SUBSTANCES WITH ANTINEOPLASTIC ACTIVITY. XLVI.*

CONTRIBUTION TO THE PREPARATION OF N-(δ-(6-PURINYLTHIO)VALERYL)AMINO ACIDS AND THEIR DERIVATIVES

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An alternative method of preparation of N-[δ-(6-purinylthio)valeryl]amino acids, their esters and analogous derivatives of diglycine and triglycine *I*—*IX* is described, the procedure consisting in a condensation of 6-mercaptopurine with the corresponding N-(δ-bromovaleryl)amino acids and their derivatives *X*—*XXII* in an alkaline medium.

In the XXIXth communication of this series we described the preparation of N-[δ-(6-purinylthio)valeryl]amino acids, their esters and analogous derivatives of dipeptides and triglycine and showed the results of evaluating some of them for their antineoplastic effect with animals bearing transplantable tumours[1]. The compounds represent the transport forms of cancerostatically active 6-(4-carboxybutyl)thiopurine[2], or 6-mercaptopurine[3]; some of them differ markedly in their affinity for certain organs and in their specific therapeutical effect on animals with certain experimental tumours. Of the compounds synthesized here one of the most interesting ones was the ethyl ester of N-[δ-(6-purinylthio)valeryl]glycine (*I*) which is at present tested on patients with blastic crisis of chronic myeloid leukemia[4,5].

The original method of preparation of N-[δ-(6-purinylthio)valeryl]amino acids and their derivatives consisted in a condensation of the reactive derivatives of 6-(4-carboxybutyl)thiopurine (*e.g.* chloride and azide) with amino acids or with their esters. In view of the fact that during synthesis of 6-(4-carboxybutyl)thiopurine one proceeds from 6-mercaptopurine, the above preparation procedure for N-[δ-(6-purinylthio)valeryl]amino acids and their derivatives is generally less suitable on account of losses of the purine component of the molecule.

In the present work we describe the preparation of amino acids *I*—*IX* by direct condensation of 6-mercaptopurine with corresponding N-(δ-bromovaleryl)amino

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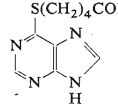
acids and their esters *X*–*XXII* which is analogous to the preparation procedure for alkylthiopurines (see *e.g.*[2,6]). In a similar way, some N-[(6-purinylthio)acetyl] amino acids were prepared recently[7]. Condensation of 6-mercaptopurine with the esters of N-(δ-bromovaleryl)amino acids was carried out in the presence of 1·1 molar equivalents of sodium hydroxide at room temperature (method *A*); the condensation is terminated after some 3–4 h. An analogous condensation of N-(δ-bromovaleryl)-amino acids was carried out in the presence of triethylamine, using 2 molar equivalents for neutral amino acids and 3 molar equivalents for acid amino acids, at 80–100°C (method *B*). The preparation procedure for *I*–*IX*, some of their properties and the reaction yields are summarized in Table I. The uniformity of the compound prepared was monitored by paper chromatography and by thin-layer chromatography on silica gel and spectrophotometrically in the UV region of the spectrum. At pH 1 and pH 13 one observes peaks in 50% aqueous methanol (Table I) corresponding with the previously described analogous data[1].

The required ethyl esters of N-(δ-bromovaleryl)amino acids were prepared by a reaction of δ-bromovaleryl chloride with the corresponding ethyl esters of amino acids, either with 2 molar equivalents of the free ester or with 1 molar equivalent of the ester in the presence of 1 molar equivalent of triethylamine, or else with 1 molar equivalent of the hydrochloride of the ethyl ester of the amino acid in the presence of 2 molar equivalents of triethylamine, in benzene or chloroform, at 0° – +5°C (method C). N-(δ-Bromovaleryl)amino acids were prepared either analogously by a reaction of δ-bromovaleryl chloride with the amino acids in the presence of 2 molar equivalents of sodium hydroxide in the case of neutral and in the presence of 3 molar equivalents of sodium hydroxide in the case of acidic amino acids (method *D*), or finally by saponification of the corresponding esters of N-(δ-bromovaleryl)amino acids with 1·1 molar equivalent of aqueous sodium hydroxide at 0° – +5°C (method *E*). The ethyl ester of N-(δ-bromovaleryl)triglycine (*XV*) was prepared in a yield of 72% from N-(δ-bromovaleryl)diglycine (*XII*) and glycine ethyl ester using N,N′-carbonyldiimidazole. Other methods were found to be less suitable: when using N,N′-dicyclohexylcarbodiimide the ester *XV* was obtained from the same starting compounds in a yield of only 30% since the N,N′-dicyclohexylurea formed during the reaction separated poorly on crystallization. When applying the chloride method to the conversion of the acid *XII* to the corresponding chloride by a reaction with thionyl chloride in dimethylformamide the starting compound was decomposed. The N-(δ-bromovaleryl)amino acids prepared here and their derivatives *X*–*XXII*, the way of their preparation, yields and some of their physical properties are shown in Table II.

The antineoplastic effect of N-[δ-(6-purinylthio)valeryl]amino acids and of their derivatives *I*–*VII* and *IX* is shown in our previous communication[1]; the results of evaluating the other compounds will be published elsewhere.

TABLE I

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



Compound	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	$CH_2COOC_2H_5$	*A* (90)	154—156 (water)	$C_{14}H_{19}N_5O_3S$ (337·4)	49·83 / 49·87	5·68 / 5·77	20·76 / 20·94	9·50 / 9·73	221 (4·03) 293 (4·23)	225 (4·17) 292 (4·21)
II	$CH_2CONHCH_2COO.C_2H_5$	*A* (95)	227—229 (aqueous ethanol)	$C_{16}H_{22}N_6O_4S$ (394·5)	48·72 / 48·77	5·62 / 5·75	21·31 / 21·17	8·13 / 8·04	219 (4·11) 294 (4·26)	224 (4·19) 293 (4·23)
III	$CH_2CO(NHCH_2CO)_2.OC_2H_5$	*A* (67)	236—239 (aqueous ethanol)	$C_{18}H_{25}N_7O_5S$ (451·5)	47·88 / 47·71	5·58 / 5·78	21·72 / 21·54	7·10 / 6·92	217 (4·08) 291 (4·23)	219 (4·21) 289 (4·23)
IV	$-CHCOOH-$ DL, $CH(CH_3)_2$	*B* (83)	103—105 (water)	$C_{15}H_{21}N_5O_3S$ (351·4)	51·26 / 50·98	6·03 / 5·99	19·93 / 19·67	9·13 / 9·06	221 (4·02) 292 (4·25)	221 (4·25) 291 (4·25)
V	$-CHCOOC_2H_5$-DL, $CH(CH_3)_2$	*A* (90)	116—118 (acetone)	$C_{17}H_{25}N_5O_3S$ (379·5)	53·80 / 53·87	6·64 / 6·94	18·46 / 18·25	8·45 / 8·73	218 (4·06) 291 (4·25)	220 (4·23) 289 (4·26)

VI	$-CHCOOC_2H_5$-L[a] $CH_2CH(CH_3)_2$	*A* (81)	144—145 (acetone–hexane)	$C_{18}H_{27}N_5O_3S$ (393·5)	54·94 54·84	6·92 6·79	17·80 17·61	8·15 8·37	218 (4·08) 292 (4·26)	220 (4·24) 290 (4·27)
VII	$-CHCOOH$-L[b] CH_2COOH	*B* (73)	197—199 (water)	$C_{14}H_{17}N_5O_5S$ (367·4)	45·77 45·65	4·66 4·63	19·06 19·14	8·73 8·93	217 (4·08) 291 (4·25)	219 (4·20) 289 (4·26)
VIII	$-CHCOOH$-L[c] CH_2CH_2COOH	*B* (80)	205—206 (water)	$C_{15}H_{19}N_5O_5S.H_2O$[d] (399·4)	45·10 45·10	5·30 5·52	17·53 17·61	8·03 8·13	219 (4·02) 291 (4·22)	222 (4·17) 291 (4·25)
IX	$-CHCOOC_2H_5$-L[e] $CH_2CH_2COOC_2H_5$	*A* (60)	100—101 (acetone–hexane)	$C_{19}H_{27}N_5O_5S$ (437·5)	52·16 51·97	6·22 6·34	16·01 15·99	7·33 7·49	219 (4·06) 291 (4·23)	221 (4·19) 289 (4·24)

[a] $[\alpha]_D^{20}$ −19·8° (*c* 1, ethanol). [b] $[\alpha]_D^{20}$ +13° (*c* 1, 0·1M-NaOH). [c] $[\alpha]_D^{20}$ +2° (*c* 1, 0·1M-NaOH). [d] A monohydrate of the given m.p. crystallizes from water; for analysis the compound was dried in air at room temperature. The crystal water is lost on drying at 100°C/0·2 Torr. For $C_{15}H_{19}N_5O_5S.H_2O$ (399·4) calculated: 4·51% H_2O; found: 4·83% H_2O. The dried compound is hygroscopic. For $C_{15}H_{19}N_5O_5S$ (381·4) calculated: 47·23% C, 5·02% H, 18·36% N, 8·41% S; found: 46·95% C, 5·29% H, 18·43% N, 8·32% S. [e] $[\alpha]_D^{20}$ −13° (*c* 1, ethanol).

TABLE II

N-(δ-Bromovaleryl)amino Acids and Their Derivatives $Br(CH_2)_4CONHR$

No	R	Method (yield, %)	M.p., °C (solvent)	Formula (m. w.)	Calculated/Found			
					% C	% H	% Br	% N
X	CH_2COOH	*E D* (68, 64)	68–70 (water)	$C_7H_{12}BrNO_3$ (238·1)	35·31 35·39	5·08 5·06	33·57 33·61	5·88 6·04
XI	$CH_2COOC_2H_5$	*C* (90)	57–59 (ether–hexane)	$C_9H_{16}BrNO_3$ (266·2)	40·61 40·87	6·06 6·16	30·03 30·25	5·26 5·02
XII	$CH_2CONH\,CH_2COOH$	*E* (94)	135–136 (acetone)	$C_9H_{15}BrN_2O_4$ (295·1)	36·62 36·82	5·13 5·13	27·08 26·96	9·49 9·59
XIII	$CH_2CONH\,.\,CH_2COOC_2H_5$	*C* (64)	161–162 (acetone)	$C_{11}H_{19}BrN_2O_4$ (323·2)	40·88 41·22	5·93 5·76	24·73 24·68	8·67 8·51
XIV	$CH_2CO(NHCH_2CO)_2OH$	*E* (78)	190–192 (aqueous acetone)	$C_{11}H_{18}BrN_3O_5$ (352·2)	37·51 38·12	5·15 5·27	22·69 22·83	11·93 11·76
XV	$CH_2CO(NHCH_2CO)_2\,.\,OC_2H_5$	—	221–223 (aqueous ethanol)	$C_{13}H_{22}BrN_3O_5$ (380·3)	41·06 41·65	5·83 5·90	21·02 20·90	11·05 11·00
XVI	$-CH(CH(CH_3)_2)COOH$-DL	*D E* (90, 67)	121–123 (benzene)	$C_{10}H_{18}BrNO_3$ (280·2)	42·86 42·93	6·48 6·51	28·52 28·65	5·00 4·88

XVII	$-CHCOOC_2H_5$-DL[a] $CH(CH_3)_2$	*C* (87)	—	$C_{12}H_{22}BrNO_3$ (308·2)	46·76 46·71	7·20 7·19	25·93 26·22	4·55 4·41
XVIII	$-CHCOOH$-L[b] $CH_2CH(CH_3)_2$	*D* (93)	134—135 (benzene)	$C_{11}H_{20}BrNO_3$ (294·3)	44·92 44·93	6·85 6·83	27·16 27·24	4·76 4·68
XIX	$-CHCOOC_2H_5$-L[c] $CH_2CH(CH_3)_2$	*C* (91)	—	$C_{13}H_{24}BrNO_3$ (322·3)	48·45 48·90	7·50 7·50	24·80 25·37	4·35 4·14
XX	$-CHCOOH$-L[d] CH_2COOH	*D* (74)	136—138 (water)	$C_9H_{14}BrNO_5$ (296·1)	36·50 36·36	4·77 4·77	26·99 27·23	4·73 4·72
XXI	$-CHCOOH$-L[e] CH_2CH_2COOH	*D* (76)	126—128 (water)	$C_{10}H_{16}BrNO_5$ (310·2)	38·72 38·65	5·20 5·29	25·77 25·61	4·52 4·55
XXII	$-CHCOOC_2H_5$-L[f] $CH_2CH_2COOC_2H_5$	*C* (81)	—	$C_{14}H_{24}BrNO_5$ (366·3)	45·91 46·23	6·61 6·52	21·82 21·72	3·92 4·08

[a] n_D^{20} 1·4852; [b] $[\alpha]_D^{20}$ −8·2° (*c* 1, ethanol); [c] $[\alpha]_D^{20}$ −28° (*c* 1, ethanol); n_D^{20} 1·4798; [d] $[\alpha]_D^{20}$ −7° (*c* 1, 0·5M-HCl); [e] $[\alpha]_D^{20}$ −15° (*c* 1, 0·5M-HCl); [f] $[\alpha]_D^{20}$ −13° (*c* 1, ethanol); n_D^{20} 1·4820.

EXPERIMENTAL

The melting points shown were determined in Kofler's block and are not corrected. Unless stated otherwise, the samples for analysis were dried at 0·2 Torr over phosphorus pentoxide at a temperature increased in proportion to the melting point of the substance. The UV spectra were measured in a Unicam SP-700 spectrophotometer in 1 cm silica cuvettes at a concentration of about 1 mg substance/100 ml aqueous–methanolic (1 : 1) 0·1M-HCl, or 0·1M-NaOH. The values of specific rotations refer, with the exception of acid *VIII*, to compounds free of crystal solvent and were determined with an accuracy of $\pm 1°$. Paper chromatography of purine derivatives *I*—*IX* was done by using the previously described technique and solvent systems, mostly in 1-butanol–acetic acid–water (4 : 1 : 5), 1-butanol saturated with 1·5M ammonia, 2-propanol–ammonia–water (9 : 1 : 2) or chloroform–benzene (formamide-impregnated paper); detection under UV light (Chromatolite) or with a mixture of bromophenol blue with silver nitrate[8]. Thin-layer chromatography was done using silica gel G (Merck, Darmstadt) in chloroform–ethanol (9 : 1) or 2-propanol–ammonia–water (10 : 1 : 1); detection was carried out by spraying with equal parts of 0·4% bromophenol blue in acetone and 2% aqueous silver nitrate with subsequent bleaching of the background with ethanol. Paper chromatography of N-(δ-bromovaleryl)amino acids and of their derivatives *X*—*XXII* was done in 1-butanol–acetic acid–water (4 : 1 : 5), 1-butanol–pyridine–water (6 : 4 : 3), or benzene, or else benzene–cyclohexane (1 : 1 to 7 : 3) (formamide-impregnated paper); detection with UV light (Chromatolite) or 1% solution of ninhydrin in acetone, or a mixture of 0·1M-$AgNO_3$ and 5M ammonia (1 : 1). The yields shown in Tables I and II refer to crude, relatively rather pure compounds.

Ethyl Esters of N-[δ-(6-purinylthio)valeryl]amino Acids *I*, *V*, *VI* and *IX*, -diglycine *II* and triglycine *III* (Method *A*)

A solution of 0·11 mol ethyl ester of N-(δ-bromovaleryl)amino acid in 11 ml ethanol (or 0·011 mol of ester *XIII* in 15 ml 80% aqueous ethanol at 20°C, or 0·0125 mol of ester *XV* in 475 ml 50% aqueous ethanol at 60°C) was added to a solution of 1·7 g (0·01 mol) 6-mercaptopurine monohydrate in 11 ml (0·011 mol) 1M-NaOH at 20—25°C. The mixture was stirred for 4 h and left to stand overnight at room temperature. In the case of ester *I* and *II*, the precipitated crystalline compounds were filtered and purified by crystallization. In the case of ester *III* the reaction mixture was stirred for 15 min to remove a small amount of nonreacted 6-mercaptopurine with 5 g Dowex 1 X 2 in the OH form; the resin was removed by filtration, the filtrate was concentrated in water-pump vacuum and the precipitated compound was purified by crystallization. In the case of esters *V*, *VI* and *IX*, most of the ethanol was removed from the reaction mixture by distillation in water-pump vacuum, the separated oily product was extracted with a mixture of chloroform with ethanol (8 : 2), the chloroform extracts were washed with water to disappearance of alkaline reaction, dried with sodium sulfate and the solvents were distilled off. The residue which usually solidifies at room temperature, was purified by crystallization (Table I).

N-[δ-(6-Purinylthio)valeryl]amino Acids *IV*, *VII* and *VIII* (Method *B*)

A solution of 5·5 mmol N-(δ-bromovaleryl)amino acid in 8·5 ml aqueous solution of triethylamine (0·53 g, *i.e.* 5·25 mmol triethylamine in the case of acid *XVI*, or 1·07 g, *i.e.* 10·5 mmol triethylamine in the case of acids *XX* and *XXI*) was added to a solution of 0·85 g (5 mmol) 6-mercaptopurine monohydrate in 8·5 ml water and 0·53 g (5·25 mmol) triethylamine at 20—25°C. The mixture was heated for 2 h on a boiling-water bath and, after cooling to room temperature, the solution was made acid with hydrochloric acid to pH 3, the precipitated compound was filtered and stirred for 30 min with 50 ml 0·1M-$NaHCO_3$ in the case of compound *IV*, or with

10 ml 1M-$NaHCO_3$ in the case of compounds *VII* and *VIII*. The small amount of nonreacted 6-mercaptopurine was filtered, the filtrate was made acid with hydrochloric acid to pH 2—3 and the precipitated acid was purified by crystallization (see Table I).

Ethyl Esters of N-(δ-Bromovaleryl)amino Acids *XI*, *XVII*, *XIX*, *XXII* and -diglycine *XIII* (Method *C*)

2·0 g (0·01 mol) δ-bromovaleryl chloride was added dropwise and under stirring at 0°C to a solution of 0·02 mol ethyl ester of the amino acid (or 0·01 mol ethyl ester of amino acid with 0·01 mol triethylamine, or 0·01 mol hydrochloride of the amino acid ethyl ester with 0·02 mol triethylamine) in 25 ml chloroform. The reaction mixture was stirred for 30 min at the temperature indicated, extracted successively with 0·1M-HCl, 0·1M-$NaHCO_3$ and water, the chloroform solution was dried with anhydrous sodium sulfate and the solvent was distilled at water-pump vacuum. The residue was purified either by crystallization (ester *XI* and *XIII*) or by column chromatography on silica gel using chloroform for ester elution (compounds *XVII*, *XIX* and *XXII*) (Table II).

N-(δ-Bromovaleryl)amino Acids

Method D — *acids* X, XVI, XVIII, XX *and* XXI: 2·2 g (0·011 mol) δ-bromovaleryl chloride with 11 ml (0·011 mol) 1M-NaOH were added dropwise over a period of 30 min under stirring at 0— + 5°C to a solution of 0·01 mol of the corresponding amino acid in 10 ml (0·01 mol) 1M-NaOH when preparing *X*, *XVI* and *XVIII*, or in 20 ml (0·02 mol) 1M-NaOH when preparing *XX* and *XXI*, keeping the reaction mixture always alkaline. After adding the chloride the mixture was stirred for further 2 h at the given temperature, extracted with ether and the aqueous phase was made acid with hydrochloric acid to pH 2; the precipitated compound was purified by crystallization (Table II).

Method E — *acids* X, XII, XIV *and* XVI: 0·01 mol of the corresponding ethyl ester of N-(δ-bromovaleryl)amino acid was introduced into 10 ml (0·01 mol) 1M-NaOH at 0°C and the mixture was stirred at 0°C until the solid dissolved (about 1 h with *X*, 2 h with *XII*, 4 h with *XIV* and 24 h with *XVI*). The mixture was then made acid with hydrochloric acid to pH 2 and the precipitated acid was crystallized (Table II).

Ethyl Ester of N-(δ-Bromovaleryl)glycylglycylglycine (*XV*)

1·48 g (5 mmol) N-(δ-bromovaleryl)glycylglycine was added under stirring at room temperature to a solution of 0·885 g (5·5 mmol) N,N′-carbonyldiimidazole in 15 ml chloroform. Under evolution of carbon dioxide, the acid dissolved rapidly and after several min the corresponding acylated imidazole precipitated. After 30 min, a solution of 0·57 g (5·5 mmol) freshly redistilled glycine ethyl ester in 5 ml chloroform was added dropwise at 15—20°C, the mixture was stirred for 1 h and was left to stand overnight at room temperature. The precipitated ethyl ester *XV* was filtered and purified by crystallization (Table II).

The analyses were done at the analytical department of this Institute by Mr K. Havel, Mrs J. Komancová and Mrs V. Šmídová under the direction of Dr J. Kőrbl. Paper chromatography was done by Miss D. Dosedlová under the direction of Dr V. Rábek, the UV spectra were registered by Dr J. Vachek, all of this Institute.

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