulting in complete suppression of test microbe development. The activity of the prepared compounds was compared with that of mercuric dichloride (corrosive sublimate) [13, 16] and ethacridine lactate (acrinol), an antimicrobial preparation used in medicine [13].

The antiviral activity of the prepared compounds was studied by comparison with induced influenzas of types A and B conducted in experiments on developing chicken embryos and on white mice charged with a virus group according to the scheme given in "Method Directions" approved by the USSR Ministry [10]. The activity of the compounds was evaluated by determining the number of culture tubes with cytopathogenic action (CPA), judged by the protection factor, the index of effectiveness, and comparison with known preparations such as remantadine and adapromin [10] (Tables 2 and 3).

The anti-inflammatory activity was studied on the basis of the acute inflammation edema produced by subplantar introduction of 0.1 ml of 1% aqueous solution of carragenin in the posterior foot of white rats weighing 180-200 g (according to "Method Recommendations for Experimental Study of Nonsteroidal Anti-inflammatory Materials" approved by the Pharmacological Committee of the Ministry of the SSSR, 11 November 1982, Protocol No. 22). The anti-inflammatory activity was evaluated by the stage of inhibition of exudation (in % of control) after intraperitoneal introduction of the compound in a dose of 50 mg/kg as a suspension in 2% starch paste; the effect was compared with amidopyrine [8].

The antispasmodic activity was evaluated by means of the maximum electroshock method [15].

The acute toxicity of the studied compounds exceeded 500 mg/kg; they were of extremely low toxicity compared with preparations of similar antimicrobial activity such as mercuric dichloride (LD_{50} 3.9 mg/kg [16]), ethacridine lactate (LD_{50} 70.0 mg/kg), as well as amidopyrine, possessing anti-inflammatory activity (LD_{50} 249 mg/kg) [8].

The antimicrobial study of compounds I-XI and XIII (Table 1) established that they possessed weak bacteriostatic activity with a MIC of 250 to 1000 μg/ml.

Compounds I, IV, and V were practically non-toxic (white mice well endured a one-time oral introduction of a 1000 mg/kg dose), and possessed significant antiviral activity against viruses of group types A and B (Tables 2 and 3). The highest anti-inflammatory activity was shown by compound I, which was active in a dose of 50 mg/kg (half as much as amidopyrine); some were lower in effectiveness (inhibition of exudation 43.4%; for amidopyrine 60.0% [8]). Acylhydrazides I-IV and phosphoranes XI and XII did not show antispasmodic activity in doses up to 600 mg/kg.

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

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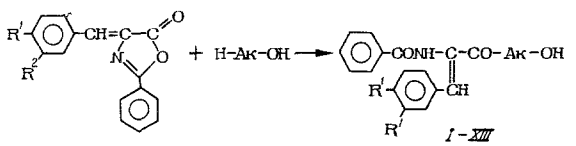
Some derivatives of α,β-dehydroaminoacids possess antitumor activity or are inhibitors of the CNS [12]. In an attempt to explore new physiologically-active materials we synthesized the N-benzoyl-α,β-dehydrodipeptides I-XV, containing phenylalanine or tyrosine residue and studied their pharmacological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

A. L. Mndzhoyan Institute of Fine Organic Chemicals, Armenian Academy of Sciences, Erevan. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 26, Nos. 7-8, pp. 31-34, July, 1992. Original article submitted May 28, 1991.

