SOME ω-(2-AMINO-6-HYDROXY-4-OXO-3,4-DIHYDRO-5-PYRIMIDINYL) ALKANOIC ACIDS, THEIR DERIVATIVES AND ANALOGUES*

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Condensation of 1,1,ω-alkanetricarboxylic acids triethyl esters *XIV*—*XXI* with guanidine gave ω-(2-amino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl) alkanoic acids *I*—*IV*, *VI*—*VIII*, *XIII* of which some were converted to derivatives (ethyl ester *V*, N-acyl derivatives *XI* and *XII*). Similarly, triester *XVII* gave by condensation with urea or thiourea the analogous 5-substituted derivatives of barbituric acid *X*, and thiobarbituric acid *IX*, respectively. The structures of selected compounds of this group (*IV*, *V*, *IX*—*XI* and *XIII*) were determined by spectral methods. Of interest, from the pharmacological point of view, has proved compound *IV*, which exhibited a significant antineoplastic effect on some experimental tumours in mice and rats, and enhanced the action of some current cytostatics.

Within the framework of our study of antimetabolites of the purine and pyrimidine bases of nucleic acids, we have dealt with the syntheses of a number of ω-(2-amino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl) alkanoic acids *I*—*IV* and *VI*—*VIII*, and investigated their biological, mainly antineoplastic, effects. For the sake of comparison with 5-(2-amino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl)pentanoic acid (*IV*), which has proved to be the most interesting one of the group, we also synthetized its 2-mercapto and 2-hydroxy analogues, *IX* and *X*, ethyl ester *V*, 2-acetyl- and 2-propionyl derivatives *XI* and *XII*, and 5-ethyl derivative *XIII*.

The acids *I*—*IV* and *VI*—*VIII* can structurally be regarded both as isocytosine derivatives, with a possible antineoplastic effect of the antimetabolite type, and as barbituric acid derivatives, which might influence the toxicity or action of some clinically used cytostatics or other drugs[1-3].

The compounds can be formulated as derivatives of 3,4-dihydropyrimidine *I*—*XII*, 1,4-dihydropyrimidine *Ia*—*XIIa* or pyrimidine *Ib*—*XIIb*, or as derivatives of 2-iminobarbituric, 2-thiobarbituric or barbituric acid *Ic*—*Xc*. The spectral data of selected compounds and the conclusions drawn from them are described below.

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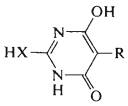
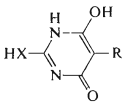
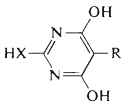


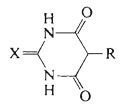
I–XII



Ia–XIIa



Ib–XIIb

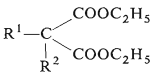


Ic–Xc

(For the substituents X and R see Table I)

Compounds *I–IV*, *VI–X* and *XIII* were synthetized by the procedure described for the preparation of 5-substituted derivatives of barbituric acid and their 2-thio- and 2-iminoanalogues[4–6]. Condensation of triesters of 1,1,ω-alkanetricarboxylic acids, *XIV–XX*, with guanidine gave compounds *I–IV* and *VI–VIII*, the condensation medium being ethanol containing sodium ethylate. Likewise, compounds *IX* and *X* were obtained by condensation of triester *XVII* with thiourea and urea, respectively. The obtained ethyl esters of the acids *I–IV* and *VI–X* were hydrolysed, without previous isolation, by aqueous sodium hydroxide at room temperature.

Compound *XIII* was prepared analogously from triethyl ester *XXI* and guanidine.



XIV–XXI

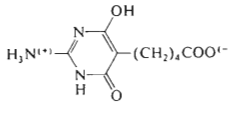
XIV, $R^1 = CH_2COOC_2H_5$, $R^2 = H$
XV, $R^1 = (CH_2)_2COOC_2H_5$, $R^2 = H$
XVI, $R^1 = (CH_2)_3COOC_2H_5$, $R^2 = H$
XVII, $R^1 = (CH_2)_4COOC_2H_5$, $R^2 = H$
XVIII, $R^1 = CH(C_3H_7)COOC_2H_5$, $R^2 = H$
XIX, $R^1 = (CH_2)_5COOC_2H_5$, $R^2 = H$
XX, $R^1 = (CH_2)_6COOC_2H_5$, $R^2 = H$
XXI, $R^1 = (CH_2)_4COOC_2H_5$, $R^2 = C_2H_5$

Condensation of guanidine with the triesters *XIV–XX* in the presence of sodium ethylate gave good yields even at room temperature, and was effected even in the absence of the condensation agent; *e.g.* compound *IV* was obtained by prolonged boiling of guanidine carbonate with the triester *XVII* in ethanol. With compounds *IX* and *X* it proved rewarding to conduct the condensation of the triester *XVII*, with thiourea and urea respectively, in the presence of sodium ethylate at the boiling temperature of the mixture. In contrast to the reported data[4], the corresponding ω-carboxamides were not formed to an appreciable extent.

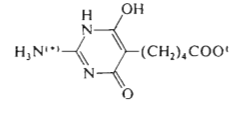
The needed triesters of 1,1,ω-alkanetricarboxylic acids, *XIV–XXI*, were prepared, as previously described[7–17], by alkylation of diethyl ester of propanedioic acid, or 2-ethylpropanedioic acid in the case of the triester *XXI*, with ethyl esters of ω-halogenoalkanoic acids, in presence of sodium ethylate.

The ethyl ester *V* was obtained in two ways: by condensation of guanidine with the triethyl ester *XVII*, followed by isolation of the product, any by esterification of the acid *IV*. The latter esterification was accomplished according to Brenner and coworkers[18-20]: thionyl chloride was treated with ethanol at −20° to −40°C and the reactive agent thus formed was allowed to react with the acid *IV* at the boiling temperature of the mixture. The acylamino derivatives *XI* and *XII* were obtained by 1 hour's boiling of the acid *IV* with acetic and propionic anhydrides respectively, followed by hydrolysis of the intermediates (probably anhydrides of the acyl derivatives *XI* and *XII*) with water; the position of the acyl group was inferred from spectral data (see further).

The products *IV*, *V*, *IX*−*XI* and *XIII* were selected as representative compounds for studying the tautomeric forms, in the solid state and in solutions, by spectral methods. In the UV region the compound *IV* had a marked absorption peak at 266 nm in both a neutral and an acid medium; in the alkaline region the absorption peak exhibited a moderate bathochromic shift to 270 nm. The intensity of the absorption was almost identical in all cases and comparable with that of the sodium and disodium salts of this compound. The infrared spectrum of the compound *IV* in solid state in a KBr pellet gave no convincing evidence of the presence of an imino group or a free amino group. If, however, in view of the high melting point and other findings (given below), the formation of an inner salt is considered, the structures *IV* and *IVa* are not at variance with the infrared spectra in the solid state. The ^{1}H-NMR spectrum of the compound in an alkaline medium, where no proton signal of the methine group at position 5 was observed, shows that the molecule of the compound contains at least one hydroxyl group. On the basis of the given spectral data we identify the compound *IV* as 5-(2-amino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl) pentanoic acid, or 5-(2-amino-6-hydroxy-4-oxo-1,4-dihydro-5-pyrimidinyl)pentanoic acid (*IV*, *IVa*). In the solid state or in a neutral solution the compound is probably in the form of an inner salt (*IV'*, *IV'a*).



IV'



IV'a

The spectra do not make it possible to distinguish between the forms *IV* and *IVa*. The structure proposed is also supported by reported data on the structures of some simpler substituted pyrimidines: 4,6-dihydroxypyrimidine has the structure of a cyclic monoamide (it contains one carbonyl group and one hydroxyl group[21]), aminopyrimidines generally occur in the amino form rather than in the imino form (although

TABLE I

ω-(2-Amino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl) Alkanoic Acids, their Derivatives and 2-Mercapto and 2-Hydroxy Analogues

Number	X R	M.p., °C (yield, %)	Formula (mol. mass)	Calculated/Found % C	 % H	 % N	UV spectra λ_{max}, nm (log ε) medium *A*	 *B*
I	NH CH_2COOH[a]	290—292[b] (65)	$C_6H_7N_3O_4$ (185·1)	38·92 38·76	3·81 3·87	22·70 22·71	259 (4·23)	266 (4·12)
II	NH $(CH_2)_2COOH$[c]	320—325[b] (56)	$C_7H_9N_3O_4$ (199·2)	42·21 42·40	4·56 4·66	21·10 20·72	262 (4·20)	267 (41·15)
III	NH $(CH_2)_3COOH$[d]	310—312[b] (52)	$C_8H_{11}N_3O_4$ (213·2)	45·07 44·78	5·20 5·24	19·71 19·45	263 (4·17)	267 (4·13)
IV	NH $(CH_2)_4COOH$[e]	318—320[b] (82)	$C_9H_{13}N_3O_4$ (227·2)	47·57 47·75	5·77 5·68	18·49 18·67	266 (4·22)	270 (4·16)
V	NH $(CH_2)_4COOC_2H_5$[g]	277—279[f] (93)	$C_{11}H_{17}N_3O_4$ (255·2)	51·75 51·49	6·71 6·69	16·46 16·41	267 (4·19)	269 (4·11)
VI	NH $CH(C_3H_7)COOH$[h]	244—245[b] (50)	$C_9H_{13}N_3O_4$ (227·2)	47·57 47·42	5·77 5·88	18·49 18·78	263 (4·24)	269 (4·18)
VII	NH $(CH_2)_5COOH$[i]	291—293[b] (51)	$C_{10}H_{15}N_3O_4$ (241·2)	49·78 49·71	6·27 6·05	17·42 17·24	266 (4·22)	270 (4·17)
VIII	NH $(CH_2)_6COOH$[j]	296—298[b] (73)	$C_{11}H_{17}N_3O_4$ (255·2)	51·75 51·48	6·71 6·67	16·46 16·32	267 (4·20)	270 (4·15)
IX	S $(CH_2)_4COOH$[k,l]	206—208[m] (76)	$C_9H_{12}N_2O_4S$ (244·3)	44·25 44·26	4·95 4·96	11·47 11·76	287 (4·33) 212 (4·02)	289 (4·12) 237 (4·25)
X	O $(CH_2)_4COOH$[n,o]	203—204[f] (69)	$C_9H_{12}N_2O_5$ (228·2)	47·37 47·43	5·30 5·45	12·28 12·63	263 (3·41) 210 (3·95)	270 (4·23) 233 (3·76)
XI	$NCOCH_3$ $(CH_2)_4COOH$[p]	300—310[q] (94)	$C_{13}H_{15}N_3O_5$ (269·3)	49·07 48·80	5·61 5·76	15·61 15·74	281 (3·79) 249 (3·76)	269 (4·09) 232 (3·70)
XII	$NCOC_2H_5$ $(CH_2)_4COOH$[r]	300—310[q] (90)	$C_{12}H_{17}N_3O_5$ (283·3)	50·88 50·57	6·05 6·23	14·83 14·80	281 (4·00) 249 (3·97)	265 (3·99) 231 (4·16)

manifestation of the amino group is strongly affected by interactions due to the hydrogen bonds[21,22]), and isocytosine (2-amino-4-hydroxypyrimidine) in a neutral medium is a mixture of 2-amino-1,4-dihydro-4-oxopyrimidine and 2-amino-1,6-dihydro-6-oxopyrimidine[23].

To substantiate the structures proposed, *IV* and *IVa*, we further analysed the spectra of the ethyl ester *V* and the acetyl derivative *XI*. In contrast to the spectrum of the acid *IV*, the ^{1}H-NMR spectrum of the ester *V* in hexadeuteriodimethyl sulphoxide exhibited a marked two-proton signal, associated undoubtedly with an NH_2 group, but again no signal of the methine group at position 5. The fact that the UV spectra of the ester *V* and the acid *IV* are nearly identical testifies to the tautomeric

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[a] 18·0 g (0·1 mol) of guanidine carbonate, 6·9 g (0·3 mol) of sodium, 24·6 g (0·1 mol) of 1,1,2-ethanetricarboxylic acid triethyl ester[7,8] (*XIV*; b.p. 168—169°C/3·2 kPa), 600 ml of ethanol, reflux for 2 h; *b*) the compound was purified by dissolution in dilute aqueous ammonia (1 : 100) and separation from the solution of the salt by acidification with dilute hydrochloric acid (1 : 1) to pH ~ 3; [c] 18·0 g (0·1 mol) of guanidine carbonate, 6·9 g (0·3 mol) of sodium, 26·0 g (0·1 mol) of 1,1,3-propanetricarboxylic acid triethyl ester[9] (*XV*; b.p. 172—174°C/2·5 kPa), 600 ml of ethanol 3 hours' reflux; [d] 27·0 g (0·15 mol) of guanidine carbonate, 10·4 g (0·45 mol) of sodium, 41·1 g (0·15 mol) of 1,1,4-butanetricarboxylic acid triethyl ester[10] (*XVI*; b.p. 182—183°C/2·0 kPa), 900 ml of ethanol, 4 h reflux; [e] UV spectrum (phosphate buffer pH 7·0): 268 (4·18); ^{1}H-NMR spectrum (in NaOD/D_2O): 1·45 (4 H, m; C$\underline{H}_2$CH$_2$CH$_2$C$\underline{H}_2$COONa), 2·10 (4 H, m; CH$_2$.C$\underline{H}_2$C$\underline{H}_2$CH$_2$COONa); pKa_1 9·37, pKa_2 10·66; IR spectrum (1% solution in dimethyl sulphoxide): 3400 (C—NH, OH), 1720 (COOH), 1640 (amide I); [f] crystallized from aqueous ethanol; [g] IR spectrum: 3580 (OH), 3420, 3340 (NH, NH_2), 1730 (COOR), 1670 (amide), 1600 (NH_2), 1550 (amide II); ^{1}H-NMR spectrum in hexadeuteriodimethyl sulphoxide: 6·32 (2 H, bs; N$\underline{H}_2$), 3·80 → 5·50 (flat band of N$\underline{H}$, $\underline{H}$O), 4·02 (2 H, q; OC$\underline{H}_2$CH$_3$, J = 7·0 Hz), 2·20 (4 H, m; C$\underline{H}_2$CH$_2$CH$_2$C$\underline{H}_2$COOC$_2$H$_5$), 1·90 (4 H, m; CH$_2$C$\underline{H}_2$C$\underline{H}_2$CH$_2$ 1·20 (3 H, t; OCH$_2$C$\underline{H}_3$, J = 7·0 Hz); pKa_1 10·85; [h] 7·9 g (0·0825 mol) of guanidine hydrochloride, 3·8 g (0·165 mol) of sodium, 21·6 g (0·075 mol) of 1,1,2-pentanetricarboxylic acid triethyl ester (*XVIII*, prepared as reported[12], b.p. 168—170°C/1·86 kPa, n_D^{20} 1·4325; for $C_{14}H_{24}O_6$ (288·35) calculated: 58·32% C 8·39% H, found: 58·63% C, 8·53% H), 500 ml of ethanol, 4 h reflux; [i] 18·0 g (0·1 mol) of guanidine carbonate, 6·9 g (0·3 mol) of sodium, 30·2 g (0·1 mol) of 1,1,6-hexanetricarboxylic acid triethyl ester[13,14] (*XIX*; b.p. 211—213°C/2·7 kPa), 900 ml of ethanol, 4 h reflux; [j] 4·63 g (0·0486 mol) of guanidine hydrochloride, 1·49 g (0·064 mol) of sodium, 5·1 g (0·016 mol) of 1,1,7-heptanetricarboxylic acid triethyl ester[15,16] (*XX*; b.p. 201—202°C/1·5 kPa), 200 ml of ethanol, 4 h reflux; [k] 6·85 g (0·09 mol) of thiourea, 2·07 g (0·09 mol) of sodium, 8·64 g (0·03 mol) of triester *XVII*, 180 ml of ethanol, 3 h reflux; [l] calculated 13·13% S, found: 13·14% S; IR spectrum: 3520 (OH), 1590 (C=N), 1635 (tert-amide), 1690 (COOH), 2500 (SH, COOH); UV spectrum (50% ethanol): 269 (4·18), 238 (3·90); [m] crystallized from a mixture of ethanol and n-hexane; [n] 1·80 g (0·03 mol) of urea, 0·69 g (0·03 mol) of sodium, 2·86 g (0·01 mol) of triester *XVII*, 60 ml of ethanol, 3 h reflux; [o] IR spectrum: 3480 (OH), 3150 (NH), 1728 (CO—NH), 1600 (COO$^-$); UV spectrum (50% ethanol): 265 (4·27), 227 (3·71); [p] IR spectrum: 3180 (NH, OH), 1720 (COOH), 1640 (amide I), 1560 (amide II); ^{1}H-NMR spectrum (hexadeuteriodimethyl sulphoxide): 11·40 (4 H, bs; N$\underline{H}$, O$\underline{H}$, COO$\underline{H}$), 2·20 (4 H, bm; C$\underline{H}_2$CH$_2$CH$_2$C$\underline{H}_2$COOH), 2·18 (3 H, s; COC$\underline{H}_3$), 1·48 (4H, bm; CH$_2$C$\underline{H}_2$C$\underline{H}_2$CH$_2$COOH); pKa_1 10·85, pKa_2 9·33; [q] crystallized from dilute acetic acid; [r] UV spectrum (phosphate buffer pH 6·8, 50% methanol): 263 (4·02), 221 (4·14).

structures *V* and *Va* as the probable ones. This is not at variance with the IR spectrum (in KBr pellet), where the absorption due to the hydroxyl group is more marked (probably as a result of esterification of the carboxyl group) and the absorption due to the primary amino group is also greater, since no inner salt can be formed.

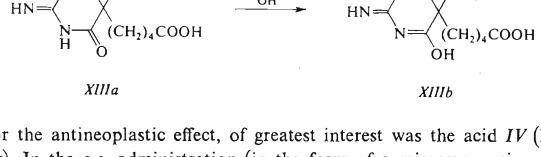
The spectra of the acetyl derivative of compound *IV* allow us to draw the conclusion that acetylation occurred at the primary amino group. The IR spectra in the solid state (KBr pellet) had an absorption band at 1720 cm^{-1} (−COOH) and a flat band at 1640 cm^{-1}, which we assign to an amidic carboxyl group. The absence of the band characteristic of an O-acetyl derivative (the C=O group of the enol acetate absorbs at a frequency higher than 1750 cm^{-1}) and of a C—O bond of the ester at 1240 cm^{-1} excludes acetylation of the hydroxyl group. The ^{1}H-NMR spectra in hexadeuterodimethyl sulphoxide exhibited, in addition to the CH_2-bands of the aliphatic chain, a complex band of the NH- or OH-hydrogens and a three-proton singlet of the acetyl group. Even here the band that would correspond to the hydrogen of the methine group at position 5 was missing. In view of these findings and the fact that the compound behaves like a diacid (see further) we assume that the acetyl derivative has the structure *XI*.

The titration curve of the compound *IV* is suggestive of an acidic amino acid with two dissociation constants: pK_1 = 9·37 (proton of a carboxyl group) and pK_2 = = 10·66 (proton of a hydroxyl group). The basic character of the compound, due to the amino groups at position 2, is strongly suppressed, which can be accounted for by the formation of an inner salt. On blocking the amino group the acidity of the compound is increased. This has been found by titrating the amino acid *IV* in the presence of formaldehyde (dissociation constants pK_1 = 9·11 and pK_2 = = 10·32) and is also evident from the dissociation constants of the acetyl derivative *XI* (pK_1 = 8·5 and pK_2 = 9·33). The ethyl ester *V* behaves like a weak acid of pK = = 10·85 (proton of a hydroxyl group).

Judging by the UV and IR spectra, the 2-mercapto and 2-hydroxy analogues of the compound *IV* seem to have the tautomeric forms *IX* and *X*: they have practically identical courses of the absorption UV spectra in 50% aqueous ethanol with a peak at 265–269 nm. The compound *IX* exhibited a band at 2500 cm^{-1}, which we assign to the SH-group, the compound *X* two intense absorption bands at 3480 cm^{-1} (OH) and 3150 cm^{-1} (NH). The two compounds exhibited relatively high absorption between 1600 and 1620 cm^{-1} (conjugated C=C and C=N). As, however, the evidence for a cyclic amide group is not convincing the forms *IXb* and *Xb* cannot be ruled out as also possible.

By contrast, the UV spectrum of the 5,5-disubstituted derivative *XIII* in an acid medium had an only peak at 215 nm and a low maximum at 245–255 nm, which rules out a conjugated system comparable with the compounds discussed above. Consequently, this compound seems to have the imine structure *XIIIa*, converted in an alkaline medium to the tautomeric form *XIIIb*. This conversion is apparent

from the UV spectrum of the compound in 0·1M-NaOH, where the absorption peak is shifted to 264 nm. The course of the spectrum is nearly identical with that of the compound *IV* in an alkaline medium and is not at variance with the IR spectrum of this compound in solid state. Also in agreement with the structure proposed are the results of potentiometric titration, in which two pK_a constants were found ($pK_1 = 6{\cdot}75$, $pK_2 = 9{\cdot}30$), in analogy to the compound *IV*



XIIIa *XIIIb*

As for the antineoplastic effect, of greatest interest was the acid *IV* (known as Damvar). In the *s.c.* administration (in the form of a microsuspension in a dose of 100 mg/kg) it significantly inhibited the growth of some experimental transplatable tumours and/or extended the survival of the animals. This was observed with the murine sarcoma S 37, the solid Krebs tumour 2 in mice and with the Yoshida ascitic sarcoma in rats. Besides, when combined with some of the clinically used cytostatic (such as cyclophosphamide, 5-fluorouracil, butocin, methotrexate, adriamycin and others) the compound *IV*, if administered *s.c.* or *p.o.*, had an additive effect or enhanced the action of the other cytostatics. It has been demonstrated by experiment, using some labelled cytostatics (*e.g.* [6-^{3}H] 5-fluorouracil) that the compound increases penetration of these cytostatics into the tumour cells. Damvar is now passing the first stage of clinical application and is supposed to find use both in monotherapy and in combination with various, clinically used cytostatics. Details concerning the results of the biological tests will be described elsewhere.

EXPERIMENTAL

The melting points were determined on the Kofler block and are not corrected. The analytical samples were dried over phosphorus pentoxide *in vacuo* (at a pressure of 27 Pa) and at temperatures proportional to their melting points. The ultraviolet spectra [with λ_{max}, nm, (log ε)] of the synthetized compounds were measured in a spectrophotometer Unicam SP 8000 at a concentration of about 0·001% in 0·1M-HCl in 50% methanol (medium *A*) or in 0·1M-NaOH in 50% methanol (medium *B*). The infrared spectra ($\tilde{\nu}$, cm^{-1}) were recorded, unless otherwise specified, in KBr pellets, a Hilger Watts apparatus being used. The ^{1}H-NMR spectra were measured with a spectrometer Tesla BSC 487 (80 MHz) at a concentration of about 10% in hexadeuterodimethyl sulphoxide or in deuterated sodium hydroxide in D_2O, tetramethylsilane or sodium salt of [$^2H_4$]-β.-(trimethylsilyl)propionic acid being used as internal standard; the values are given in ppm units. The values of pK_a were determined, unless otherwise stated, in 80% aqueous dimethyl sulphoxide. The purity of the compounds was assessed by thin-layer chromatography on silica gel plates

(DC-Fertigplatten Kieselgel F_{254}, Merck) or on silica gel reflex sheets with a luminiscent indicator (Silufol UV_{254}, Kavalier) in systems chloroform–methanol–25% ammonia (2 : 1 : 1), 1-propanol–25% ammonia–water (7 : 1 : 2), or chloroform–methanol–acetic acid–water (65 : 25 : 8 : 4). The spots were detected in a UV light of 254 nm.

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

a) To a solution of sodium (8·65 g, 0·375 mol) in ethanol (250 ml) was added, under stirring in a nitrogen atmosphere at 40—45°C, guanidine hydrochloride (23·9 g, 0·25 mol) and triethyl ester of 1,1,5-pentanetricarboxylic acid[11] (36·2 g, 0·125 mol); (*XVII*: b.p. 184°C/2 kPa). The mixture was stirred for 4 h at room temperature and left standing overnight. The ethanol was distilled off under a reduced pressure and the dry residue was dissolved in 0·5M-NaOH (250 ml, 0·125 mol). The mixture was left standing overnight at 20°C, then brought to the boiling temperature and filtered with active carbon. The hot filtrate was acidified with dilute hydrochloric acid (1 : 1) to pH 3. After cooling, 23·5 g (82%) of the acid *IV* separated. This was dissolved in a 50-fold amount of boiling dilute ammonia (1 : 100) and released from the salt by acidification of the solution with dilute hydrochloric acid (1 : 1) to pH ~ 3.

b) To a solution of the ester *XVII* (14·4 g, 50 mmol) in ethanol (70 ml) was added guanidine carbonate (4·5 g, 25 mmol) and the suspension, stirred under nitrogen, was brought to the boil and refluxed until the solid had dissolved (15 h). The solvent was distilled off under reduced pressure and the crude ester *V* was hydrolysed by exposure to 0·5M-NaOH (100 ml, 50 mmol) at room temperature for 2 h. The solution of the sodium salt was then heated to the boiling temperature discolourled with active carbon and filtered. The hot filtrate was brought to pH ~ 3 with dilute hydrochloric acid (1 : 1). After cooling, 3·3 g (29%) of the acid *IV* separated. It had the same properties as that obtained by procedure *a*).

The conditions used in liberating the acid *IV* from a solution of its ammonium salt by dilute hydrochloric acid (1 : 1) made it possible to obtain the acid in two crystalline forms, differing by the content of the solvent. At temperatures between 60 and 95°C the acid *IV* was liberated in a solvent-free form (form *A*), whereas precipitation at temperatures below 50°C gave a product with 1·5 to 2 molecules (10·6—13·6%, w/w) of bound water (form *B*). The two forms could be interconverted by choice of conditions in recovering the acid from alkaline solutions. Drying the form *B* at 140°C and 27 Pa to constant weight also resulted in the form *A*. Crystallization of both the form *A* and *B* from hot water gave the form *A* (according to the IR spectra). The IR spectra of the forms *A* and *B* in KBr pellets were not identical, whereas in dimethyl sulphoxide they were. The physico-chemical properties of the compound *IV* (common for the forms *A* and *B*) are given in Table I.

Monosodium salt of the acid *IV* was prepared by dissolving the acid (5·68 g, 25 mmol) in boiling 0·1M-NaOH (250 ml, 25 mmol) under nitrogen, followed by concentration of the solution under reduced pressure to a volume of about 30 ml. Yield 5·4 g of the salt, m.p. 200—205°C (decomp.). For $C_9H_{12}N_3NaO_4$ (249·2) calculated 9·23% Na, found 9·03% Na. UV spectrum 266 (4·25) in medium *A*, 270 (4·16) in medium *B*.

Disodium salt of the acid *IV* was prepared by dissolving the acid (5·68 g, 25 mmol) in 1M-NaOH (50 ml, 50 mmol) at room temperature under nitrogen, followed by evaporation of water under reduced pressure; m.p. 260—265°C (decomp.). For $C_9H_{11}N_3Na_2O_4$ (271·2) calculated: 16·96% Na; found: 16·76% Na. UV spectrum: 266 (4·03) in medium *A*, 270 (4·05) in medium *B*.

Monopotassium salt of the acid *IV* was prepared like the monosodium salt, from the acid and an equivalent of potassium hydroxide; m.p. 210—212°C. For $C_9H_{12}KN_3O_4$ (265·3) calculated: 14·74% K; found: 14·40% K.

Di(2-hydroxyethyl)ammonium salt of the acid *IV* was prepared by dissolving the acid (2·27 g, 10 mmol) under nitrogen, in boiling water (100 ml) containing bis(2-hydroxyethyl)amine (1·58 g, 15 mmol). After evaporation of the solution under reduced pressure, to a volume of *c.* 20 ml, 200 ml of hot ethanol was added; m.p. 167—169°C (decomp.). For $C_{13}H_{24}N_4O_6$ (332·4) calculated: 46·98% C, 7·28% H, 16·86% N; found: 46·84% C, 7·10% H, 17·11% N.

Acids *I*—*III* and *VI*—*X*

These were obtained from the corresponding constituents by procedures analogous to that used to prepare the acid *IV*. The reaction conditions, yields and physico-chemical properties are given in Table I.

5-(4,6-Dioxo-5-ethyl-2-imino-1,2,3,4,5,6-hexahydro-5-pyrimidinyl)pentanoic Acid (*XIII*)

The acid *XIII* (4·1 g, yield 32%) was synthetized by condensation of guanidine carbonate 19·0 g, 0·05 mol) with triethyl ester of 3,3,7-heptanetricarboxylic acid (*XXI*) (ref.[17]) (15·8 g, 0·05 mol; b.p. 195—200°C/2·7 kPa) in ethanol (200 ml) containing sodium ethylate, (prepared from 3·45 g, 0·15 mol of sodium), as described for the acid *IV*; m.p. 278—280°C (water) (decomp.). For $C_{11}H_{17}N_3O_4$ (255·2) calculated: 51·75% C, 6·71% H, 16·46% N; found: 51·54% C, 6·86% H, 16·76% N; UV spectrum 215 (4·367) in medium *A*, 226 (4·35), 264 (4·04) in medium *B*. IR spectrum: 3290 (NH, OH), 1740 (COOH), 1650 (amide I), 1580 (amide II). $pKa_1 = 6·75$, $pKa_2 = 9·30$ in 80% aqueous methylcellosolve.

Ethyl Ester of 5-(2-Amino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl)pentanoic Acid (*V*)

a) Thionyl chloride (12·1 g, 0·11 mol) was added dropwise, under stirring and cooling to —20 to —40°C, to ethanol (130 ml), then the acid *IV* (22·72 g, 0·1 mol) was gradually added. The suspension was stirred 2 h at 40°C, then refluxed for another 2 h. The volatile constituents were distilled off under reduced pressure. The residue was stirred with 230 ml of water, the suspension was neutralised with sodium hydrogen carbonate and the separated substance was collected on a filter; yield 23·8 g of the ester *V*. For its physicochemical properties see Table I.

b) Condensation of guanidine (liberated from 7·88 g of its hydrochloride) with the triester *XVII* (11·9 g) in the presence of sodium ethylate (prepared from 2·85 g of sodium and 85 ml of ethanol) was carried out as described for the compound *IV*. The mixture was left standing overnight, then, under stirring and cooling to —5 to 0°C, dilute acetic acid (1 : 10, 110 ml) was added dropwise and the separated ester *V* (8·5 g, 81%) was recrystallised from 50% aqueous ethanol. The product was identical with the ethyl ester obtained by procedure *a*).

5-(2-Acetylamino-6-hydroxy-4-oxo-3,4-dihydro-5-pyrimidinyl)pentanoic Acid (*XI*)

A suspension of the acid *IV* (5·0 g) in acetic anhydride (50 ml) was refluxed for 1 h under stirring. The volatile constituents were then distilled off under reduced pressure. The residue was stirred 1 h at 20°C with 500 ml of a 0·2% aqueous solution of sodium hydrogen sulphite and the suspension was left standing for 3 days at room temperature. The separated acetyl compound *XI* (5·58 g, 94%) was dissolved in acetic anhydride (25 ml) and the solution was poured into 500 ml of water under stirring; thy physico-chemical properties are given in Table I.

5-(6-Hydroxy-4-oxo-2-propionylamino-3,4-dihydro-5-pyrimidinyl)pentanoic Acid (*XII*)

A suspension of the acid *IV* (5·0 g) in propionic anhydride (50 ml) was refluxed for 1 h and worked up as described for compound *XI*; for properties see Table I.

The analyses were performed by Mrs J. Komancová and Mrs V. Šmídová. the dissociation constants of selected compounds were determined by Mr E. Kraus (Analytical Department, head Dr J. Körbl). Thin layer chromatography was carried out by Miss D. Dosedlová under the direction of Dr V. Rábek. The antineoplastic effects of the compounds and their potentiation of the current cytostatics were evaluated by Dr K. Řežábek.

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