SOME DERIVATIVES OF 5-(2-AMINO-6-HYDROXY-4-OXO-3,4-DIHYDRO-5-PYRIMIDINYL)PENTANOIC ACID*

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Using the chloride method esters *II*—*X*, amides *XI*—*XIV*, and condensates with amino acid esters *XV*—*XVII* were prepared from 5-(2-amino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl)-pentanoic acid (*I*); the amides *XIII* and *XIV* were also prepared by aminolysis of ester *II*. The derivative of glycine, *XVIII*, was obtained on saponification of ester *XV*, also obtained by condensation of triethyl ester of N-(6,6-dicarboxyhexanoyl)glycine (*XXIII*) with guanidine hydrochloride in a medium containing sodium ethylate. Hydrazinolysis of ethyl ester *XXIV* gave hydrazide *XIX*. Disubstituted ureas *XX*—*XXI* were obtained on reaction of esters *VI* and *XXIV* with 2-chloroethyl isocyanate; saponification of the ester function in the urea derivative *XX* led to the free acid *XXII*. Reaction of acid *I* with an excess of diazomethane gave a mixture of compounds in which compound *XXV* (a product of esterification and O-methylation) and *XXVI* (a product of esterification, O-methylation, and N-methylation) predominated. None of the substances prepared displayed a clear anti-tumour activity. Some of the substances tested affected the weight of experimental tumours (*XV*, *XVI*, *XX*, *XXV*) or protracted the survival time of experimental animals (*XXVI*, *XX*). Substance *XX* had the broadest spectrum of activity.

In connection with the study of biological activity of pyrimidine derivatives, structurally related to derivatives of isocytosine with potential antineoplastic activity of the antimetabolite type, we described the synthesis and the fundamental pharmacological properties of ω-(2-amino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl) alkanoic acids[1] among which 5-(2-amino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl)-pentanoic acid (*I*) displayed the ability to modulate the biological responses of other drugs administered together with it[1-4]. Compound *I* was submitted to the first phase of clinical tests under the name Damvar, but there its further development was stopped in view of its side effects and a non-distinct antitumour activity.

In this communication we describe the preparation of some derivatives of compound *I* (Table I, substances *II*—*XXII*) and the results of a preliminary testing of the antineoplastic activity of selected compounds in animals with experimental transplanted tumours. These compounds were synthetized with the aim of preparing potential "prodrugs" of compound *I*, by substituting the carboxyl function by the

* Part XCI in the series Substances with Antineoplastic Activity; Part XC: Česk. Farm. *33*, 72 (1984).

TABLE I

Derivatives of 5-(2-amino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl)pentanoic acid (*I*)

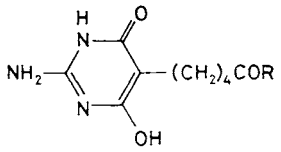
Compound	Yield (method)	M.p., °C (solvent)[a]	Formula (mol. weight)	Calculated/found			UV, λ_{max}, nm[b] (log ε)	
				% C	% H	% N	M_1	M_2
II	89	267–270	$C_{10}H_{15}N_3O_4$	49·78	6·26	17·42	266·5[c]	271[c]
CH_3O	(*A*)	(S_2)	(241·0)	49·76	6·43	17·71	(4·14)	(4·12)
III	62	276–278	$C_{12}H_{19}N_3O_4$	53·52	7·11	15·60	266·5	270
C_3H_7O	(*A*)	(S_2)	(269·4)	53·55	7·42	15·85	(4·19)	(4·15)
IV	65	272–274	$C_{13}H_{21}N_3O_4$	55·11	7·47	14·83	267	271
C_4H_9O	(*A*)	(S_2)	(283·3)	55·07	7·39	14·71	(4·17)	(4·15)
V	41	282–284	$C_{13}H_{21}N_3O_4$	55·11	7·47	14·83	266	270·5
i-C_4H_9O	(*A*)	(S_2)	(283·3)	55·02	7·53	15·03	(4·19)	(4·15)
VI	63	269–272	$C_{16}H_{27}N_3O_4$	59·05	8·36	12·91	266·5	271
$C_7H_{15}O$	(*A*)	($CHCl_3$)	(325·4)	58·64	8·42	12·91	(4·17)	(4·14)
VII	79	263–265	$C_{12}H_{17}N_3O_4$	53·92	6·41	15·72	267	270
CH_2 $CHCH_2O$	(*A*)	(S_2)	(267·3)	53·95	6·44	16·02	(4·16)	(4·13)
VIII	61	233–234	$C_{11}H_{17}N_3O_5$	48·70	6·31	15·49	266	269
$HOCH_2CH_2O$	(*A*)	(water)	(271·3)	48·75	6·56	15·73	(4·35)	(4·10)
IX	28	250–252	$C_{12}H_{19}N_3O_5$	50·52	6·71	14·73	266·5	271
$CH_3OCH_2CH_2O$	(*A*)	(S_3)	(285·3)	50·41	6·74	14·94	(4·18)	(4·14)
X	78	288–290	$C_{14}H_{21}N_3O_4$	56·93	7·17	14·23	265	268
cyclo-C_5H_9O	(*A*)	(DMF)	(295·3)	56·85	7·02	14·50	(4·16)	(4·10)
XI	53	290–292	$C_9H_{14}N_4O_3$	47·78	6·24	24·76	266	270
NH_2	(*B*)	(water)	(226·2)	47·46	6·16	25·04	(4·02)	(4·06)
XII[c]	46	263–264	$C_{10}H_{20}N_4O_5$	43·46	7·29	20·28		
CH_3NH	(*B*)	(water)	(276·3)	43·75	6·91	20·16		
XIII[d]	21 88	263–265	$C_{13}H_{24}N_4O_4$	51·98	8·05	18·66	268	270
C_4H_9NH	(*B*) (*C*)	(C_2H_5OH)	(300·4)	51·79	7·85	18·44	(4·25)	(4·18)
XIV[d]								
cyclo-	32 72	292–294	$C_{15}H_{26}N_4O_4$	55·19	8·03	17·17	266	268
-$C_6H_{11}NH$	(*B*) (*C*)	(C_2H_5OH)	(326·4)	55·03	7·92	17·25	(4·35)	(4·27)
XV[e]	82	247–249	$C_{13}H_{24}N_4O_7$	44·82	6·94	16·08	266	270
Gly(OEt)	(*B*)	(water)	(348·4)	44·80	6·68	16·30	(4·11)	(4·07)
XVI[e]	43	250–253	$C_{17}H_{28}N_4O_5$	55·41	7·66	15·21	266	270
L-Leu(OEt)	(*B*)	(C_2H_5OH–H_2O)	(368·4)	55·18	7·66	15·04	(4·15)	(4·13)
XVII	74[f]	181–183	$C_{15}H_{23}N_5O_6$	48·77	6·28	18·96	266[f]	271[f]
GlyGly(OEt)	(*B*)	(water)	(369·4)	48·88	6·24	18·57	(4·21)	(4·18)

TABLE I
(*Continued*)

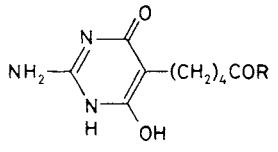
Compound	Yield (method)	M.p., °C (solvent)[a]	Formula (mol. weight)	Calculated/found			UV, λ_{max} nm[b] (log ε)	
				% C	% H	% H	M_1	M_2
XVIII	90	274—276	$C_{11}H_{16}N_4O_5$	46·48	5·67	19·71	266	270
GlyOH	—	(water)	(284·3)	46·14	5·85	19·73	(4·13)	(4·10)
XIX	25	285—287	$C_9H_{15}N_5O_3$	44·81	6·27	29·03	267	271
$NHNH_2$	—	(water)	(241·2)	44·47	6·31	29·33	(4·20)	(4·14)
XX[g]	84	240—242	$C_{14}H_{21}ClN_4O_5$	46·60	5·87	15·53	—	—
OC_2H_5	—	(S_2)	(360·8)	47·09	5·98	15·30	—	—
XXI[h]	35	233—235	$C_{19}H_{31}ClN_4O_5$	52·96	7·25	13·00		
OC_7H_{15}	—	(S_2)	(430·9)	52·79	7·46	12·84	[i]	
XXII[j]	70	234—236	$C_{12}H_{17}ClN_4O_5$	43·31	5·15	16·84	266	279
OH	—	(S_3)	(332·8)	43·48	5·22	17·23	(4·07)	(4·10)
							242	
							(4·05)	

[a] Systems for crystallization: DMF–dimethylformamide; S_2 DMF–methanol; S_3 DMF–water; [b] infrared spectra (ν, cm^{-1}, in KBr): *II* 1 700 (ester), 1 690 (lactam), 1 600 (NH_2), 3 400, 3 340, 3 220 (NH_2, NH, OH); *III* 1 720 (ester), 1 660 (lactam), 1 620 (NH_2), 3 160, 3 060 (NH_2, NH, OH); *IV* 1 720 (ester), 1 660 (lactam), 1 620 (NH_2), 3 460, 3 320, 3 240, 3 180 (NH_2, NH, OH); *V* 1 720 (ester), 1 660 (lactam), 1 620 (NH_2), 3 450, 3 320, 3 220, 3 180 (NH_4, NH, OH); *VI* 1 720 (ester), 1 660 (lactam), 1 610 (NH_4), 3 460, 3 330, 3 100 (NH_2, NH, OH); *VII* 1 695 (ester), 1 665 (lactam), 1 600 (NH_2), 3 450, 3 330, 3 240, 3 050 (NH_4, NH, OH); *VIII* 1 730 (ester), 3 530 (OH), 3 420, 3 220 (NH_4, NH), 1 700 (lactam), 1 605 (NH_2); *IX* 1 720 (ester), 1 660 (lactam), 1 620 (NH_2), 1 130 (aliphat. ether), 3 450, 3 320, 3 220, 3 180 (NH_2, NH, OH); *X* 1 730 (ester), 1 700 (lactam), 1 630 (NH_2), 3 300, 3 250, 3 140 (NH_2, NH, OH); *XI* 1 680 (lactam), 3 200, 3 400 (NH_2, NH), 1 670 (prim. amide), 1 620 (NH_2); *XIII* 1 545, 1 620 (sec. amide), 1 590 (NH_2), 1 640 (lactam), 3 360, 3 240 (NH, NH_2); *XIV* 1 540, 1 620 (sec. amide), 1 590 (NH_2), 1 680 (lactam), 3 250, 3 340, 3 400 (NH, NH_4); *XV* 1 760 (ester), 1 645, 1 560 (sec. amide), 1 650 (lactam), 3 360 (NH_2); *XVI* 1 660 (lactam), 1 630, 1 540 (sec. amide), 1 720 (ester), 3 180 (NH_2, NH, OH), 1 600 (NH_2); *XVII* 3 380 (OH), 3 260, 3 210 (NH), 1 720 (ester), 1 640 (lactam), 1 625, 1 620, 1 550, 1 540 (sec. amide); *XVIII* 3 500, 3 320, 3 100 (NH_2, NH), 1 700 (COOH), 1 660 (lactam), 1 640, 1 560 (sec. amide); *XIX* 1 660 (lactam), 1 690, 1 540 (N-subst. amide), 1 600 (NH_2), 3 310, 3 200, 3 110 (NH_2, NH, OH); *XXI* 1 740 (ester), 1 685 (lactam), 3 200, 3 080 (NH, OH), 1 560, 1 640, 3 300 (1,3-disubst. urea); *XXII* 1 680 (COOH), 1 675 (lactam), 1 630, 1 550 (1,3-disubst. urea), 3 240, 3 120 (NH, OH); [c] dihydrate; [d] monohydrate; [e] $[\alpha]_D^{20}$ −17·6° (*c* = 1, pyridine); [f] trihydrate; [g] calculated: 9·85% Cl; found: 9·69% Cl; [h] calculated: 8·23% Cl; found: 8·28% Cl; [i] UV (in 50% methanol): 276 (4·25), 247 (4·01) nm; [j] calculated: 10·66% Cl; found: 10·42% Cl.

more lipophilic ester (compounds *II*–*X*) or amide residue (compounds *XI*–*XVIII*). Further we prepared hydrazide *I* (compound *XIX*) and derivatives carrying on the amino group in position 2 of the pyrimidine nucleus the 2-chloroethylcarbamoyl residue (*XX*–*XXII*) as precursors of compounds with the alkylating character of their effect, of the type of N-(2-chloroethyl)-N-nitrosoureas. The aim of the above mentioned substitutions of compound *I* was the determination of the effect of the substituents on the antineoplastic activity.

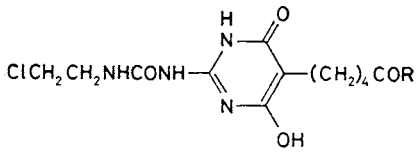


A

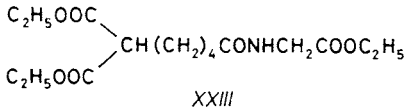


B

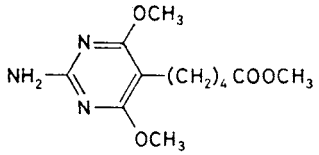
I, R = OH
II–*XIX*, R (see Table I)
XXIV, R = OC_2H_5



XX–*XXII*, R (see Table I)



XXIII

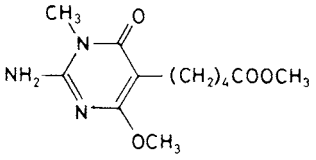


XXV

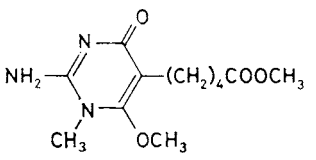
The esters *II*–*X* were synthetized according to the method of Brenner and coworkers[5–7]. The amides *XI*–*XIV* were obtained from the chloride of acid *I* on reaction with an excess of ammonia or primary amines; in the case of the synthesis of cyclohexylamide *XIV* the hydrogen chloride formed was bound by an excess of triethylamine. Amides *XIII* and *XIV* were also prepared by aminolysis of methyl ester *II* with butylamine resp. cyclohexylamine. The condensates of compound *I* with the esters of amino acids (compounds *XV*–*XVII*) were also prepared by the chloride method, using an excess of ethyl ester of glycine as a base (compound *XV*), or using hydrochlorides of amino acid esters and an excess of triethylamine (*XVI* and *XVII*). The glycine derivative *XVIII* was synthetized both by saponification of ester *XV* in aqueous alkaline medium at room temperature and by condensation of triethyl ester of N-(6,6-dicarboxyhexanoyl)glycine (*XXIII*) obtained by malonic ester synthesis from the sodium salt of malonic ester and ethyl ester of N-(5-bromopentanoyl)glycine (in analogy with ref.[8]), with guanidine hydrochloride. Reaction of ethyl ester of 5-(2-amino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl)pentanoic acid (*XXIV*) with hydrazine hydrate gave hydrazide *XIX*. In the synthesis of derivatives with a 2-chloroethylcarbamoyl residue in the molecule (compounds *XX*–*XXI*) we allowed ethyl ester *XXIV* (ref.[1]) or heptyl ester *VI* to react with 2-chloroethyl isocyanate in dimethylformamide at elevated temperature. Using acid hydrolysis, compound *XX* was converted to compound *XXII* with a free carboxyl group. The structure of the prepared esters and amides (Table I) is in agreement with our previous findings[1] according to which we assign the substances in solid state and in neutral solution the structure *A* [derivative of 5-(2-amino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl)pentanoic acid] or *B* [derivative of 5-(2-amino-6-hydroxy-4-oxo-1,4-dihydro-5-pyrimidinyl)pentanoic acid]. In contrast to acid *I*, in the case of esters and amides the formation of internal salts is prevented, which is manifested by the relatively easy reaction of esters *VI* and *XXIV* with 2-chloroethyl isocyanate (compounds *XX*, *XXI*). In the reaction of esters *VI* and *XXIV* with 2-chloroethyl isocyanate two sites come into consideration for the attack: one on the hydroxyl group in position 6 and the other on the amino group in the position 2. We have demonstrated[1] that acetylation of compound *I* takes place only on the amino group in position 2, where a flat band at 1 640 cm^{-1} (KBr pellet) was observed in the IR spectrum, while the characteristic band of the carbonyl group of the enol acetate was not present. The same situation also occurs in the case of the reaction of 2-chloroethyl isocyanate with the esters of compound *I*, where in the IR spectrum of compound *XXI* the bands at 1 560, 1 640, and 3 300 cm^{-1} are evident, which belong to the disubstituted urea, and a distinct band of the hydroxy group in position 6 at 3 200 cm^{-1}. On hydrolysis of compound *XX* with dilute hydrochloric acid disubstituted urea *XXII* is formed instead of compound *I* which could be formed only in the case of the hydrolysis of 2-chloroethyl carbamate bound in position 6.

For the sake of chemical checking of the tautomeric form in which acid *I* occurs

we analysed the course of its reaction with an excess of diazomethane. Using TLC analysis we identified the formation of two products in addition to three further admixtures which could not be isolated. Both isolated products had the same elemental composition ($C_{12}H_{19}N_3O_4$). On the basis of the analysis of spectral data the less polar substance was assigned the structure of methyl 5-(2-amino-4,6-dimethoxy-5-pyrimidinyl)pentanoate (*XXV*) and the more polar substance the struc-



XXVI



XXVI a

ture of methyl 5-(2-amino-6-methoxy-3-methyl-4-oxo-3,4-dihydro-5-pyrimidinyl)-pentanoate (*XXVI*) or its isomer *XXVIa*. According to the position of the singlet of the N-methyl group at $\delta = 3{\cdot}40$ the structure *XXVI* seems more probable. Compounds *XXV* and *XXVI* are formed in the reaction mixture in approximately the same quantity (densitometric determination at 269 nm for compound *XXV* and at 283 nm for compound *XXVI*), *i.e.* each in an about 20% yield. The mass spectra of compounds *XXV* and *XXVI* are very similar and they contain several common fragments (*m/z*: 168 – $C_7H_{10}N_3O_2$, 136 – $C_6H_8N_3O$, 112 – $C_5H_6NO_2$, 98 – $C_3H_4NO_2$, 71 – C_3H_7N). The fragmentation in the spectra differs in that compound *XXVI* gives fragments *m/z* 182 – $C_8H_{12}N_3O_2$ and 196 – $C_9H_{14}N_3O_2$ which are absent in the spectrum of compound *XXV*. From this fact it may be judged that the distribution of the methyl groups and the double bonds in the pyrimidine nucleus in compound *XXVI* contributes to the higher lability of the bonds between the methylene groups of the side chain in position 5, in contrast to compound *XXV*. The assumption of the structure correlates with the results of ^{1}H NMR spectroscopy.

Some of the substances prepared were tested for their antitumour activity in animals with experimental tumours, *i.e.* SAK, HK, S37, Kr2, and Y (ref.[9]), some also with LsG, S180, and L1210 (ref.[10]). The substances were administered subcutaneously in the form of an aqueous suspension, with the exception of compounds *XXVI* and *XXV* which were also administered orally. The antitumour effect, *i.e.* a simultaneous decrease of the average weight of the tumour (or the average value of the total ascitocrite) and the increase of the average value of the survival time of the animals in comparison with the control group, was not proved in any of the substances tested ($\alpha = 0{\cdot}05$). However, favourable effect on at least one of the several measured parameters was observed, in substance *XV* (decrease of the average weight of the tumour Kr2 by 23% in a 112 mg/kg dose, *s.c.*) and substance *XVI* (decrease of the weight of the tumour S37 by 27% in a 40 mg/kg dose, *s.c.*). The total ascitocrite

in Kr2 was decreased after addition of compound *XV* (by 15% in a 112 mg/kg dose, *s.c.*), *XI* (by 30% in a 80 mg/kg dose, *s.c.*), in S37 after addition of compounds *XI* (by 16% in a 40 mg/kg dose, *s.c.*), *XVI* (by 18% in a 40 mg/kg dose, *s.c.*), and *XXV* (by 18%in a 100 mg/kg dose, *s.c.*). Compound *XXVI* prolonged the survival time of the animals with L1210 (by 16% in a 100 mg/kg dose, *p.o.*). Compound *XX* prolonged the survival time of the animals in a 50 mg/kg dose, *s.c.*, by 19% in S180 and by 56% in the case of S37. In the case of S37 and a 100 mg/kg dose, *s.c.*, a decrease of the average weight of the tumour by 41% was observed and by 28% in the case of total ascitocrite. In animals with Kr2 this compound decreased the value of the total ascitocrite in a 200 mg/kg dose, *p.o.*, by 20%, in a 100 mg/kg dose, *s.c.*, by 16%, and in a 50 mg/kg dose, *s.c.*, by 18%. The broadest activity spectrum in experimental tumours was found in compound *XX*, probably in consequence of the introduction of the 2-chloroethylcarbamoyl residue with an alkylating character into the pyrimidine molecule. In similar tests the starting compound, ester *XXIV*, was completely inactive.

EXPERIMENTAL

The melting points were determined on a Kofler block and they are not corrected. The samples for analyses were dried over phosphorus pentoxide in a vacuum (27 Pa) at temperatures proportional to their melting points. The ultraviolet spectra of samples (λ_{max}, nm, log ε are indicated) were recorded on a Pye Unicam SP 8000 spectrometer, at an about 0·001% (w/v) concentration in 0·1M-HCl (medium M_1) and in 0·1M-NaOH (M_2), in both instances in 50% (v/v) methanol. The IR spectra were recorded on a Hilger Watts spectrometer (KBr technique). The ^{1}H NMR spectra were taken with a Tesla BSC 487 spectrometer (80 MHz) at about 10% concentration (w/v) in deuteriochloroform, using tetramethylsilane as internal reference. The mass spectra were measured on a MAT 44S instrument. The uniformity of the substances was checked by thin-layer chromatography on silica gel plates (DC-Fertigplatten Kieselgel F_{254}, Merck) or on reflecting foils with a luminiscence indicator (Silufol UV_{254}, Kavalier) in the following systems: chloroform–methanol (95 : 5) (S_1), chloroform–methanol–25% ammonia (2 : 2 : 1) or 1-propanol–25% ammonia–water (7 : 1 : 2) or chloroform–methanol–acetic acid–water (65 : 25 : 8 : 4), detection in the UV light of 254 nm.

Esters of 5-(2-amino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl)pentanoic Acid *II*–*X*

Method A: Thionyl chloride (2·0 g, 17 mmol) was added dropwise under stirring and exclusion of air humidity to an excess of corresponding alcohol (15–20 ml) cooled at −20 to −40°C, and then 3·4 g (15 mmol) of acid *I* were added in several portions to it. The suspension was stirred at 0°C for 15 min, then for 2 h at 40°C and 2 h at 80 to 100°C. From the solution formed volatiles were evaporated under reduced pressure, the residue was triturated with water and neutralized by addition of sodium hydrogen carbonate. The separated product was filtered off, washed with water, dried and crystallized; the physico-chemical properties are shown in Table I.

Amides of 5-(2-Amino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl)-pentanoic Acid (*XI*–*XVII*)

Method B: Thionyl chloride (1·4 g; 12 mmol) was added at 30–25°C to a suspension of 2·3 g (10 mmol) of acid *I* in 40 ml of dimethylformamide and the mixture was stirred at 50°C for 1 h.

The solution of acid chloride *I* obtained was saturated in the case of *XI* with gaseous ammonia at 20–25°C, and in the case of *XII*, *XIII*, and *XV* it was allowed to react with excess amine [25 ml (0·32 mol) of 40% (m/v) of aqueous methylamine solution for *XII*; 2·2 g (30 mmol) of n-butylamine for *XIII*; 4·54 g (44 mmol) of ethyl ester of glycine for *XV*], or in the case of *XIV*, *XVI*, and *XVII* with the amine or its hydrochloride in the presence of triethylamine [1·49 g (15 mmol) of cyclohexylamine and 3·03 g (30 mmol) of triethylamine for *XIV*; 2·34 g (12 mmol) of hydrochloride of L-leucine ethyl ester and 6·0 g (60 mmol) of triethylamine, 2 h at 60°C, for *XVI*; 2·16 g (11 mmol) of hydrochloride of ethyl ester of diglycine and 5·6 g (56 mmol) of triethylamine for *XVII*]. After 4 to 6 h stirring at room temperature dimethylformamide was distilled off, the residue stirred with water and the compound separated was filtered off after cooling at +5°C, washed with water, dried and crystallized; the yields and the physico-chemical properties of the products are given in Table I.

Method C: A suspension of methyl ester *II* (1·21 g, 6 mmol) in butylamine (6 ml, 0·06 mol), or in cyclohexylamine (6 ml, 0·052 mol) was heated for 4 h in a bath of 55–60°C in the first case, and at 100–110°C for 8 h in the second. The volatile components were distilled off from the mixture under reduced pressure and the residue stirred with 25 ml of water, the suspension was acidified with acetic acid to pH about 6, the crude amide was filtered off and crystallized; for the yields and the physico-chemical properties see Table I.

N-[5-(2-Amino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl)-pentanoyl]-glycine (*XVIII*)

a) Ester *XV* (7·7 g of dihydrate, 0·022 mol) was introduced at room temperature and under stirring into 1M-NaOH solution (66 ml), containing about 0·5 g of sodium sulfite. After dissolution of the solid material the mixture was allowed to stand at room temperature for 2 h and then acidified with dilute hydrochloric acid (1 : 1) to pH 3. After 2 h of cooling with ice the separated compound was filtered off, washed with water, dried (yield 6·40 g of dihydrate *XVIII*) and crystallized (see Table I).

b) Guanidine hydrochloride (0·45 g, 4·64 mmol) was added to a solution of sodium (0·16 g, 6·96 mmol) in ethanol (5 ml) and the mixture was stirred at room temperature for 10 min. A solution of triethyl ester *XXIII* (0·80 g, 2·32 mmol) in ethanol (5 ml) was then added and the stirring at 20 to 23°C continued for another 4 h. The mixture was then allowed to stand at room temperature overnight. Ethanol was distilled off under reduced pressure, the residue was dissolved in 1M-NaOH (2·35 ml, 2·35 mmol) containing 0·1 g of sodium sulfite at room temperature and the mixture was allowed to stand overnight. After acidification with dilute hydrochloric acid to pH 3·5 and cooling with ice the separated compound was filtered off, washed with water and dried in a vacuum at room temperature. Yield, 0·37 g (50%) of dihydrate *XVIII*.

Triethyl Ester of N-(6,6-Dicarboxyhexanoyl)glycine (*XXIII*)

Diethyl malonate (3·2 g, 0·02 mol) and a solution of ethyl ester of N-(5-bromopentanoyl)glycine[11] (5·4 g, 0·02 mol) in ethanol (10 ml) was added to a solution of sodium (0·51 g, 0·022 mol) in ethanol (50 ml) and the mixture was refluxed for 20 h. Ethanol was distilled off in a vacuum, the residue was extracted with a mixture of water and ether, and the organic phase was dried and the solvent evaporated. The crude product (4·15 g) was first precipitated from a benzene solution with hexane and then crystallized repeatedly from hexane. Yield, 1·3 g of needles with m.p. 52–53°C. For $C_{16}H_{27}NO_7$ (345·4) calculated: 55·64% C, 7·88% H, 4·05% N; found: 55·58% C, 8·12% H, 4·13% N. ^{1}H NMR spectrum (deuteriochloroform): δ 6·15 (bt, 1 H, NH), 4·15 (q, J = 7·0 Hz, 2 H, $NHCH_2COOCH_2CH_3$), 4·13 (q, J = 7·0 Hz, 4 H, $CH(COOCH_2CH_3)_2$),

3·95 (d, $J = 5·0$ Hz, $NHCH_2COOC_2H_5$), 3·25 (t, $J = 7·0$ Hz, 1 H, $CH(COOC_2H_5)_2$), 2·18 (t, $J = 7·0$ Hz, 2 H, CH_2CONH), 1·30—2·00 (m, 6 H, $CH(CH_2)_3CH_2$), 1·25 (t, $J = 7·0$ Hz, 3 H, $NHCH_2COOCH_2CH_3$), 1·23 (t, $J = 7·0$ Hz, 6 H, $CH(COOCH_2CH_3)_2$).

Hydrazide of 5-(2-Amino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl)pentanoic Acid (*XIX*)

100% Hydrazine hydrate (3 ml) was added to a suspension of ethyl ester *XXIV* (1·3 g, 5 mmol) in 50 ml ethanol and the mixture was stirred at room temperature for one week. The compound separated was filtered off, washed with ethanol and purified by crystallization from water (see Table I).

Ethyl 5-[2-(2-Chloroethylcarbamoyl)amino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl]pentanoate (*XX*)

A suspension of ethyl 5-(2-amino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl)pentanoate (6·5 g, 20 mmol) in 150 ml of dimethylformamide was heated at 100°C till dissolution, then 2-chloroethyl isocyanate (2·9 g, 22 mmol) was added and the mixture heated for 5 h. After cooling and standing at room temperature overnight dimethylformamide was distilled off, the residue was stirred with 100 ml of ethanol and the substance separated was filtered off dried and crystallized. Yield, 6·1 g (see Table I).

Heptyl 5-[2-(2-Chloroethylcarbamoyl)amino-6-hydroxy-5-oxo-3,4-dihydro-5-pyrimidinyl]pentanoate (*XXI*)

It was prepared in the same manner as compound *XX* on reaction of heptyl ester *VI* (3·3 g, 10 mmol) in dimethylformamide (100 ml) with 2-chloroethyl isocyanate (1·2 g, 11 mmol) for 5 h. Yield, 1·5 g (see Table I). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide, 100°C): δ 3·99 (t, 2 H, $COOCH_2$), 3·60 (m, 4 H, $CONHCH_2$—CH_2—Cl), 2·20 (m, 4 H, —$CH_2CH_2CH_2CH_2COO$—), 1·60—1·80 (m, —CH_2), 0·81 (def. t, 3 H, CH_3). Mass spectrum (*m/z*): 430 ($C_{19}H_{31}ClN_4O_5$).

5-[2-(2-Chloroethylcarbamoyl)amino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl]pentanoic Acid (*XXII*)

A suspension of ethyl ester *XX* (1 g, 3 mmol) in 10 ml concentrated hydrochloric acid was heated at 50°C until dissolution. The mixture was allowed to stand at room temperature for 3 days and the separated material was filtered off, washed with water, dried and recrystallized (see Table I). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide, 60°C): δ 7·98 (bt, 1 H, —CH_2NH—CO—), 3·50 (m, 4 H, NH—CH_2—CH_2—Cl), 2·18 (m, 4 H, CH_2—CH_2CH_2—CH_2—COOH), 1·40 (m, 4 H, CH_2—CH_2—CH_2—CH_2—COOH).

Reaction of 5-(2-Amino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl)pentanoic Acid with Diazomethane (*XXV*, *XXVI*)

An ethereal diazomethane solution (116 ml, 54 mmol) was added under stirring to a suspension of acid *I* (2·0 g, 9 mmol) in 116 ml of dioxane at 0°C and the mixture was stirred at 0—5°C until dissolution (26 h) and then allowed to stand at room temperature for 3 days. After distillation off of volatile components in a vacuum (water pump) the residue was dried to constant weight. The crude product (2·2 g) was dissolved in 10 ml chloroform and chromatographed on a silica gel (Merck) column (45 g) with chloroform with increasing amount of methanol (0—5% v/v).

The combined foreruns (0·6 g) containing methyl 5-(2-amino-4,6-dimethoxy-5-pyrimidinyl)-

pentanoate (*XXV*) with R_F 0·82 (in S_1) were combined and evaporated. Crystallization from acetone (20 ml) gave 0·5 g of a compound, m.p. 118–119°C; for $C_{12}H_{19}N_3O_4$ (269·3) calculated: 53·52% C, 7·11% H, 15·61% N; found: 53·65% C, 7·21% H, 15·67% N. UV spectrum: 281 (4·03), 236 (4·07) nm (M_1); 268 (3·89), 238 (4·13) nm (M_2). IR spectrum ($CHCl_3$): 1 725 (ester), 1 125, 1 155 (ether), 3 520, 3 420 (NH_2) cm^{-1}. ^1H NMR spectrum (deuteriochloroform): δ 4·90 (bs, 2 H, NH_2), 3·80 (s, 6 H, $(OCH_3)_2$), 3·60 (s, 3 H, $COOCH_3$), 2·30 (m, 4 H, —$CH_2CH_2CH_2CH_2COO$—), 1·50 (m, 4 H, —$CH_2CH_2CH_2CH_2COO$—). IR spectrum (KBr): 3 520, 3 420 (NH_2), 1 725 (ester), 1 125, 1 155 (ether), 1 610, 1 570 (pyrimidine nucleus) cm^{-1}. Mass spectrum (*m/z*): 269 (M^+, $C_{12}H_{19}N_3O_4$).

The further combined fractions (1·2 g) containing predominantly a compound of R_F 0·3 (S_1) were repeatedly crystallized from a mixture of acetone and hexane, to yield 0·55 g of methyl 5-(2-amino-6-methoxy-3-methyl-4-oxo-3,4-dihydro-5-pyrimidinyl)pentanoate (*XXVI*) with m.p. 109–110°C. For $C_{12}H_{19}N_3O_4$ (269·3) calculated: 53·52% C, 7·11% H, 15·61% N; found: 53·34% C, 7·23% H, 15·73% N. UV spectrum: 280 (4·07), 238 (3·85) nm (M_1); 281 (4·12), 238 (3·90) nm (M_2). IR spectrum ($CHCl_3$): 1 720 (ester), 1 650 (lactam), 1 160 (ether), 3 420, 3 520 (NH_2) cm^{-1}. ^1H NMR spectrum (deuteriochloroform): δ 5·70 (bs, 2 H, NH_2), 3·80 (s, 3 H, —OCH_3), 3·62 (s, 3 H, $COOCH_3$), 3·40 (s, 3 H, N—CH_3), 2·35 (m, 4 H, CH_2CH_2——CH_2CH_2COO—), 1·55 (m, 4 H, $CH_2CH_2CH_2CH_2COO$—). Mass spectrum (*m/z*): 269 (M^+, $C_{12}H_{19}N_3O_4$).

The analyses of the compounds were carried out by Mrs J. Komancová (Analytical department of the Institute, head Dr J. Körbl). The mass spectra were recorded by Dr M. Ryska and the ^1H NMR *spectra by Dr J. Holubek (Physico-chemical department of the Institute, head Dr B. Kakáč).*

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