5,5-DISUBSTITUTED BARBITURIC ACIDS AND THEIR ANALOGUES*

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Received March 26th, 1981

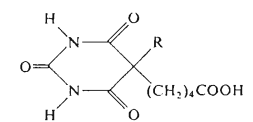
Condensation of 2-substituted triethyl esters of 2-carboxy-1,7-heptanedioic acids *XIV*–*XIX* with urea afforded 5-substituted 5-(4-carboxybutyl)barbituric acids *I*–*VI*. Similarly, thiourea and the triethyl esters *XIV* and/or *XVI* gave the analogous 5-substituted 5-(4-carboxybutyl)-2-thiobarbituric acids, *VII* and *VIII* respectively. Condensation of guanidine with triethyl esters *XIX*–*XXI* produced 5-substituted derivatives of 5-(4-carboxybutyl)-2-amino-1,4,5,6-tetrahydro-4,6-dioxopyrimidine, *IX*–*XI*. Reactions of the acids *II* and *IV* with glycine ethyl ester, using the method of mixed anhydrides, gave rise to ethoxycarbonylmethylamides, *XII* and *XIII* respectively. Some of the compounds prepared exhibited enhancing effects on 5-fluorouracil in curing leukemic mice, and antineoplastic activity, manifesting itself by reducing the size of transplanted tumours in experimental animals.

The present paper continues the study of antimetabolites of the purine and pyrimidine bases of nucleic acids. It deals with the synthesis and study of the biological, mainly antineoplastic, effects of 5-substituted 5-(4-carboxybutyl)barbituric acids, *I*–*VI*, and 2-thiobarbituric acids, *VII* and *VIII*, as well as the ethoxycarbonylmethyl amides *XII* and *XIII* of acids *II* and *IV*. For the sake of comparison with 5-(2-amino-4-oxo-6-hydroxy-3,4-dihydro-5-pyrimidinyl)pentanoic acid[1] (Damvar), which had shown the most promising antineoplastic activity, we prepared its 5-substitution derivatives *IX*, *X* and *XI*. By analogy with the known compounds and in accordance with the measured UV and IR spectra, the compounds prepared can be ascribed the structures of hexahydro-2,4,6-trioxopyrimidines (*I*–*VI*, *XII*, *XIII*), hexahydro-4,6-dioxo-2-thioxopyrimidines (*VII*, *VIII*) and 2-amino-1,4,5,6-tetrahydro-4,6-dioxopyrimidines (*IX* to *XI*).

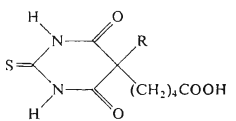
Compounds *I*–*XI* were synthetized by the procedure described[2–4] for the preparation of 5-substitution derivatives of barbituric acids and their analogues, *i.e.* by condensation of triethyl esters of 2-substituted 2-carboxy-1,7-heptanedioic acids *XIV*–*XIX* with urea (to prepare compounds *I*–*VI*) or thiourea (compounds *VII* and *VIII*). Compounds *IX*–*XI* were obtained by condensation of the triethyl esters *XIX*–*XXI* with guanidine in ethanol containing sodium ethoxide as condensation agent. The formed ethyl esters of the acids *I*–*XI* were not isolated, but directly hydrolysed to the free acids with aqueous sodium hydroxide.

* Part LXXXII in the series Compounds with Antineoplastic Activity; Part LXXXI: This Journal *47*, 2525 (1982).

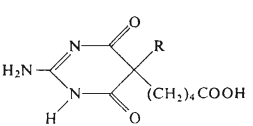
Triethyl esters of 2-substituted 2-carboxy-1,7-heptanedioic acids, *XIV–XX*, were prepared by the described procedure[5–15] (alkylation of diethyl esters of 2-substituted 1,3-propanedioic acids with ethyl 5-bromopentanoate), or, in the case of ester *XXI*, by dialkylation of diethyl 1,3-propanedioate with ethyl 5-bromopentanoate, always with the use of sodium ethoxide. Compounds *XII–XIII* were obtained by reaction of the acids *II* and *IV* with hydrochloride of glycine ethyl ester by the method of mixed anhydrides[16–18], using ethyl chloroformate and triethylamine in dichloromethane.



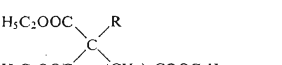
I–VI, XII, XIII



VII, VIII



IX–XI



XIV: R = CH_3, *XV*: R = C_2H_5,
XVI: R = C_3H_7, *XVII*: R = C_4H_9
XVIII: R = C_6H_5, *XIX*: R = CH_2–CH=CH_2
XX: R = CH_2–C≡CH,
XXI: R = $(CH_2)_4COOC_2H_5$

In tests for the action on leukemia La, the acid *IX* in a dose of 200 mg/kg *s.c.* enhanced the action of 5-fluorouracil by 22%. In screening tests for antineoplastic effect on animals with experimental tumours, some of the compounds in doses of 100 to 200 mg/kg *p.o.* reduced the size of the tumours, but did not extend the survival of the animals. The strongest effects were observed with compound *XIII*, administered to mice with sarcoma S 37 in a dose of 200 mg/kg *p.o.*, which reduced the size of the tumours by 27%, and with compound *IV* which in the same way of administration reduced the size of tumours in mice with sarcoma S 37 by 25%. The compounds tested appeared to be non-toxic.

EXPERIMENTAL

The melting points, determined on the Kofler block, are not corrected. The analytical samples were dried at a pressure of 27 Pa over P_2O_5 at elevated temperatures, adequate to their melting points. The ultraviolet spectra were measured on a spectrophotometer Unicam SP-700 at a concentration of about 1 mg of a compound in 100 ml of aqueous methanol (1 : 1), which was

0·1M in HCl (A) or in NaOH (B). The structures of the compounds were corroborated by their infrared spectra (in KBr pellets, in the region 400—4 000 cm^{-1}). The purity of the compounds was verified by TLC in a system chloroform–methanol–25% ammonia (40 : 40 : 20), or propanol–24% ammonia–water (70 : 10 : 20), using F-P-Kieselgel F_{254} Merck, migration distance 15 cm, detection with ultraviolet light, or reflex silica gel foils with a luminiscent indicator (Silufol UV_{254}, Kavalier).

5-Ethyl-5-(4-carboxybutyl)barbituric Acid (*II*)

To a solution of 3·45 g (0·15 mol) of sodium in 150 ml of ethanol was added 9·00 g (0·15 mol) of urea. The mixture was stirred for 10 min, 15·82 g (0·05 mol) of triethyl 2-ethyl-2-carboxy--1,7-heptanedioate[15] was added and the stirred mixture was boiled under a reflux condenser for 4h, then it was left standing overnight at room temperature. Following the removal of ethanol under reduced pressure (using a water pump), 100 ml of water and 5 ml of 10M-NaOH were added to the residue and the mixture was stirred 1 h at room temperature, then left standing at this temperature overnight. The solution was brought to the boil, filtered with activated carbon, and the hot filtrate was brought to pH 2 with dilute hydrochloric acid. After cooling there separated 8·58 g of the acid *II*, which was purified by crystallization.

Acids *I* and *III*—*VIII*

These were prepared analogously from the corresponding components. The yields and the physico-chemical properties are given in Table I.

5-Allyl-5-(4-carboxybutyl)-2-amino-1,4,5,6-tetrahydro-4,6-dioxopyrimidine (*IX*)

To a stirred solution of 3·45 g (0·15 mol) of sodium in 150 ml of ethanol was added 9·00 g (0·05 mol) of guanidine carbonate and, 10 min later, 16·42 g (0·05 mol) of triethyl 2-allyl-2-carbo-xy-1,7-heptanedioate (*XIX*), which had been prepared analogously to[15]; b.p. 207—208°C/4·3 kPa; for $C_{17}H_{28}O_6$ (328·4) calculated: 62·17% C, 8·59% H; found: 62·42% C, 8·91% H. The mixture was stirred at room temperature for 4 h and the ethanol was distilled off under reduced pressure (a water pump). One hundred ml of water and 5 ml of 10M-NaOH were added to the residue and the mixture was stirred for 1 h at room temperature. After standing overnight it was briefly boiled and brought to pH 3 with dilute (1 : 1) hydrochloric acid. The separated product was collected on a filter and purified; yield 5·64 g of the acid *IX*.

The same procedure afforded 5-propargyl-5-(4-carboxybutyl)-2-amino-1,4,5,6-tetrahydro--4,6-dioxopyrimidine (*X*); the needed triethyl 2-propargyl-2-carboxy-1,7-heptanedioate (*XX*) had been prepared analogously to[15], b.p. 199—201°C/2·4 kPa; for $C_{17}H_{26}O_6$ (326·4) calculated: 62·55% C, 8·03% H; found: 62·64% C, 8·03% H.

Diethyl 2,2-Bis(4-ethoxycarbonylbutyl)-1,3-propanedioate (*XXI*)

To a solution of 2·3 g (0·1 mol) of sodium in 100 ml of ethanol was added 28·8 g (0·1 mol) of triethyl 2-carboxy-1,7-heptanedioate and, after refluxing the mixture for 1 h, 23 g (0·11 mol) of ethyl 5-bromopentanoate. The mixture was refluxed for 6 h, the ethanol distilled off under reduced pressure (a water punp), water was added and the organic phase was taken into chloroform. The chloroform extract was dried with anhydrous sodium sulphate and the chloroform was removed under reduced pressure. The crude product was distilled *in vacuo*, the fraction boiling at 190 to 192°C/53 Pa being collected. For $C_{21}H_{36}O_8$ (384·5) calculated: 65·59% C, 9·43% H; found: 65·06% C, 9·15% H.

TABLE I

5,5-Disubstituted barbituric acids and their analogues

No	R	M.p., °C yield %	Formula (mol.mass)	Calculated/Found			UV spectra λ_{max}, nm (log ε)	
				% C	% H	% N	A	B
I	CH_3[a,b,c]	185–187[d] (40)	$C_{10}H_{14}N_2O_5$ (242·2)	49·58 49·52	5·82 5·86	11·57 12·02	213·5 (3·57) 237 i (3·31)	262·5 (4·00)
II	C_2H_5[a,c]	205–207[d] (67)	$C_{11}H_{16}N_2O_5$ (256·3)	51·55 51·52	6·29 6·44	10·93 11·12	208 i (4·02)	241·5 (4·03)
III	C_3H_7[a,f,g]	182–184[h] (54)	$C_{12}H_{18}N_2O_5$ (270·3)	53·32 53·20	6·71 6·81	10·37 11·01	210 (3·90) 240 (3·02)	240 (4·02)
IV	C_4H_9[a,i,j]	192–194[h] (48)	$C_{13}H_{20}N_2O_5$ (28·43)	52·92 54·86	7·09 7·44	9·85 10·12	211·5 (3·95)	241 (3·97)
V	C_6H_5[a,k,l]	206–208[m] (51)	$C_{15}H_{16}N_2O_5$ (304·3)	59·20 59·02	5·30 5·42	9·21 9·50	215 (3·77) 235·5 (3·45)	240 (3·97)
VI	CH_2—CH=CH_2[a,n]	150–152[h] (42)	$C_{12}H_{16}N_2O_5$ (268·3)	53·72 53·67	6·01 6·02	10·44 10·60	220 (3·51)	242 (3·82)
VII	CH_3[a,b,o]	201–203[p] (17)	$C_{10}H_{14}N_2O_4S$ (258·3)	46·50 46·70	5·46 5·51	10·85 11·38	238 (4·20) 286 (3·90)	253 (3·97) 305 (4·02)
VIII	C_3H_7[a,f,q]	137–139[h] (36)	$C_{12}H_{18}N_2O_4S$ (286·4)	50·33 49·96	6·33 6·52	9·79 9·96	237 (3·99) 286·5 (4·35)	253·5 (3·91) 306 (4·40)
IX	CH_2—CH=CH_2[a,r]	293–295[s] (37)	$C_{12}H_{17}N_3O_4$ (267·3)	53·92 53·62	6·41 6·44	15·72 15·86	217 (4·03)	262 (3·00) 226 (4·21)
X	CH_2—C≡CH[a,t]	266–268[s] (40)	$C_{12}H_{15}N_3O_4$ (265·3)	54·32 54·25	5·70 5·70	15·84 15·94	216 (3·96)	263 (3·98) 228 (4·20)
XI	$(CH_2)_4COOH$[a,c]	269–271[x] (68)	$C_{14}H_{21}N_3O_6$ (327·3)	51·37 51·65	6·47 6·30	12·84 12·97	207 (4·32)	263 (4·00) 224 (4·37)

TABLE I
(*Continued*)

No	R	M.p., °C yield %	Formula (mol.mass.)	Calculated/Found % C	% H	% N	UV spectra λ_{max}, nm (log ε) A	B
XII	C_2H_5 [y,z]	155–157 [aa] (66)	$C_{15}H_{23}N_3O_6$ (341·4)	52·77 52·71	6·79 7·03	12·31 12·37	[bb]	—
XIII	C_4H_9 [y,cc]	173–174 [h] (74)	$C_{17}H_{27}N_3O_6$ (369·4)	55·27 55·25	7·37 7·61	11·38 11·38	[dd]	—

[a] $R^2 = (CH_2)_4COOH$. [b] Triethyl ester of 2-methyl-2-carboxy-1,7-heptanedioic acid (*XIV*) was prepared analogously to[15], b.p. 191°C/2 kPa; for $C_{15}H_{26}O_6$ (302·4) calculated: 59·58% C, 8·67% H; found: 58·74% C, 8·33% H. [c] IR spectrum: 2 850, 2 960, 2 940 (CH_2, CH_3), 3 440 to 3 080 (wide band: NH, OH), 1 750 (COOH), 1 685 (wide band: amide carbonyl, C=N), 1 250 (COOH) cm^{-1}. [d] Crystallized from water. [e] IR spectrum is consistent with barbituric acid (hexahydro-2,4,6-trioxopyrimidine), no bands in the region 1 500–1 650 cm^{-1}. [f] Triethyl ester of 2-propyl-2-carboxy-1,7-heptanedioic acid (*XV*) was prepared analogously to[15], b.p. 200 to 212°C/1·7 kPa; for $C_{17}H_{30}O_6$ (330·4) calculated: 61·79% C, 9·15% H; found: 62·16% C, 9·00% H. [g] IR spectrum: 3 420–2 800 (wide band: OH, NH), 2 920 (CH_2), 1 735–1 685 (multiplet of COOH, C=N, ketone) cm^{-1}. [h] Crystallized from aqueous ethanol (1 : 1). [i] Triethyl ester of 2-butyl-2-carboxy-1,7-heptanedioic acid (*XVII*) was prepared analogously to[15], b.p. 212–217°C/2 kPa; for $C_{18}H_{32}O_6$ (344·5) calculated: 62·76% C, 9·37% H; found: 62·53% C, 9·58% H. [j] IR spectrum: 1 680 (CO), 1 710 (CO), 1 760 (COOH), 3 080 (NH), 2 600–3 200 (hydrogen bond), 3 200 (NH), 3 420 (OH) cm^{-1}. [k] Triethyl ester of 2-phenyl-2-carboxy-1,7-heptanedioic acid (*XVIII*) was prepared analogously to[15], b.p. 217–221°C/1 to 1·2 kPa, m.p. 41–43°C (acetone–hexane, 1 : 2); for $C_{20}H_{28}O_6$ (364·4) calculated: 65·91% C, 7·74% H; found: 66·00 C, 7·78 H%. [l] IR spectrum: 1 600, 1 502 (benzene bands), 2 860, 2 940, 2 975 (CH_2), CH_3), 3 100 to 3 200 (wide band: OH, NH), 1 750–1 680 (multiplet of COOH, amide carbonyl), 1 237 (COOH), 1 650 (C=N) cm^{-1}. [m] Crystallized from ethanol. [n] IR spectrum: 3 200, 3 060, (OH), COOH, NH), 1 740, 1 310 (COOH), 2 920, 2 840, 1 460 (CH_2, CH), 1 710 (CO) cm^{-1}. [o] IR spectrum: 2 600 (SH), 3 200–2 950 (wide band: NH, OH), 2 960, 2 920, 2 890 (CH_2, CH_3), 1 740 (COOH), 1 685 (wide band: amide carbonyl, C=N) cm^{-1}. [p] Crystallized from acetone–hexane 1 : 1, calculated: 12·41% S; found: 12·64% S. [q] IR spectrum: 2 600 (SH), 3 100 (wide band: NH, OH), 2 970, 2 940, 2 880 (CH_2, CH_3), 1 738 (COOH), 1 690 (wide band; amide carbonyl, C=N) cm^{-1}, calculated: 11·20% S; found: 11·16% S. [r] IR spectrum: 3 200, 3 180 (NH, amide, COOH), 3 020 (CH_2, NH_3^+), 1 710, 1 310 (COOH), 2 920, 2 850, 1 415 (CH_2), 1 560, 1 640 (C=C), 1 620, 1 500 (amino acid bands), 1 705 (CO) cm^{-1}. [s] Purified by dissolving in dilute aqueous ammonia (1 : 100) and precipitation by acidification to c. pH 3 with dilute (1 : 1) hydrochloric acid. [t] IR spectrum: 3 280 (C=C), 3 025 (NH_3^+), 1 620, 1 500 (amino acid bands), 1 565, 1 640 (sec-amide bands), 1 700 (CO), 1 740 (COOH), 2 920, 2 850, 1 524 (CH_2), 3 240 (NH, COOH) cm^{-1}. [u] $R^1 = R^2 = (CH_2)_4$ COOH. [v] IR spectrum: 3 240, 3 180 (NH), 2 700 (wide band, 1 720 (COOH), 1 620 (lactam), 1 570 (amide band), 1 650 (C=N) cm^{-1}. [x] Crystallized from dimethyl sulphoxide–methanol (1 : 1) [y] $R^2 = (CH_2)_4$ $CONHCH_2COOC_2H_5$. [z] IR spectrum: 1 630 (CONH), 1 695 (CO), 1 720 (CO), 1 760 (ester), 3 300 (NH), 3 220 (NH), 3 120 (NH) cm^{-1}. [aa] Crystallized from ethanol–cyclohexane 1 : 1. [bb] UV spectrum in methanol: λ_{max} 212·5 nm, log ε 3·87. [cc] IR spectrum: 1 755 (ester), 1 552, 1 632 (aliphatic amide), 1 698, 1 722 (cyclic amide), 3 340 (NH) cm^{-1}. [dd] UV spectrum in methanol: λ_{max} 212·5 nm, log ε 3·98.

5,5-Bis(4-carboxybutyl)-2-amino-1,4,5,6-tetrahydro-4,6-dioxopyrimidine (*XI*)

To a solution of 0·69 g (0·03 mol) of sodium in 20 ml of ethanol was added 1·91 g (0·02 mol) of guanidine hydrochloride and 4·16 g (0·01 mol) of the ester *XXI*. The mixture was boiled under a reflux condenser for 3 h, then left standing overnight at room temperature. The ethanol was removed under reduced pressure. The residue was dissolved in aqueous sodium hydroxide (0·8 g NaOH in 20 ml of water) and hydrolysed at the boiling temperature for 1 h. The hot mixture was brought to pH 5 with dilute (1 : 1) hydrochloric acid and the precipitate was collected on a filter, washed with water, dried and recrystallized; yield 2·2 g.

Ethoxycarbonylmethylamide of 5-Butyl-5-(4-carboxybutyl)barbituric Acid (*XIII*)

To a solution of 1·14 g (0·004 mol) of the acid *IV* in a mixture of dichloromethane (20 ml) and triethylamine (0·41 g, 0·004 mol) at −10°C was added 0·46 g (0·004 mol) of ethyl chloroformate and, 15 min later, 0·56 g (0·004 mol) of glycine ethyl ester hydrochloride and 1·62 g (0·016 mol) of triethylamine. The mixture was stirred 1 h at room temperature and left standing overnight. The volatile constituents were removed under reduced pressure (a water pump) and the residue was mixed with 20 ml of water and 3 ml of acetic acid. The precipitate was collected on a filter, washed with water and dried; yield 1·09 g of the product *XIII*, which was purified by crystallization.

Analogously, using the acid *II*, we prepared compound *XII*. The properties of the compounds are given in Table I.

The elemental analyses wer performed by Mrs J. Komancová and Mrs V. Šmídová (Analytical Department, head: Dr J. Körbl). TLC was carried out by Miss D. Dosedlová under the direction of Dr V. Rábek. The IR *and* UV *spectra were measured by Dr J. Vachek. Antineoplastic activity of the compounds and their enhancing effects on the generally used cytostatics were assessed under the direction of Dr K. Řežábek.*

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Translated by J. Salák.