SOME AMIDES OF β-4-PENTOXYBENZOYL-β-BROMOACRYLIC ACID*

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N-Substituted amides of *cis*-β-4-pentoxybenzoyl-β-bromoacrylic acid (*cis*: Br, H) *I*–*XIII* were synthesized and their occurrence in the linear or cyclic form was examined with the aid of spectra. Amides *I*–*XIII* were orientatively evaluated for antineoplastic activity; most of them either inhibited growth of transplantable tumours in experimental animals or extended the survival of the animals.

In the context of studying the relationship between structure and antineoplastic activity we prepared N-substituted amides of the antineoplastically active *cis*-β-4-pentoxybenzoyl-β-bromoacrylic acid[1] (*cis*: Br, H) *I*–*XIII* (Table I).

Amides *I*–*V* and *VII*–*XIII* were obtained in a reaction of γ-4-pentoxyphenyl-γ-acetoxy-β-bromo-$\Delta^{\alpha,\beta}$-crotonolactone[2] with the appropriate amino compound in benzene at 20°C, or (in the case of amide *I*) at 0°C (see ref.[2,3]). In some cases the amino compounds were employed in the form of hydrochlorides and, before the reaction with the mixed anhydride, bases were liberated from them with triethylamine. Amide *VI* was prepared in a reaction of amide *I* with formaldehyde. The crude amides were obtained in a fine yield and were purified by crystallization from organic solvents; some physical properties of compounds *I*–*XIII* are shown in Table I.

In earlier papers on amides of the analogous β-4-methoxybenzoyl-β-chloroacrylic acid and of its β-bromo analogue[3–5] we recognized the possibility of existence of amides of these acids in the form of linear or cyclic structures. The UV spectra of *I*–*IX* show an extended inflexion in the region 260–280 nm while compounds *XII*, *XIII* under the same conditions show absorption maxima at 301, 295 and 221 nm (log ε 4·19, 4·23, 4·25 and 4·17, 4·18, 4·30, respectively). Hence, in agreement with earlier papers[3–5], compounds *XII* and *XIII* were considered to be linear (*A*) while *I*–*IX* were cyclic, with a γ-hydroxylactam ring (*B*).

Model experiments showed similarly that *XII* and *XIII* cannot exist as cyclic due to steric reasons. In contrast with the above compounds, *X* and *XI* showed relatively low UV absorption maxima in the region of the linear form *A*, on the basis of which

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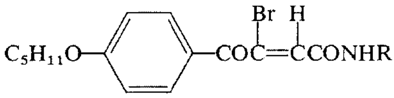
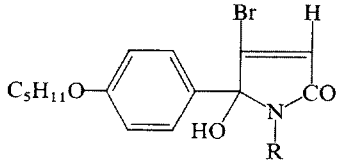
it can be assumed that the compounds are mixtures of both forms, there being some 23% *A* in *X* and 36% *A* in *XI*.



A



B

In agreement with these findings were the results of NMR and IR spectra. The ^{1}H-NMR spectra of the γ-hydroxylactam *B* structure are characterized by a lower value of the chemical shift of aromatic protons adjacent to the lactam substituent (7·15 p.p.m.) in comparison with form *A* where the protons are screened by the carbonyl group and hence shifted lower (7·63 p.p.m.). The shape of the band at 6·98 to 7·11 p.p.m. (a sharp singlet) excludes the possibility of an amide proton (—NH—) which, in the case of the linear form, would exhibit a broad triplet due to the neighbourhood of the —CH_2— group in *III*–*IX*, or a quadruplet due to the vicinity of the —CH_3 group in the case of *II*. This band is ascribed, in agreement with the UV-established cyclic structure, to the —OH group, a direct proof of which by deuterization could not be carried out due to low solubility of the compounds. With *XII* and *XIII*, possessing the linear form *A*, the broad singlet at 8·62 and 8·28 p.p.m. corresponds to the amide proton in the vicinity of a tertiary carbon. The presence of the linear form *A* in *X* and *XI* is supported by the presence of low bands at 7·66 p.p.m. (aromatic protons) and 8·64 or 8·75 p.p.m, (doublets corresponding to the presence of an amide proton adjacent to the —CH—group). Likewise, IR spectra recorded in KBr pellets display the following bands in compounds with the linear *A* structure: for —NH— at 3340 cm^{-1}, for amide II band at 1535 cm^{-1} and for amide I band at 1648 cm^{-1}, and for—CO— at 1668 cm^{-1}. On the other hand, compounds with cyclic structure *B* lack in their spectrum the —NH— group bands and the —CO— group bands. The broad band at 3200 cm^{-1} was ascribed to the associated and the sharp band at 3100 cm^{-1} to the free hydroxyl group. In the carbonyl region of the spectrum, there is a single high band corresponding, in agreement with the proposed structure, to a 5-membered lactam ring. Like the UV and NMR spectra, the IR spectra of *X* and *XI* contain absorption bands characteristic for both forms, with bands corresponding to *B* predominating.

Compounds *I*–*XIII* were orientatively evaluated as to their antineoplastic effect on H strain mice with transplantable tumours: Crocker's solid sarcome (S 180), mammary gland adenocarcinome (HK), ascitic carcinome (37 (S 37), Krebs ascitic carcinome (Kr 2); further on rats (Wistar) with Yoshida transplantable ascitic sarcome (Y). The compounds were applied *p.o.* in daily doses of 100 and 200 mg/kg in an aqueous suspension or, in the case of *X* and *XI*, in 50 and 100 mg/kg doses in a mixture of N,N-dimethylacetamide with olive oil (1 : 50).

With animals bearing solid tumours, the compounds were applied seventh days after transplantation of tumour cells, in a total of 10 daily doses; with animals bearing the ascitic tumour, one day after transplantation, in a total of five daily doses; in the case of the Kr 2 ascites, the compound was applied in a single dose one day after transplantation. In the case of rats with the Y tumour, only the survival effect was examined. The tumour size and the survival length of the untreated animals were taken as 100%. In the case of *II*, *IV*, *V*, *VI*, *XI* and *XII*, applied in a partial dose of 100 mg/kg, the survival of the treated animals with tumour Y was extended by 31, 34, 29, 55, 22 and 36%, respectively. In the case of *VIII* applied in a 200 mg/kg dose, by 39%. Growth of S 37 tumour, without pronounced concomitant effect on survival, was inhibited by *I*, *IV* and *V*, at a partial dose of 200 mg/kg by 21, 24 and 36%, respectively. In the case of HK tumour and partial dose of 200 mg/kg of *VII* and *IX*, by 21 and 26%; at a dose of 100 mg/kg of *XIII* by 21%: in the case of S 180 tumour and a partial dose of 100 mg/kg by 28%. In summary, it can be seen that none of the amides tested (*I*—*XIII*) are superior in the therapy of transplantable tumours to the parent β-4-pentoxybenzoyl-β-bromoacrylic acid[1].

EXPERIMENTAL

The melting points were determined in a capillary and are not corrected. For analysis, the compounds were dried at 0·1 Torr at a temperature raised in proportion to their melting points. The purity of the compounds was checked by thin-layer chromatography on Silufol UV_{254} using chloroform with 1% acetic acid for *I*, *III*, *X*, *XI*, *XII* and *XIII*; chloroform with 5% acetic acid for *II*, *VI* and *VII*; chloroform with 5% ethanol for *IV*, and *V*; chloroform for *VIII* and *IX*. The IR spectra were recorded in an Infrascan (Hilger and Watts) spectrophotometer (KBr pellets, *c* 2 mg/600 mg KBr); UV spectra in an OPTICA Milano spectrophotometer (*c* 2 mg% in ethanol); NMR spectra in a Tesla BS 487C (80 MHz) spectrometer at *c* 8—10% in hexadeuteriodimethyl sulfoxide with tetramethylsilane as standard.

Amide I: A solution of 3·83 g (0·01 mol) γ-4-pentoxyphenyl-γ-acetoxy-β-bromo-$\Delta^{\alpha,\beta}$-crotonolactone[2] in 60 ml benzene was saturated at 0°C for 2 h with dry ammonia. The precipitated product was filtered, the filtrate was shaken with water, dried with Na_2SO_4 and the solvent was removed by distillation in water-pump vacuum. The residue together with the filtered fraction (2·95 g, 86%) was purified by crystallization (Table I).

Amides II, IV, V, VII: A solution of 3·83 g (0·01 mol) γ-4-pentoxyphenyl-γ-acetoxy-β-bromo-$\Delta^{\alpha,\beta}$-crotonolactone[2] in 40 ml benzene was combined with a solution of 0·021 mol methylamine (in the case of amide *II*), or propylamine (amide *IV*), or butylamine (amide *V*) or ethanolamine (amide *VII*) in 10 ml benzene and the mixture was left to stand at 20°C for 24 h, 70 h, 120 h, and 80 h, respectively. Crude amide *VII* was obtained by filtration and precipitation from an acetone solution by a dition of water (9·3 g, 80%). In the remaining case, the reaction mixture was shaken with water, 1M-$NaHCO_3$ and water, dried with Na_2SO_4 and freed of the solvent by distillation under water-pump vacuum. Crude amide *II* (2·51 g, 71·6%), *IV* (3·8 g, 100%) and *V* (3·6 g, 91%) as well as amide *VII* were purified by crystallization (Table I).

Amides III, VIII—XIII: A mixture of 0·021 mol ethylamine hydrochloride (for amide *III*), glycine ethyl ester (for amide *VIII*), β-alanine ethyl ester (for amide *IX*), L-aspartic acid diethyl ester (for amide *X*), L-glutamic acid diethyl ester (for amide *XI*), 1-aminocyclopentanecarboxylic acid ethyl ester (for amide *XII*), or 1-aminocyclohexanecarboxylic acid ethyl ester (for amide *XIII*) with 0·021 mol triethylamine in 50 ml benzene was combined with 3·83 g (0·01 mol) γ-4-pentoxyphenyl-γ-acetoxy-β-bromo-$\Delta^{\alpha,\beta}$-crotonolactone and the mixture was stirred for 72 h at 20°C. After extracting the mixture with water, 1M-$NaHCO_3$ and water, the dried organic phase (Na_2SO_4)

TABLE I
Amides of β-4-Pentoxybenzoyl-β-bromoacrylic Acid

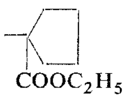
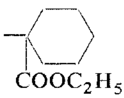
Compound	R	M.p., °C (solvent)
I	H	above 130 decomposition (benzene–cyclohexane)
II	CH_3	154–156 (benzene–cyclohexane)
III	C_2H_5	142–143 (ethanol)
IV	$(CH_2)_2CH_3$	143–144 (benzene)
V	$(CH_2)_3CH_3$	134–135 (benzene)
VI	CH_2OH	154–156 (benzene)
VII	CH_2CH_2OH	134–135 (acetone)
VIII	$CH_2COOC_2H_5$	94–95 (benzene–hexane)
IX	$CH_2CH_2COOC_2H_5$	93–95 (cyclohexane)
X	$CHCH_2COOC_2H_5$ (L)[a] │ $COOC_2H_5$	viscous
XI	$CHCH_2CH_2COOC_2H_5$ (L)[b] │ $COOC_2H_5$	93–95 (cyclohexane)
XII		130–131 (benzene)
XIII		141–143 (benzene)

TABLE I

(*Continued*)

General structure	Formula (mol.wt.)	Calculated/Found			
		% C	% H	% Br	% N
B	$C_{15}H_{18}BrNO_3$ (340·2)	52·95 53·22	5·33 5·12	23·49 23·42	4·11 3·95
B	$C_{16}H_{20}BrNO_3$ (354·3)	54·24 54·04	5·69 5·83	22·56 22·60	3·95 3·88
B	$C_{17}H_{22}BrNO_3$ (368·3)	55·44 55·50	6·02 6·13	21·70 21·70	3·80 3·91
B	$C_{18}H_{24}BrNO_3$ (382·3)	56·54 56·01	6·33 6·58	20·90 21·02	3·66 3·98
B	$C_{19}H_{26}BrNO_3$ (396·3)	57·58 57·10	6·61 6·72	20·16 20·47	3·53 3·59
B	$C_{16}H_{20}BrNO_4$ (370·3)	51·90 52·23	5·44 5·63	21·58 21·48	3·78 3·64
B	$C_{17}H_{22}BrNO_4$ (384·3)	53·13 53·56	5·77 5·96	20·79 20·46	3·64 3·55
B	$C_{19}H_{24}BrNO_5$ (426·3)	53·52 53·68	5·67 6·09	18·75 18·40	3·28 3·12
B	$C_{20}H_{26}BrNO_5$ (440·4)	54·55 54·50	5·95 6·02	18·15 18·08	3·18 3·11
A (23%) *B* (77%)	$C_{23}H_{30}BrNO_7$ (512·4)	53·91 53·94	5·90 5·98	15·60 15·50	2·73 2·55
A (36%) *B* (64%)	$C_{24}H_{32}BrNO_7$ (526·4)	54·75 54·65	6·12 6·20	15·18 15·01	2·66 2·74
A	$C_{23}H_{30}BrNO_5$ (480·4)	57·50 57·69	6·29 6·57	16·63 16·43	2·91 2·86
A	$C_{24}H_{32}BrNO_5$ (494·4)	58·30 58·19	6·52 6·49	16·16 16·41	2·83 2·74

[a] $[\alpha]_D^{20}$ −26·8° (c = 1·5, ethanol); [b] $[\alpha]_D^{20}$ −7·4° (c = 0·54, ethanol).

was freed of solvent by distillation under water-pump vacuum and the crude amides *III* (3·7 g, 100%), *VIII* (4·2 g, 98%), *IX* (13 g, 98%), *X* (10 g, 96%), *XI* (9·9 g 94%), *XII* (4 g, 84%), *XIII* (4 g, 82%) were recrystallized (Table I).

Amide VI: A mixture of 3·4 g (0·01 mol) amide *I*, 0·12 g sodium carbonate and 1·8 g (0·02 mol) 37% aqueous formaldehyde was heated for 1 h on a boiling-water bath; after adding 10 ml water, the mixture was heated for 10 min, left to stand overnight at 0°C and the filtered product (3·7g, 99%) was purified by crystallization (Table I).

The analyses reported here were done in the analytical department of this institute by Mrs J. Komancová and Mrs V. Šmídová under the direction of Dr J. Körbl.

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