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Keyphrases

Hyperglycemic mechanism—chlorpromazine, epinephrine
Chlorpromazine potentiation—epinephrine hyperglycemia
β-Adrenergic receptors—relation to hyperglycemia

Potential Antineoplastic Agents: *N*-(2-Benzoxazolyl)amino Acid Esters

By SHYAM B. ADVANI* and JOSEPH SAM

The investigation of the preparation of *N*-(2-benzoxazolyl)aminoacids and esters from the reaction of aminoacids and esters with either 2-chlorobenzoxazole or 2-benzoxazolinone is described.

TUMORS, because of their parasitic nature, depend upon the host for nutrition and growth. Moreover, because of a limited blood supply, tumor cells depend solely on highly effective transport mechanisms for nutrients. Transport mechanisms involving the accumulation of aminoacids in tumor cells have been studied extensively (1). Tumor cells, being more dependent than the normal tissues on the surrounding fluids for an adequate supply of amino acids, are susceptible to attack by substances which interfere with amino acid transport. Ethionine not only was found to concentrate to a greater extent in tumor cells than methionine, but also was found to possess antineoplastic activity (2). Cycloleucine (1-aminocyclopentanecarboxylic acid) was reported to inhibit growth of tumors by inhibiting transport of other amino acids (3–5). Nitrogen mustards of phenylalanine were found to possess high antitumor activity, probably because of increased transport to the tumor cells (6).

The marked antineoplastic activity of some amino acids prompted the preparation of *N*-(2-benzoxazolyl)amino acids and esters (III, Table I) from 2-chlorobenzoxazole (I) and aminoacids or ethyl esters of aminoacids (II). No reaction was observed when 2-chlorobenzoxazole (I) was treated with either tyrosine or leucine in dry ethanol or benzene according to the method of Montgomery (7). Glycine, on the other hand, gave the desired product (III*c*). The esterification of III*c* in ethanol gave ethyl *N*-(2-benzoxazolyl)glycinate (III*b*). When phenylalanine in ethanol was treated with I in the presence of triethylamine, the ester (III*d*) rather than the acid was isolated. Whereas most amino acids did not react with I, the esters of the amino acids gave satisfactory results. When dimethylformamide (DMF) was used as a solvent in the reactions above, 2-dimethylaminobenzoxazole was isolated as a product (8).

The condensation of I with ethyl *p*-aminobenzoate gave ethyl *N*-(2-benzoxazolyl)-*p*-aminobenzoate (III*h*). The corresponding acid (III*i*) was obtained either from the sodium hydroxide hydrolysis of III*h* or from the reaction of I with

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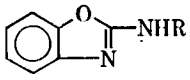
TABLE I—*N*-(2-BENZOXAZOLYL)AMINO ACIDS AND ESTERS



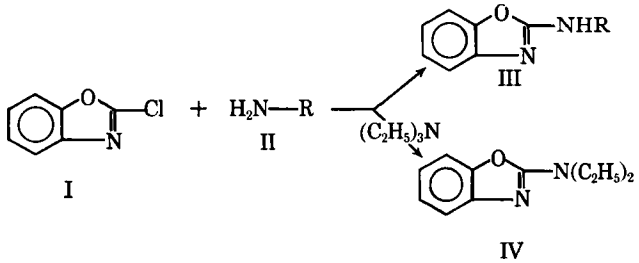
No.	R	Method	Yield, %	M.p., °C.	Molecular Formula	Anal., % Calcd.	Found
III*a*	—CH($CO_2C_2H_5$)CH_2-*p*-C_6H_4OH	A, B[a]	52, 71	161–162[b]	$C_{18}H_{18}N_2O_4$	C, 66.25 H, 5.56 N, 8.58	C, 66.38 H, 5.68 N, 8.69
III*b*	$CH_2CO_2C_2H_5$	D, E	38	104–104.5[c]	$C_{11}H_{12}N_2O_3$	C, 59.99 H, 5.49 N, 12.72	C, 60.20 H, 5.61 N, 12.56
III*c*	CH_2CO_2H	F[d]	31	196–197.5[e]	$C_9H_8N_2O_3$[f]	C, 56.25 H, 4.20 N, 14.58	
III*d*	—CH($CO_2C_2H_5$)$CH_2C_6H_5$	A, F[g]	45, 51	128–129[h]	$C_{18}H_{18}N_2O_3$	C, 69.66 H, 5.85 N, 9.03	C, 69.96 H, 6.15 N, 9.25
III*e*	—CH($CO_2C_2H_5$)$CH_2C_3H_3N_2$[i]	C	70	207–208.5[b]	$C_{15}H_{17}ClN_4O_3$[j]	C, 53.57 H, 5.06 N, 16.66	C, 53.40 H, 5.23 N, 16.07
III*f*	—CH($CO_2C_2H_5$)$CH_2CH_2SCH_3$	B[k]	41	198–199[h] 139–139.5[h,l]	$C_{20}H_{21}N_5O_{10}S$[l]	C, 45.88 H, 4.01 N, 13.38	C, 45.43 H, 3.99 N, 13.11
III*g*	$CH_2CH_2CO_2H$	G	49	175.5–177[m]	$C_{10}H_{10}N_2O_3$	C, 58.25 H, 4.89 N, 13.59	C, 58.20 H, 5.34 N, 13.55
III*h*	—*p*-$C_6H_4CO_2C_2H_5$	B[a]	73	202[b]	$C_{16}H_{14}N_2O_3$	C, 68.08 H, 5.00 N, 9.92	C, 68.45 H, 5.18 N, 9.76
III*i*	—*p*-$C_6H_4CO_2H$	F,[n] H, I	36, 71, 88	328–329[b]	$C_{14}H_{10}N_2O_3$[o]	C, 66.14 H, 3.96 N, 11.02	
III*j*	—*o*-OH-*p*-$C_6H_3CO_2H$	F[n]	26	251.5–252[b]	$C_{14}H_{10}N_2O_4$	C, 62.22 H, 3.73 N, 10.37	C, 62.02 H, 3.91 N, 10.29
III*k*	—*p*-$C_6H_4CH{=}CHCO_2H$	H	70[c]	269.5–270.5[h]	$C_{16}H_{12}N_2O_3$	C, 68.57 H, 4.32 N, 9.99	C, 68.76 H, 4.36 N, 9.84

[a] Benzene used as the solvent. [b] Recryst. from ethanol. [c] Recryst. from ethanol–water. [d] Refluxed 72 hr. [e] Recryst. from benzene–ethanol. [f] Identified through III*b*. [g] Refluxed 92 hr. [h] Recryst. from benzene. [i] $C_3H_3N_2$ = 4-imidazolyl. [j] Hydrochloride. [k] Toluene used as the solvent. [l] Picrate. [m] Recryst. from chloroform. [n] Refluxed 4 hr. [o] Identified through III*h*.

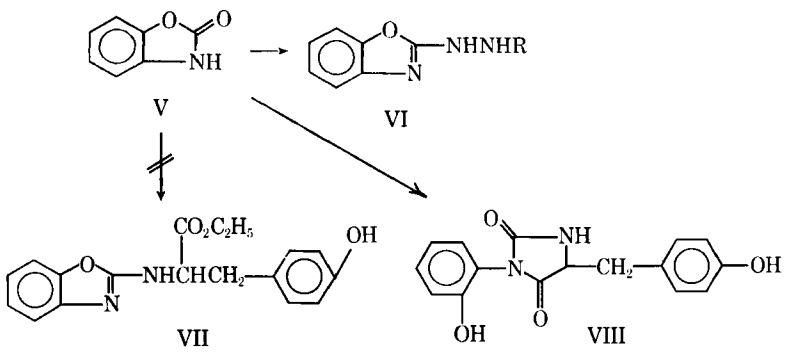
p-aminobenzoic acid. The treatment of I with histidine ethyl ester gave III*e*.

The reaction of 2-chlorobenzoxazole (I) with ethyl tyrosinate in the presence of triethylamine in benzene solution gave approximately a 50% yield of product (III*a*). When the same reaction was conducted in the absence of triethylamine the yield was increased to over 70%. Although no exhaustive study was made, it is speculated that the low yield of III (Table I) obtained with triethylamine in the reaction of I with II (Methods A, D, F, and G) was due to the competitive reaction of triethylamine with 2-chlorobenzoxazole to give 2-diethylaminobenzoxazole (IV). Some of the latter was isolated when conditions described under Method G were utilized. Also, 2-diethylaminobenzoxazole (IV) was the only product obtained when triethylamine was used to remove hydrogen chloride evolved in the reaction (Method J) between I and diethyl glutamate, ethyl valinate, ethyl leucinate, and ethyl alaninate, respectively. The results were in agreement with the observation noted earlier (9) with 2,5-dichlorobenzoxazole and trimethylamine.

An alternate method attempted for the preparation of III (VII) involved the reaction of 2-benzoxazolinone with ethyl tyrosinate. Bower and associates (10) reported favorable results in the preparation of 2-hydrazinobenzoxazoles (VI) by fusion of a hydrazine with V. The fusion of ethyl tyrosinate with 2-benzoxazolinone, however, gave 1-(2-hydroxyphenyl)-4-(4-hydroxybenzyl)imidazolidine-2,5-dione (VIII), which was identified by elemental analysis, infrared, and NMR data. Similar rearrangements have been



I IV



observed (11) in the reaction of ethyl anthranilate with 2-benzoxazolinone.

Infrared data of the compounds are reported in Table II.

Screening of the compounds described in this study currently is being coordinated by the Cancer Chemotherapy National Service Center.

EXPERIMENTAL[1]

Commercially available *dl*-aminoacids (except L-(+)-glutamic acid and *l*-leucine) were utilized in this study; the esters were prepared according to the Fisher esterification method (12).

N-(2-Benzoxazolyl)aminoacid Esters—*Method A*—A solution of 0.025 mole of 2-chlorobenzoxazole, 0.025 mole of aminoacid ester, 6.5 g. (0.065 mole) of triethylamine, and 100 ml. of dry benzene was refluxed for 24 hr. The solution was cooled and the triethylamine hydrochloride removed by filtration. The filtrate was evaporated to dryness under reduced pressure. The residual material either was crystallized and/or recrystallized from a suitable solvent.

Method B—A solution of 0.02 mole of 2-chlorobenzoxazole and 0.02 mole of aminoacid ester in 300 ml. of benzene or toluene was refluxed for 72 hr. The solution was concentrated under reduced pressure on a water bath. The residual material was recrystallized from a suitable solvent.

Method C—A solution of 0.02 mole of aminoacid ester in 200 ml. of benzene or toluene was treated dropwise, while stirring, with a solution of 0.02 mole of 2-chlorobenzoxazole in 10 ml. of ethanol and then refluxed for 48 hr. The solution was allowed to stay at room temperature for 12 hr. and then filtered. The filtrate was concentrated under reduced pressure. The residual material was triturated with acetone, removed by filtration, and then recrystallized.

Ethyl N-(2-Benzoxazolyl)glycinate (IIIb)—*Method D*—The procedure described by Sheehan *et al.* (13) was followed. To a solution of 2.5 g. (0.02 mole) of ethyl glycinate hydrochloride, 3.8 g. (0.025 mole) of 2-chlorobenzoxazole, and 60 ml. of anhydrous chloroform, maintained at −15° (ice and sodium nitrite), was added over a period of 40 min. with vigorous stirring a solution of 4.0 g. (0.04 mole) of triethylamine in 40 ml. of chloroform. Thereafter, while stirring, the temperature was maintained at −15° for 10 min. and then slowly raised to 0°, held at this temperature for another 10 min., and then maintained at room temperature for 1 hr. The solvent was distilled under reduced pressure; the residue was washed with cold water to remove triethylamine hydrochloride and any unreacted ethyl glycinate hydrochloride and then recrystallized.

The nuclear magnetic resonance spectrum showed a four-proton aromatic multiplet (7.1–7.15), a four-proton methylene multiplet (4.1–4.5), and a three-proton methyl triplet (1.2–1.5).

Method E—A solution of 100 mg. of III*c* in 10 ml. of ethanol was treated with an excess of hydrogen chloride and then refluxed for 2 hr. Distillation of the solvent under reduced pressure left an oil which was dissolved in tetrahydrofuran and treated with an excess of triethylamine. The mixture was filtered to remove triethylamine hydrochloride and then again concentrated under reduced pressure. A solution of the residual oil in 5 ml. of ether was allowed to evaporate slowly at room temperature; 25 mg. of solid was obtained, m.p. 104–106°. A mixture of the products obtained by Methods D and E showed no depression of the melting point.

N-(2-Benzoxazolyl)aminoacids—*Method F*—A mixture of 0.05 mole of aminoacid, 10.0 g. (0.1 mole) of triethylamine, and 0.05 mole of 2-chlorobenzoxazole in 200 ml. of ethanol was refluxed for 4–96 hr. The unreacted aminoacid was removed by filtration and the filtrate concentrated under reduced pressure on a water bath. The residual material was treated with

TABLE II—INFRARED SPECTRAL DATA (cm.$^{-1}$)

No.	N—H Str.	C=N C=O Str.	C=C Str.	1,2-Disubst. C=C Str.
III*a*	3,250	1,730	1,650	
			1,585	
III*b*	3,300	1,730	1,640	740
			1,570	
III*d*	3,330	1,725	1,575	745
III*e*	3,330	1,740	1,575	745
III*f*	3,350	1,740	1,675	760
			1,630	715
III*g*	3,330	1,700	1,650	740
			1,575	
III*i*	3,350	1,670	1,600	740
			1,550	
III*j*	3,450	1,650	1,575	
IV	3,350	1,700	1,640	740
			1,575	

[1] All melting points were taken on a Fisher-Johns melting-point apparatus and are uncorrected. Infrared spectra were determined on a Perkin-Elmer model 137 Infracord spectrophotometer using KBr pellets. Nuclear magnetic resonance spectra were determined on a Varian A-60A spectrometer using tetramethylsilane as the internal standard. Chemical shifts are recorded as δ values.

acetone and filtered. Concentration of the filtrate under reduced pressure on a water bath and crystallization and/or recrystallization of the residual material gave product.

Method G—The above procedure was utilized with the exception that benzene was used as the solvent in place of ethanol. A 20% yield of 2-diethylaminobenzoxazole also was obtained.

Method H—A mixture of 0.1 mole of 2-chlorobenzoxazole, 0.1 mole of aminoacid, 200 ml. of benzene, and 20 ml. of ethanol was refluxed on a steam bath for 24 hr. The solid was removed by filtration and washed with warm ethanol to remove any unreacted aminoacid.

N-(2-Benzoxazolyl)-p-aminobenzoic Acid (IIIi)—*Method I*—A mixture of 1.0 g. (0.004 mole) of ethyl *N*-(2-benzoxazolyl)-*p*-aminobenzoate and 50 ml. of 10% sodium hydroxide solution was refluxed on a steam bath for 1 hr. The clear solution was cooled and treated with 10% hydrochloric acid. A white microcrystalline precipitate was obtained.

2-Diethylaminobenzoxazole (IV)—*Method J*—A solution of 7.7 g. (0.05 mole) of 2-chlorobenzoxazole, 10.0 g. (0.05 mole) of diethyl glutamate, and 10.0 g. (0.1 mole) of triethylamine in 200 ml. of dry benzene was refluxed for 24 hr. and then allowed to stay at room temperature for 2 hr. The solution was concentrated under reduced pressure on a water bath. The residue was treated with 50 ml. of acetone and filtered to remove triethylamine hydrochloride. The filtrate was distilled at 140°/1.5 mm. to give 14.1 g. (88%) of oil, $n_D^{25} = 1.5527$. The hydrochloride was prepared in the usual manner and recrystallized from toluene, m.p. 136–137°. The NMR spectrum showed a four-proton aromatic multiplet (6.7–7.4), a four-proton methylene quartet (3.1–3.6), and a six-proton methyl triplet (1.0–1.4).

Anal.—Calcd. for $C_{11}H_{15}ClN_2O$: C, 58.31; H, 6.63; N, 12.39. Found: C, 58.18; H, 6.69; N, 12.40.

Similar results were obtained with ethyl valinate, ethyl leucinate, and ethyl alaninate.

Method K—A solution of 7.7 g. (0.05 mole) of 2-chlorobenzoxazole and 7.3 g. (0.1 mole) of diethylamine in 100 ml. of benzene was refluxed for 24 hr. The diethylamine hydrochloride was removed by filtration; the filtrate was treated as in Method A to yield 9.4 g. (83%) of product, m.p. 135–137°. A mixture of the products obtained by Methods J and K showed no depression of the melting point.

Method L—A solution of 7.7 g. (0.05 mole) of 2-chlorobenzoxazole and 10.0 g. (0.1 mole) of triethylamine in 100 ml. of benzene was refluxed for 2 hr. The triethylamine hydrochloride was removed by filtration; the filtrate was treated as in Method A to give 9.1 g. (82%) of product, m.p. 136–137°. A mixture of the products obtained by Methods J, K, and L showed no depression of the melting point.

1 - (2 - Hydroxyphenyl) - 4 - (4 - hydroxybenzyl)-imidazolidine-2,5-dione (VIII)—A mixture of 3.5 g. (0.017 mole) of ethyl tyrosinate and 2.0 g. (0.015 mole) of 2-benzoxazolinone was fused in an oil bath, preheated to 180° for 15 min. The fused material was allowed to cool to room temperature and then recrystallized from a benzene–alcohol mixture to give 2.3 g. (52%) of product, m.p. 201–201.5°.

The infrared absorption spectrum showed carbonyl absorption at 1775 $cm.^{-1}$ and 1680 $cm.^{-1}$.

Anal.—Calcd. for $C_{16}H_{14}N_2O_4$: C, 64.42; H, 4.73; N, 9.39. Found: C, 64.26; H, 4.98; N, 9.27.

The NMR spectrum showed a one-proton imino singlet (8.3), an eight-proton aromatic multiplet (6.3–7.5), a one-proton methine triplet (4.4–4.6), a two-proton methylene doublet (3.1–3.2), and a broad hydroxyl peak (2.5–3.5).

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Keyphrases

N-(2-Benzoxazolyl)amino acids, esters—synthesis
IR spectrophotometry—structure
NMR spectroscopy—identity