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SYNTHESIS AND BIOLOGICAL PROPERTIES OF DERIVATIVES OF IMIDAZO[1,2-f]PURINE

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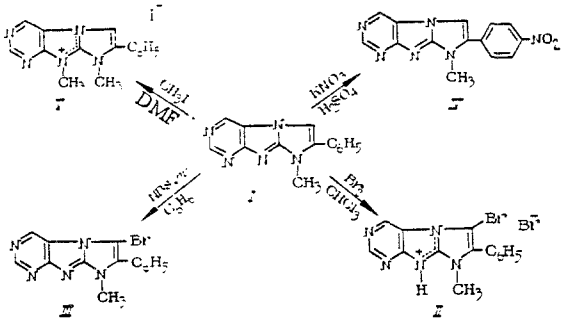
Derivatives of imidazo[1,2-f]xanthine have distinct biological activities [4, 6, 11, 12]. It seemed of interest to study the complex of biological properties and their changes in compounds in which the tricyclic system was changed from a xanthine to a purine derivative. In continuation of our systematic research into the synthesis of derivatives of imidazo[1,2-f]-purine [3, 10] we have studied the behavior of 1-methyl-2-phenylimidazo[1,2-f]purine (I) in some electrophilic reactions and investigated the biological properties of the synthesized compounds.



On reaction of (I) with bromine in chloroform the hydrobromide of 1-methyl-2-phenyl-3-bromoimidazo[1,2-f]purine (II) is formed. The IR and PMR spectra of the compound give evidence in favor of the proposed structure. In the IR spectrum there is a rather strong C—Br band at 500 cm^{-1}, and in addition there is also an absorption band at 1685 cm^{-1}, which may arise from the protonated heteroaryl nitrogen atom [8]. In the PMR spectrum the singlet at 8.09 ppm of the proton at C(3) of (I) disappears. The rest of the PMR spectrum shows a characteristic series of signals corresponding with the assigned structure. In the PMR spectrum of compound II the proton at N(9) is exchanged for deuterium and consequently not recorded.

In the mass spectrum of compound II the M^+ peak corresponding with the calculated molecular weight is not found. This is explained by the fact that hydrohalide and alkylhalide salts under the conditions of electron impact form the ions HHal and AlkHal, corresponding with the process M^+—HHal → $(M-HHal)^+$ or M^+—AlkHal → $(M-AlkHal)^+$ [1, 9, 13]. In the case under consideration these are the recorded peaks of the ions of m/z 327 and 329 (the isotopic distribution of Br is 1:1). Moreover, in the spectrum ions which correspond with HHal and HAlk are recorded. In the mass spectrum this is a series of ions of m/z 79-82.

Bromination experiments with I in glacial acetic acid at 45-50°C did not give the expected results. This is most likely explained by deactivation of the system as a result of protonation.

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On bromination of I with N-bromosuccinimide (irradiation with UV light) the 3-bromo derivative (III) is formed, in the PMR spectrum of which the singlet of the proton at the C(3) atom is also absent.

The recorded mass number of M^+ confirms the presence of a bromine atom in molecule III and corresponds with a monosubstituted product with the fragmentation ions $M-CH_3-N^+\equiv CC_6H_5$ (m/z 209 and 211) and $CH_3N^+\equiv C-C_6H_5$ (m/z 118); this is evidence in favor of the fact that in starting molecule I only the 3-position is substituted. In addition that fact is confirmed by the registration of the ion $(Br-C\equiv C-C_6H_5)^+$ (m/z 180 and 182). The presence of the methyl substituent at the N(1) atom is responsible for the removal of the CH_3 group directly from M^+ (ions of m/z 312 and 314). Just as for the majority of halogen substituted heteroaryls, the elimination of bromine from M^+ (m/z 248) takes place also. Repeated elimination of neutral HCN particles from the ion $(M-Br)^+$ is characteristic of purine derivatives – ions of m/z 221 and 194 [14, 15].

The ion of m/z 129 arises from removal of the particle $CH_3-N^+\equiv C-C_6H_5$ from the ion $(M-HBr)^+$.

Nitration of compound I with potassium nitrate in concentrated sulfuric acid at 0-5°C leads to the formation of 1-methyl-2(p-nitrophenyl)imidazo[1,2-f]purine (IV). In the IR spectrum the absorption bands caused by the vibrations of the NO_2 group are weakly displayed because they are superimposed on the vibrations of the imidazo[1,2-f]purine nucleus [10]. The presence of the NO_2 group can indirectly be judged by the CNO absorption band at 640 cm^{-1}.

PMR spectral data give evidence of the fact that nitration of I leads to the p-nitrophenyl derivative (compound IV). In the mass spectrum of IV an M^+ peak is recorded which corresponds with the molecular weight of a mononitro substituted compound (m/z 294). Presence of the ions $(CH_3-N^+\equiv C-C_6H_4-NO_2=p)$ (m/z 163), $(HC\equiv C-C_6H_4NO_2)^+$ (m/z 147), and $(M-CH_3N^+\equiv C-C_6H_4-NO_2-p)$ (m/z 131) fully confirms the data of the PMR spectra.

Reaction of (I) with methyl iodide in DMF yields the methoiodide of 1-methyl-2-phenylimidazo[1,2-f]purine (V).

In the mass spectrum of compound V an M^+ peak is recorded of the ion $(M-CH_3I)^+$ (m/z 249) [2, 9, 13]. The ions of m/z 142 $(CH_3I)^+$ and of m/z 127 (I^+) point to the formation of salt V. PMR spectral data do not contradict structure V. Quaternization of the nitrogen N(9) of imidazole, but not of the nitrogen atoms of the pyrimidine ring can explain the much higher basicity of the pyrimidine nitrogen atom that is present at the imidazole ring. In the case of proposed structure V it is possible that delocalization of the positive charge with partial transfer of it to atom N(4) is possible and considerably stabilizes the produced salt, which is practically impossible in case of quaternization of the nitrogen atoms of the pyrimidine ring.

In the IR spectra of compounds II-V absorption bands are found that are characteristic of the vibration of the imidazo[1,2-f]purine ring: νCH_{atom} 3030-3100; $\nu C=N$ 1640-1610; $\nu C=C$ 1600-1610, 1540, and 1530 cm^{-1}, and also a characteristic group of absorption bands at 1465, 1360, and 1280 cm^{-1}, which is specific for the purine ring.

In the UV spectra of compounds I and II there are two intensive absorption bands with maxima in the regions 204-208 and 256-262 nm.

In the UV spectrum of compound V there is a maximum at 222 nm (log ε 4.33) in addition to the absorption in the regions mentioned. A shoulder with a maximum at 292 and 238 nm in compounds II and V, respectively, arises in connection with the quaternization of the purine nitrogen atom.

EXPERIMENTAL CHEMICAL

IR spectra were recorded from KBr disks on a UR-20 (GDR) instrument. UV spectra were recorded on a Specord UV-VIS instrument from $5\cdot10^{-5}$ M solutions in 95% ethanol. PMR spectra were taken with a Hitachi R-20A spectrometer operating at 60 MHz from solutions in DMSO-d_6 with TMS as internal standard. The temperature of the ampul during recording was 130°C. Mass spectra were recorded on a Variant MAT-311A instrument with direct injection of the sample into the ion source. Recording conditions were standard: accelerating voltage 3 kV, cathode emission current 300 μA, ionization voltage 70 eV (in the mass spectra ion peaks with an intensity of ≥ 10% of I_{max} are listed). TLC was carried out on Silufol UV-254 plates and were visualized with iodine vapor.

1-Methyl-2-phenylimidazo[1,2-f]purine was prepared by the method of [10]. IV spectrum, λ_{max}, nm (log ε): 204 (4.26), 259 (4.37). See [10] for the mass spectrum.

Hydrobromide of 1-Methyl-2-phenyl-3-bromoimidazo[1,2-f]purine (II). This was prepared with quantitative yield by reaction of an equivalent of bromine with a chloroform solution of I at room temperature. mp 251-252°C (dec.). UV spectrum, λ_{max}, nm (log ε): 208 (4.41), 256 (4.38), 292 (4.05). Found, %: C 41.35, H 3.01; N 17.27, Br 39.51. $C_{14}H_{10}BrN_5 \cdot HBr$. Calculated, %: C 41.2, H 2.7, N 17.16, Br 39.16. PMR spectrum, δ, ppm: 3.6 [s, 3H, N(1)—CH_3], 9.03 [s, 1H, C(5)H], 9.27 [s, 1H, C(7)H], 7.58 (broad s, 5H, C_6H_5). Mass spectrum (evaporation temperature of the sample 175°C), m/z (I, %): 50 (13), 51 (44), 63 (19), 65 (12), 67 (12), 75 (14), 76 (16), 77 (78), 79 (33), 80 (100), 81 (35), 87 (100), 89 (41), 91 (13), 102 (14), 103 (44), 104 (49), 115 (13), 118 (46), 129 (29), 150 (22), 151 (20), 163.5 (15), 164.5 (16), 207 (10), 209 (38), 211 (34), 247 (15), 248 (16), 312 (15), 314 (15), 327 (99), 328 (42), 329 (99), 330 (28); W_M = 11.3.

1-Methyl-2-phenyl-3-bromomidazo[1,2-f]purine (III). A mixture of 2.5 g (0.01 mole) of I, 1.95 g (0.011 mole) of N-bromosuccinimide, and 100 ml of benzene was refluxed for 8 h. The benzene was evaporated to dryness under vacuum. The residue was treated with an aqueous sodium bicarbonate solution, filtered, and washed with water. Yield 2.2 g (67%) of III, mp 258-259°C (dec., from a 1:1 DMF—dioxane mixture). Found, %: C 51.28, H 3.5, N 21.02, Br 24.72. $C_{14}H_{10}BrN_4$. Calculated, %: C 51.23, H 3.07, N 21.34, Br 24.35. PMR spectrum, δ, ppm: 3.71 [s, 3H, N(1)—CH_3], 8.91 [s, 1H, C(5)H], 9.13 [s, 1H, C(7)H], 7.65 (broad s, 5H, C_6H_5). Mass spectrum (evaporation temperature of the sample 165°C), m/z (I, %): 51 (38), 52 (20), 56 (16), 58 (20), 63 (23), 65 (19), 67 (17), 68 (12), 69 (83), 75 (14), 76 (14), 70 (70), 78 (14), 81 (58), 89 (21), 95 (13), 103 (39), 104 (37), 109 (16), 164 (15), 209 (34), 211 (38), 247 (15), 248 (12), 314 (15), 327 (100), 328 (40), 329 (100), 330 (40): W_M = 10.6.

1-Methyl-2-p-nitrophenylimidazo[1,2-f]purine (IV). One gram (4 mmole) of I was dissolved in 30 ml of concentrated sulfuric acid (d 1.82), cooled to 0-5°C, and 0.41 g (4 mmole) of potassium nitrate was added. The mixture was stirred at room temperature for 4 h and poured out into 200 ml of water. After neutralization with a concentrated NH_4OH solution to weak alkaline reaction the precipitate was filtered off. Yield 1.1 g (93%), mp 299-301°C (dec., from a DMF—n-propanol mixture; yellow needles). Found, %: C 57.26, H 3.8, N 28.76, $C_{14}H_{10}N_6O_2$. Calculated, %: C 57.14, H 3.4, N 28.56. PMR spectrum, δ, ppm: 3.9 [s, 3H, N(1)—CH_3], 8.6 [s, 1H, C(3)H], 8.96 [s, 1H, C(5)H], 9.21 [s, 1H, C(7)H], 7.25 (q, 4H, AB system, J 13.5 Hz). Mass spectrum (evaporation temperature of the sample 180°C), m/z (I, %): 63 (21), 65 (9), 66 (11), 75 (18), 76 (18), 77 (23), 89 (24), 101 (11), 102 (16), 103 (18), 116 (14), 117 (12), 128 (15), 130 (15), 140 (13), 221 (12), 233 (25), 247 (14), 248 (86), 249 (18), 264 (26), 294 (100), 295 (25); W_M = 10.2

Methoiodide of 1 methyl-2-phenylimidazo[1,2-f]purine (V). To a suspension of 1.02 g (4 mmole) of I in 30 ml of DMF was added 1.2 ml (2 mmole) of methyl iodide and the mixture was refluxed for 10 min. Two thirds of the volume of solvent was evaporated under vacuum and the precipitate was filtered off. Yield 1.3 g (83%), mp 241-243°C (dec., from a DMF—n-propanol mixture; gold-colored plates). UV spectrum, λ_{max}, nm (log ε): 204 (4.33), 222 (4.32), 261 (4.34), 298 (4.11). Found, %: C 46.16, H 4.1, N 17.78. $C_{15}H_{14}N_5I_1$. Calculated, %: C 46.04; H 3.6, N 17.9. PMR spectrum, δ, ppm: 3.95 [s, 3H, N(1)—CH_3], 3.40 [s, 3H, N(9)—CH_3],, 8.45 [s, 1H, C(3)H], 9.33 [s, 1H, C(5)H], 9.56 [s, 1H, C(7)H], 7.55 (broad s, 5H, C_6H_5). Mass spectrum (evaporation temperature of the sample 175°C), m/z (I, %): 77 (25), 102 (11), 104 (14), 111 (14), 118 (8), 124.5 (13), 127 (20), 234 (17), 248 (15), 249 (100), 250 (18); W_M = 16.8. R_f 0.56 in the system n-butanol—formic acid—water 77:10:13, and 0.45 in the system n-butanol—acetic acid—water 2:1:1.

EXPERIMENTAL BIOLOGICAL

Biological investigation of the synthesized compounds was carried out with the following tests: determination of the acute toxicity (LD_{50}), investigation of the neurotropic action, and study of the bacteriostatic and mycostatic activities.

The acute toxicity (LD_{50}) of the compounds studied was determined in experiments with white mice of both sexes weighing 18-27 g. The preparations were administered intraperitoneally as 3-5% fine suspensions in water stabilized with Tween-80. Each dose was tested in 5-7 animals. Observation was carried out during 14 days after administering the compound under investigation. The LD_{50} was calculated by Kerber's method [2] (see Table 1).

TABLE 1. Influence of Compounds I, IV, and V on the Prolongation of the Narcotic Sleep of White Rats

Compound	LD_{50}, mg/kg	Dose, mg/kg	Prolongation of the narcotic sleep	
			min	% of control
Control	—	—	81,3±4,84	100
I	155,0±18,39	10	45,0±5,7	55,35
		15,5	174,14±6,55	214,19
		31	199,7±6,48	245,63
IV	270,0±32,7	15	233,14±3,86	286,77
		31	188,0±6,41	231,24
V	405,0±18,39	10	134,0±4,78	164,82
		20	178,85±5,32	219,98
		40	153,3±3,63	188,56
Aminazine	—	5	111,95±4,7	137,7
Caffeine sodium benzoate	—	10	68,0±6,44	83,64

The investigations carried out demonstrated the relatively low toxicity of the studied imidazo[1,2-f]purine derivatives.

The neurotropic activity was studied by the method of prolongation of the action of subnarcotic doses of barbiturates [5].

The influence of the new imidazopurine derivatives on the duration of the pentobarbital sodium induced sleep was studied with intact male white rats of the Wistar strain weighing 170-250 g at doses averaging 1/10-1/20 of the LD_{50}. Each dose of the preparation was tested in seven animals. The preparations under investigation were injected intraperitoneally as 3-5% fine suspension in water stabilized with Tween-80 at 30 min before administering pentobarbital sodium at a dose of 30 mg/kg. The prolongation of the narcotic sleep was judged by the time during which the animals were lying on their sides, that is from the moment they had lost the reflex to turn over.

In the applied dose all the compounds studied considerably increase the soporific action of pentobarbital sodium, on an average by 64.8-186.8% in comparison with the control. At a dose of 10 mg/kg compound I produces a sharply pronounced analeptic effect and shortens the time of the narcotic sleep by 44.7% in comparison with the control.

Comparison of the data obtained with the chemical structures of the compounds studied leads to the assumption that the mechanism of their action is connected with the blocking of a number of microsomal and nonmicrosomal enzyme systems correspoding with the inactivation of barbiturates, for example xanthine oxidase or monoamine oxidase. Low doses of the compounds studied cannot block the enzyme systems and, on the contrary, can activate them, as takes place in the case of compound I.

Together with distinct neuroleptic activity, the compounds studied also show antispasmotic activity, increasing or decreasing spasms caused by Corazol, strychnine or Cordiamin. In all cases they prolong the latent period of onset of the spasms 2-3 times.

The antimicrobial and mycostatic activities of the compounds were studied by the method of twofold serial dilutions on a liquid culture medium [7] with a spectrum including 11 strains of microorganisms. The experiments carried out showed that of the compounds studied only compound II has mycostatic activity with regard to the fungi Microsporum lanosum and Trichophyton mentagrophites at a concentration of 250 μg/ml. Thus it has been shown by the performed experiments that derivatives of imidazo[1,2-f]purine are promising in terms of search for novel biologically active compounds and potential medicines among them.

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 1α-HYDROXYVITAMIN D_3 1-BUTYRATE

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Vitamin D esters have aroused definite interest among researchers. Vitamins D_2 and D_3 are found in animal tissues [7] in the form of fatty acid esters, significant amounts of which are contained in fish liver [10]. The advantage of this form of ester is that it is marked by a greater stability compared with the free vitamin [17] and probably supplies a reserve source in the organism. A series of investigations has, however, established that esterification of D vitamins leads to a decrease in their biological activity. Thus, the antirachitic activity of the acetate, palmitate, and butyrate of ergocalciferol for growing rats corresponds to 59, 61, and 72% of the activity of ergocalciferol [5]. Similar results were obtained for rats, chickens, and Japanese quail in studies of the activity of cholecalciferol and its various fatty acid esters [14]. One of the main reasons for the decrease of biological activity of the vitamin after its esterification may be its weakened capability for absorption in the small intestine of animals [6]. This conclusion is supported by data [8] according to which vitamin D_3 palmitate or the free vitamin, when introduced parenterally, shows equal activity. There is little published data regarding the esters of vitamin D metabolites and their analogs.

There are indications that the active hormonal form of vitamin D_3 – 1α,25-dihydroxyvitamin D_3, as well as its analogs 1α-hydroxyvitamin D_3 and 1α,24,25-trihydroxyvitamin D_3 – are converted into esters in the organism [12, 15]. The structure of these esters, however, is not clear, and their biological activity has not been investigated.

To address the question of whether blockage of the 1α-hydroxy group by a radical of fatty acid preserves the biological activity of the secosteroid, we synthesized 1α-hydroxyvitamin D_3 1-butyrate (I) and studied its biological activity.

In studying the effect of this ester on chickens, we compared it with the effect of 1α-hydroxyvitamin D_3 (1α-OH-D_3), taking into consideration changes in the process of ab-

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