SUBSTANCES WITH ANTINEOPLASTIC ACTIVITY. XLIX.*
N-[δ-(6-PURINYLTHIO)VALERYL]GLYCYLGLYCYLAMINO ACIDS AND THEIR DERIVATIVES

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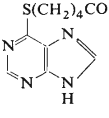
Condensation of N-[δ-(6-purinylthio)valeryl]glycylglycine with ethyl esters of amino acids and an ester of diglycine, using N,N′-carbonyldiimidazol, or N,N′-dicyclohexylcarbodiimide, led to esters *Ia*—*Va*. Compounds *Ia*—*IIIa* and *Va* were saponified to tripeptides *I*—*III* and to the tetraglycine derivative *V*. Compounds *Ia* and *IVa* showed an antineoplastic effect in animals with a transplantable tumour.

In connection with an investigation of relationships between structure and efficiency of N-[δ-(6-purinylthio)valeryl]amino acids, -dipeptides and their derivatives it had been observed before that the amino acid or peptide moiety of the molecule influences significantly the affinity of the compounds for tissues of certain organs and their therapeutical efficiency toward various transplantable tumours in mice and rats[1]. In view of the pronounced biological activities of the ethyl ester of N-[δ-(6-purinylthio)valeryl]triglycine[1] we were interested to see what neoplastic activity might be found in analogues formed by replacing the terminal glycine with a residue of another amino acid, or to what extent this activity might be altered by extending the triglycine chain by another molecule of glycine. In this connection we prepared the tripeptides *I*—*III*, the tetraglycine derivative *V* and the ethyl esters *Ia*—*Va* (Table I).



For synthesizing the above esters we used first the carbodiimide method (Method A) employing N-[δ-(6-purinylthio)valeryl]glycylglycine[1] (*VI*) as the starting compound. Condensation of *VI* with the ethyl ester of L-leucine, or with the diethyl ester of L-glutamic acid by means of N,N′-dicyclohexylcarbodiimide in dimethylformamide

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TABLE I

N-[δ-(6-Purinylthio)valeryl]glycylglycylamino Acids and Their Derivatives

No	R	Method (yield, %)	M.p., °C (solvent)	Formula (m.w.)	Calculated/Found % C	% H	% N	% S	UV Spectra λ_{max} (log ε) 0·1M-HCl	0·1M-NaOH
I	—NH—CH(CH(CH$_3$)$_2$)—COOH -DL	C (87)	162—164 (water)	$C_{19}H_{27}N_7O_5S$ (465·5)	49·02 / 49·32	5·84 / 5·94	21·07 / 21·03	6·89 / 7·21	217 (4·01) 289 (4·28)	224 (4·02) 289 (4·22)
Ia	—NH—CH(CH(CH$_3$)$_2$)—COOC$_2$H$_5$ -DL	B (75)	116—118 (ethanol)	$C_{21}H_{31}N_7O_5S$ (493·6)	51·10 / 50·92	6·33 / 6·46	19·87 / 19·63	6·49 / 6·79	217 (4·02) 289 (4·27)	222 (4·00) 289 (4·22)
II	—NH—CH(CH$_2$CH(CH$_3$)$_2$)—COOH -L[a]	C (87)	147—150 (water)	$C_{20}H_{29}N_7O_5S$ (479·6)	50·08 / 49·80	6·10 / 5·93	20·45 / 20·59	6·69 / 6·87	224 (3·84) 289 (4·25)	222 (4·08) 291 (4·25)
IIa	—NH—CH(CH$_2$CH(CH$_3$)$_2$)—COOC$_2$H$_5$ -L[b]	A (57)	155—157 (acetone)	$C_{22}H_{33}N_7O_5S$ (507·6)	52·05 / 51·89	6·55 / 6·70	19·32 / 18·99	6·32 / 6·45	222 (3·88) 294 (4·23)	224 (4·03) 291 (4·23)
III	—NH—CH(CH$_2$COOH)—COOH -L[c]	C (95)	146—147 (aqueous acetone)	$C_{18}H_{23}N_7O_7S.H_2O$ (499·5)	43·28 / 43·90	5·05 / 5·00	19·63 / 19·37	6·42 / 6·46	218 (4·11) 294 (4·20)	225 (4·07) 293 (4·18)
IIIa	—NH—CH(CH$_2$—COOC$_2$H$_5$)—COOC$_2$H$_5$ -L[d]	B (83)	121—123 (ethanol)	$C_{22}H_{31}N_7O_7S$ (537·6)	49·15 / 49·12	5·81 / 5·85	18·24 / 18·20	5·96 / 6·28	217 (4·09) 289 (4·39)	224 (4·06) 289 (4·29)
IVa	—NH—CH(CH$_2$CH$_2$COOC$_2$H$_5$)—COOC$_2$H$_5$ -L[e]	A (98) B (36)	172—174 (aqueous acetone)	$C_{23}H_{33}N_7O_7S$ (551·6)	50·08 / 50·18	6·03 / 6·30	17·78 / 17·51	5·81 / 5·99	220 (4·02) 291 (4·29)	223 (4·11) 291 (4·26)
V	—NHCH$_2$CONHCH$_2$COOH	C (80)	250—252 (water)	$C_{18}H_{24}N_8O_6S$ (480·5)	44·99 / 44·52	5·04 / 5·10	23·32 / 23·07	6·67 / 6·99	221 (3·90) 292 (4·22)	225 (4·05) 290 (4·26)
Va	—NHCH$_2$CONHCH$_2$COOC$_2$H$_5$	B (89)	258—262 dimethylsulfoxide–water	$C_{20}H_{28}N_8O_6S$ (508·6)	47·23 / 47·53	5·55 / 6·54	22·04 / 21·93	6·30 / 6·08	220 (3·89) 291 (4·22)	225 (4·03) 290 (4·23)

[a] $[\alpha]_D^{20} = 0° \pm 2°$ (*c* 0·5, 0·1M-NaOH); [b] $[\alpha]_D^{20} = -10·6°$ (*c* 1, 80% aqueous ethanol); [c] $[\alpha]_D^{20} = +8·5°$ (*c* 0·5, 0·1M-NaOH); [d] $[\alpha]_D^{20} = -3°$ *c* 1, 90% aqueous ethanol); [e] $[\alpha]_D^{20} = -5°$ (*c* 1, 95% aqueous ethanol).

afforded the crude ester *IIa* or *IVa*, which contained a considerable amount of the corresponding N-acylated urea derivative, N-[δ-(6-purinylthio)valerylglycyl-glycyl]-N,N'-dicyclohexylurea (*VII*; see formula, $R = N(C_6H_{11})CONHC_6H_{11}$) and of N,N'-dicyclohexylurea. The desired compound was separated by column chromatography on silica gel, using a mixture of chloroform and ethanol (9 : 1) for elution. The formation of acylated urea *VII* could be somewhat suppressed by carrying out the reaction at 0° – 5°C, using an excess (3 molar equivalents) of the amino acid ester. Conducting the reaction in dichloromethane or acetonitrile was practically without effect. Esters *Ia*, *IIIa* – *Va* were prepared by the N,N'-carbonyl-diimidazol method (Method B) which produced much better results with this type of compounds. It is an advantage of the method that even the crude esters are rather pure and that all the other components of the reaction mixture (imidazol, dimethyl-formamide) may be readily separated on the basis of different water solubility. Esters *Ia* – *IIIa* and *Va* were saponified to the tripeptides *I* – *III* and to the tetra-peptide *V* with sodium hydroxide: for esters *Ia* and *Va* at 20°C, for esters *IIa* and *IIIa* at 0 – 5° (Method C).

In an orientation survey of compounds *I* – *III*, *V* and *Ia* – *Va* as to their therapeutic effect on animals with transplantable tumours (V. J.) purine *Ia* was found to increase survival of Wistar rats with an ascitic Yoshida sarcome (tumour Y) by 22% in comparison with a control group of animals, using a single daily dose of 200 mg/kg *p.o.*, applied on five subsequent days, beginning on the second day after tumour transplantation. Compound *IVa* inhibited in H mice with a mammary adenocarcinome the tumour growth by 39%, the favourable effect was less pronounced in the survival of the treated animals, using the same type of application as shown above and a daily dose of 200 mg/kg applied on 12 consecutive days beginning on the third day after tumour transplantation. The other compounds did not show any more pronounced antineoplastic effect. For detailed data on the methods and evaluation of the results see ref.[2].

In summary, it may be said that the replacement of terminal glycine in the molecule of the ethyl ester of N-[δ-(6-purinylthio)valeryl]glycylglycylglycine with another amino acid, similarly to extending the chain length by another glycine residue, did not produce more effective antineoplastic agents.

EXPERIMENTAL

The melting points of compounds were determined in Kofler's block and are not corrected. Samples for analysis were dried at 0·2 Torr over phosphorus pentoxide at a temperature adequately raised. Most compounds contain a crystal solvent and the dried compounds are hygroscopic. The UV spectra of the compounds were examined in a Unicam SP-700 spectrophotometer in 1 cm quartz cuvettes at a concentration of about 1 mg compound/100 ml aqueous methanolic (1 : 1) 0·1M-HCl or 0·1M-NaOH. The values of specific rotation refer to compounds free of the crystal solvent and were determined with an accuracy of ±1°. The purity of the compounds was followed by thin-layer chromatography on silica gel G (Merck) using chloroform–ethanol (9 : 1) or 2-propanol–ammonia–water (10 : 1 : 1), detecting with bromophenol blue[3], or by paper chromatography using the previously described solvents and detection[4], mostly in 1-butanol–acetic acid–water (4 : 1 : 5), 1-butanol–pyridine–water (6 : 4 : 3) or in chloroform (formamide-impregnated paper). The yields shown in Table I refer to crude, relatively pure compounds.

Ethyl Esters of N-[δ-(6-purinylthio)valeryl]glycylglycylamino Acids *Ia—Va*

Method A: A solution of 6·15 g (0·03 mol) N,N′-dicyclohexylcarbodiimide in 5 ml dimethylformamide was added at 0°C to a solution of 10·0 g (0·027 mol) solvent-free[1] *VI* and 0·082 mol ethyl ester of the corresponding amino acid in 20 ml dimethylformamide and the mixture was left for 4 days at 0—5°C with occasional stirring. The precipitated N,N′-dicyclohexylurea was filtered, the filtrate distilled at reduced pressure at 60°C to remove most of the dimethylformamide and the residue was triturated with 50 ml 10% aqueous acetic acid. The precipitated crude ester was chromatographed on a column of silica gel (20-fold volume) using a mixture of chloroform and ethanol for elution. The first fractions contained the remaining N,N′-dicyclohexylurea, the next fractions contained acylated urea *VII* and the subsequent ones the ester of the substituted tripeptide (*IIa*, or *IVa*) which was purified by crystallization (Table I). N-[δ-(6-Purinylthio)valeryl-glycylglycyl]-N,N′-dicyclohexylurea (*VII*) was obtained pure by crystallization of the residue of the appropriate combined fractions from 80% aqueous ethanol. M.p. 206—208°C. For $C_{27}H_{40}N_8O_4S$ (572·7) calculated: 56·62% C, 7·04% H, 19·56% N, 5·60% S; found: 56·36% C, 7·14% H, 19·22% N, 5·99% S. In the UV region of the spectrum maxima were found at 291 and 220 nm (log ε 4·19 and 3·83) in 0·1M-HCl, and at 291 and 224 nm (log ε 4·21 and 4·03) in 0·1M-NaOH.

Method B: 7·33 g (0·02 mol) N-[δ-(6-purinylthio)valeryl]glycylglycine dried at 95—100°C/0·1 Torr was introduced under stirring at 20°C into a solution of 3·56 g (0·022 mol) N,N′-carbonyldiimidazol in 37 ml dimethylformamide. With development of carbon dioxide, the acid dissolved rapidly and after some time the acylated imidazol precipitated. After 1 h of standing at room temperature 0·044 mol of the appropriate amino acid ethyl ester (ethyl ester of DL-valine for *Ia*, diethyl ester of L-aspartic acid for *IIa*, diethyl ester of L-glutamic acid for *IVa*, ethyl ester of glycylglycine for *Va*) was added to the practically solidified reaction mixture and the combination was left to stand overnight at room temperature. Most of the dimethylformamide was distilled from the solution obtained under water-pump vacuum at 60°C, the viscous residue was mixed with 110 ml 10% aqueous acetic acid and the precipitated ethyl ester was purified by crystallization (Table I).

N-[δ-(6-Purinylthio)valeryl]glycylglycylamino Acids *I—III* and -tetraglycine *V*

Method C: 2 mmol esters *Ia*, *IIa* or *Va*, or 1·33 mmol ester *IIIa* was added to 8·8 ml (4·4 mmol) 0·5M-NaOH and the reaction mixture was left under occasional stirring for 2 days at 0—5°C (for *IIa* and *IIIa*) or at 20°C (for the other compounds). The solution was then acidified with dilute hydrochloric acid to pH 3 and the precipitated tripeptide *I—III*, or tetraglycine *V*, was purified by crystallization (Table I).

The analyses were done at the analytical department of this institute by Mr K. Havel, Mrs J. Komancová and Mrs V. Šmídová (the direction of Dr J. Körbl). Paper chromatography was done by Miss D. Dosedlová (direction of Dr V. Rábek), the UV spectra were measured by Dr J. Vachek, all from this institute.

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