SYNTHESIS AND RADIOPROTECTANT PROPERTIES OF SULFUR-CONTAINING DERIVATIVES OF NATURAL PURINE METABOLITES

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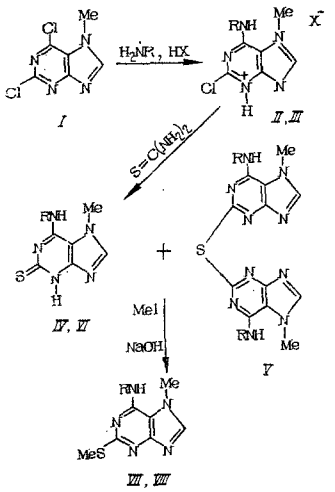
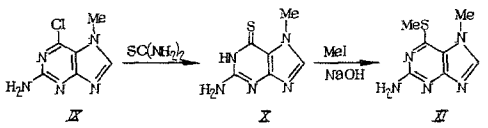
Continuing a search for potential radioprotectant compounds amongst derivatives of purine metabolites, we have synthesized and examined in mice a series of 7-methyl-2-thio-derivatives of adenine and hypoxanthine, and 6-thioguanine.

The adenine derivatives were obtained as follows:



In view of the low reactivity of the chlorine in 7-methyladenines, these compounds were converted into their salts (II, III). The formation of positively-charged carbonium ions on protonation enhances the electrophilicity of the molecule, and facilitates replacement of the chlorine by sulfur. In addition to 2-thioadenine (IV), condensation of the salt (II) with thiourea gave some of the sulfide (V). 2-Amino-6-chloro-7-methylpurine (IX) gave 6-thio-7-methylguanine (X) and 2-amino-6-methylthio-7-methylpurine (XI).



The structures of the compounds obtained were confirmed by their elemental analyses and their PMR spectra.

In order to compare the pharmacological activities of the compounds mentioned above with those of analogs differing in the positions and numbers of S- and N-methyl groups, the following compounds were synthesized and examined: 2-methylthioadenine (XII), its 9-methyl analog (XIII), 2-methylthio-3,7-dimethyladenium salt (XIV), 2,6-bismethylthiopurine (XV), and 2-amino-6-methylthio-3,7-dimethylpurinium salt (XVI).

The toxicities and radioprotectant properties of the compounds are shown in Table 1.

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TABLE 1. Toxicity and Radioprotectant Activity of 7-Methyl-2-thio Derivatives of Adenine, Hypoxanthine, and Their Analogs

Compound	LD_{50}, mg/kg	Dose of drug, mg/kg	Number of test animals	Survival, %
IV	590	100	20	0
		200	20	0
VI	490	100	20	0
		200	20	0
		300	20	10
VII	950	300	20	0
		500	20	0
VIII	1630	100	40	0
		200	20	0
		300	20	0
X	130	10	20	0
		30	20	0
XI	440	50	20	0
		100	60	30
		200	60	30
XII	560	50	20	10
		100	40	35
		300	40	40
XIII	1150	100	20	0
		200	20	5
		400	20	10
XIV	780	100	40	30
		200	40	10
		300	20	0
XV	49	10	40	20
XVI	218	10	20	0
		25	20	0
		50	20	10
XVII	1320	150	30	0
		300	20	0
		500	20	0
XVIII	1470	150	20	0
		300	20	0
		500	20	30
Control		—	170	5

It will be seen from Table 1 that the least toxic of the compounds are the sulfur-containing hypoxanthines 2-thio- and 2-methylthio-7-methylhypoxanthine (XVII and XVIII). Introduction of a mercapto-group into the 6-position increases toxicity (X). However, methylation of this compound at sulfur reduces the toxicity (XI).

Introduction of a methylthio-group into the 2-position of purine (XV) results in a marked increase in toxicity.

It will also be seen from Table 1 that (XI) and (XII) have weak protectant activity in comparison with 2-thioadenine and 6-thioguanine. The remaining compounds were inactive.

EXPERIMENTAL (CHEMICAL)

UV spectra were obtained on an SF-6 instrument (LOMO, Leningrad), in water. PMR spectra were obtained in CF_3COOH on a Tesla BS 487C instrument, 80 MHz (Chechoslovakia), internal standard HMDS. 2-Methylthioadenine (XII) and its 9-methyl analog (XIII) were obtained as described in the literature [5, 8], and 2-methylthio-3,7-dimethyladeninium salt (XIV) and the isomeric 2-amino-3,7-dimethyl-6-methylthiopurinium salt (XVI) as described in [4]. 7-Methyl derivatives of 2-thio- and 2-methylthiohypoxanthine (XVII, XVIII) were synthesized as described in [7].

2-Chloro-7-methyladenine Hydrochloride (II). A mixture of 4.25 g of 2-chloro-7-methyladenine [6] and 8.5 ml of concentrated HCl was stirred for 20 min at room temperature, then diluted with 200 ml of acetone to give 4.85 g (96%) of the salt (II), mp 277°C. λ_{max}, nm (log ε): 274-276 (4.03). Found, %: Cl 32.00; N 29.87. $C_6H_6ClN_5 \cdot HCl$. Calculated, %: Cl 32.27; N 29.41.

2-Thio-7-methyladenine (IV). A mixture of 4.85 g of the salt (II), 1.94 g of thiourea, and 242 ml of n-butanol was boiled with stirring for 6.5 h. The product, initially white

in color, became yellow, and was filtered off, washed with methanol, and treated twice with 100 ml of 25% ammonia solution. The insoluble residue (0.3 g) was isolated, and reprecipitated from hot acetic acid with ammonia to give (V), mp above 320°C, λ_{max}, nm (log ε): 2.46 (4.40), 262-268 (4.36, inflexion). Found, %: S 9.98; 10.04; C 43.01. $C_{12}H_{12}N_{10}S$. Calclated, %: S 9.76; C 42.68.

The filtrate after separation of (V) was concentrated to 80 ml, and neutralized with acetic acid. The solid which separated (2.75 g, 67%) was reprecipitated from ammonia with acetic acid to give pure (IV), mp 279°C, λ_{max}, nm (logε): 244 (4.32), 284 (4.33) (pH 1). Found, %: S 17.89. $C_6H_6N_5S$. Calculated, %: S 17.68.

2-Methylthio-7-methyladenine (VII). 2-Thioadenine (IV) (2 g) was dissolved in 22 ml of 0.5 N NaOH, 0.8 ml of MeI added, shaken until solution was complete, and the solution kept for several hours. A precipitate of (VI) separated (1.6 g, 74.2%), and this was crystallized from water with charcoal, mp 268-270°C, λ_{max}, nm (log ε): 244 (4.30), 288-290 (4.06) (pH 1), PMR, δ, ppm: 2.73 s (SCH_3), 4.43 s (N^7CH_3), 8.48 m (NH_2), 9.222 s (C^8H). Found, %: S 16.23; N 35.50. $C_7H_9N_5S$. Calculated, %: S 16.41; N 35.89.

2-Chloro-6-hydroxyethylamino-7-methylpurine Hydrobromide (III). A mixture of 6 g of 2,6-dichloro-7-methylpurine (I) [2], 3.55 ml of ethanolamine, and 56 ml of water was boiled with stirring for 2 h. The mixture was cooled, and the yellow precipitate of 2-chloro-6-hydroxyethylamino-7-methylpurine (6.18 g, 91.9%) which separated was filtered off and crystallized from water, mp 229-231°C, λ_{max}, nm (log ε): 280 (4.20). Found, %: Cl 15.02. $C_8H_{10}N_5OCl$. Calculated, %: Cl 15.60.

The base (6.18 g) was dissolved with heating in 10.2 ml of 20% HBr, 50 ml of absolute ethanol added, and the precipitated salt washed with alcohol to give 7.42 g (88.5%) of the salt (III).

2-Thio-6-hydroxyethylamino-7-methylpurine (VI). A mixture of 7.42 g of (III) hydrobromide, 2.8 g of thiourea, and 150 ml of absolute alcohol was boiled with stirring for 6 h. The product (VI) separated on cooling (4.17 g, 50.4%). Crystallization from aqueous alcohol gave mp 210°C, λ_{max}, nm (log ε): 236 (4.32), 282 (4.18). PMR spectrum, δ, ppm: 4.37 s ($N^7C\ H_3$), 4.02 (broadened signal for C^6NH), 9.04 s (C^8H). Found, %: N 30.87; S 14.70. $C_8H_{11}N_5OS$. Calculated, %: N 31.11; S 14.22.

2-Methylthio-6-hydroxyethylamino-7-methylpurine (VIII). The 2-thiopurine (VI) (4.17 g) was dissolved in 50 ml of 0.5 N NaOH, and 1.5 ml of MeI added. The mixture was shaken until the MeI dissolved, and kept at room temperature for one day. The solid which separated was crystallized from water to give 2.43 g (82%), mp 229-232°C, λ_{max}, nm (log ε): 240-242 (4.30), 288 (4.05). PMR spectrum, δ, ppm: 2.66 s (SCH_3), 4.27 s (N^7CH_3), 383 (unresolved triplet, NCH_2), 4.67 (unresolved triplet, OCH_2), 8.02 (broadened signal for C^6NH), 8.82 s (C^8H). Foung, %: N 29.70; C 45.50. $C_9H_{13}ON_5S$. Calculated, %: N 29.29; C 45.18.

6-Thio-7-methylguanine (X). A mixture of 3.25 g of 2-amino-6-chloro-7-methylpurine (IX) [3] and 2.7 g of thiourea in 61 ml of absolute alcohol was boiled with stirring for 12 h. The solid which separated was reprecipitated from 0.1 N alkali (with charcoal) with acetic acid to give 2.44 g (76%) of yellow product (X), λ_{max}, nm (log ε): 258 (3.83), 350 (4.13) (pH 1). Found, %: S 18.01. $C_6H_7N_5S$. Calculated, %: S 17.68.

2-Amino-6-methylthio-7-methylpurine (XI). A mixture of 1 g of 7-methyl-6-thioguanine (X) and 22 ml of 0.5 N NaOH was shaken at room temperature for 20 min with 0.7 ml of MeI. The starting material dissolved after 5 min, and the (XI) then gradually separated (0.67 g, 62.2%). Crystallization from alcohol with charcoal gave mp 227-229°C C, λ_{max}, nm (log ε): 276 (4.07), 318 (4.13) (pH 1). PMR, δ, ppm: 2.76 s (SCH_3), 4.25 s (N^7CH_3), 8.83 s (C^8H). Found, %: S 16.62; N 35.52. $C_7H_9N_5S$. Calculated, %: S 16.41; N 35.90.

EXPERIMENTAL (PHARMACOLOGICAL)

The radioprotectant and toxic properties of the compounds obtained were examined in mongrel female mice weighing 18-24 g. Toxicities were determined in nonirradiated mice by the method of Litchfield and Wilcoxon [1].

To examine the radioprotectant properties of the compounds, the animals were irradiated once with X-rays in a dose of 7 Gy in an RUM-17 apparatus, under the following conditions: voltage 200 kV, current strength 15 mA, filters 0.5 Cu + 1.0 Al, focussing distance 50 cm, dose rates 1.12 and 0.91 Gy/min.

The compounds were administered as aqueous solutions or Tween emulsions, intraperitoneally, 10-15 min before irradiation. Radioprotectant activity was assessed by the survival of the animals to the 30th day following irradiaton.

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF CARBOXYLIC ACID DERIVATIVES CARRYING SULFUR-CONTAINING GROUPS IN THE β-POSITION

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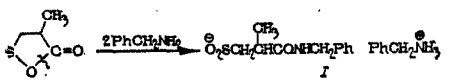
We have previously reported [3] the synthesis of new heterocyclic compounds: 1,2-oxathiolan-5-one 2-oxides and 2,1-benzoxathiol-3-one 1-oxide. In the present work, we have studied reactions involving splitting of these compounds by certain nucleophilic reagents. As in the case of cyclic anhydrides of β-sulfocarboxylic acids [9], their splitting by nucleophiles does not occur at the O–SO bond, but at the O–CO bond with the formation of the corresponding derivatives of β-(hydroxysulfinyl)carboxylic acids. Thus, reactions of 4-methyl-1,2-oxathiolan-5-one 2-oxide and 2,1-benzoathiol-3-one 1-oxide with amines led to the corresponding amides of β-(hydroxysulfinyl)isobutyric and o-(hydroxysulfinyl)benzoic acids (I-II) in the form of salts with these amines (Table 1).



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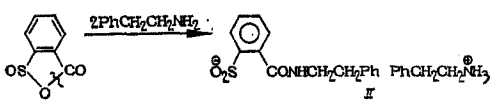
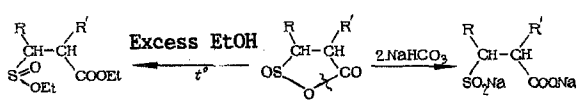


II

The splitting of 1,2-oxathiolan-5-one 2-oxide by alcohol in ether also proceeds at the O–CO bond with the formation of ethyl ester of β-(hydroxysulfinyl)propionic acid, which under more rigorous conditions in the presence of a large excess of alcohol is esterified to a diester (III). As should have been expected, anhydrides of β-(hydroxysulfinyl)carboxylic acids are readily saponified by sodium bicarbonate with the formation of the corresponding disodium salts (IV-V) (see Table 1)



III IV, V

III: R = R′ = H; IV: R = CH_3, R′ = H; V: R = H, R′ = CH_3

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