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SYNTHESIS AND BIOLOGICAL ACTIVITY OF ALLYLPYRIMIDINES AND THEIR DERIVATIVES

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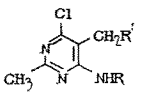
The broad spectrum of biological activity (antitumor, antifungal, and antibacterial [1, 2]) of the naturally-occurring pyrimidine antibiotic sparsomysin, which contains a vinyl group, is due to specific blocking of peptide synthesis by reaction of the double bond in sparsomycin with ribosomal proteins [3, 4]. This has led to a search for antitumor activity in pyrimidines containing a double bond in the substituent.

To study the pharmacological activity and the effect of the presence of a double bond on activity, we have synthesized some pyrimidines containing double bonds, in particular the allylpyrimidines (I-IV) and their bromination products (V-VIII) (Table 1).



I-VIII

I: R = H; R′ = CH = CH_2, II: R = CH_3, R′ = CH = CH_2; III: R = CH_2CH_2OH, R′ = CH = CH_2; IV: R = $CH_2C_6H_5$; R′ = CH = CH_2; V: R = H, R′ = $CHBrCH_2Br$; VI: R = CH_3, R′ = $CHBrCH_2Br$; VII: R = CH_2CH_2OH, R′ = $CHBrCH_2Br$; VIII: R = $CH_2C_6H_5$, R′ = $CHBrCH_2Br$.

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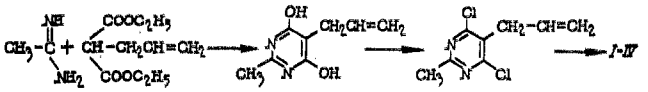
TABLE 1. Substituted 5-Allyl and 5-(2',3'-dibromo-1'-propyl)-4-amino-2-methyl-6-chloropyrimidines (I–VIII)

Compound	Yield, %	Mp, °C	R_f	Found, %					Molecular formula	Calculated, %				
				C	H	Br	Cl	N		C	H	Br	Cl	N
I	50,1	185—6	0,60	51,90	5,90	—	19,22	22,80	$C_8H_{10}ClN_3$	52,30	5,49	—	19,25	22,80
II	85,4	85—7	0,46	54,40	6,20	—	18,05	21,58	$C_9H_{12}ClN_3$	54,68	6,11	—	17,93	21,26
III	90,5	125—6	0,62	52,85	6,30	—	15,81	8,26	$C_{10}H_{14}ClN_3$	52,74	6,19	—	15,58	18,42
IV	74,8	115—7	0,67	65,94	5,62	—	13,30	15,63	$C_{15}H_{16}ClN_3$	65,81	5,89	—	12,95	15,35
V	69,3	170—1	0,66	27,63	2,79	46,85	—	12,30	$C_8H_{10}Br_2ClN_3$	27,97	2,93	46,53	—	12,23
VI	60,7	130—1	0,54	30,57	3,07	45,31	—	11,85	$C_9H_{12}Br_2ClN_3$	30,27	3,38	44,92	—	11,75
VII	87,5	100—1	0,69	30,70	3,79	41,09	—	11,11	$C_{10}H_{14}Br_2ClN_3O$	30,99	3,64	41,40	—	10,98
VIII	80,3	90—1	0,72	41,58	3,64	36,47	—	9,70	$C_{15}H_{16}Br_2ClN_3$	41,55	3,71	36,85	—	9,69

In addition, these compounds were of interest as starting materials for the synthesis of bicyclic pyrrolo[2,3-d]pyrimidines [5, 6].

The allylpyrimidines were synthesized by cyclization of allylmalonic ester with acetamidine, followed by chlorination with phosphoryl chloride and amination.



It is noteworthy that the reaction of dichloropyrimidine with a variety of amines at temperatures not exceeding 90°C gave exclusively the monoamino derivatives.

Bromination of pyrimidines (I-IV) in chloroform afforded the corresponding 4-amino-5-(2',3'-dibromo-1'-propyl)-2-methyl-6-chloropyrimidines (V-VIII).

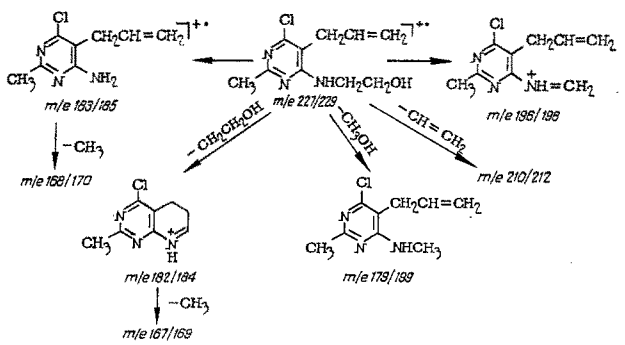
Attempts to cyclize the dibromopropylpyrimidines to pyrrolo[2,3-d]pyrimidines by reaction with bases (sodium bicarbonate and ethoxide) or by boiling in alcohol or DMF were unsuccessful, the dibromo-derivatives being recovered unchanged.

The purity and identity of the compounds obtained were checked by chromatography and elemental analysis, and their structures proved by PMR and mass spectroscopy.

The PMR spectra of the allylpyrimidines (I-IV) showed, in addition to signals for the methyl and substituted amino groups, signals assigned to the allyl group, appearing as a quadruplet for the methylene group (3.35 ppm, $J_{1,2}$ 5 Hz, $J_{1,3}$ 1 Hz), and signals for the vinyl protons (quadruplet for $=CH_2$ at 5 ppm and a multiplet for the methine group at 5.5-6.0 ppm).

Bromination resulted in the disappearance of the signals for the vinyl protons, and the appearance of a multiplet for the alkyl group protons at 3.8-4.0 ppm (CH_2Br) and 4.8 ppm (CHBr).

The mass spectra of (I-VIII) showed strong peaks for the molecular and several fragmentation ions, which enabled the compounds in this series to be identified unambiguously. Dissociative ionization of the allylpyrimidines (I-IV) occurs largely by breakdown of the allyl group and the amino group substituents, as shown by way of example for (III).



Mass-spectral breakdown of the dibromopropylpyrimidines (V-VIII) is mainly due to the elimination of a bromine atom; further fragmentation of the 4-amino- and 4-methylaminopyrimidines (V-VI) proceeds from the ion (M^+—Br). In the case of the dibromo-compounds (VII-VIII), there is also observed preliminary breakdown of the substituted amino group.

As would be expected, the spectra of the benzylaminopyrimidines (IV) and (VIII) show very intense peaks with masses 106 and 91, corresponding to the $^+NHCH_2C_6H_5$ and tropylium ($C_7H_7^+$) ions.

TABLE 2. Summary of Data on the Toxicity and Antitumor Activity (sarcoma) of Substituted 5-Allylpyrimidines (I-IV) and 5-(2',3'-Dibromo-1'-propyl)pyrimidines (V-VIII)

Compound	Acute toxicity in mice				Antitumor activity				
					mass of tumor (M±m)				
	LD_{100}	LD_{50}	maximum tolerated dose	therapeutic dose	test	control	% inhibition	a* >	K† g
	mg/kg								
I	750	550	400	75	10,1±1,57	22,2±2,88	54,5	0,99	+8,11
II	650	450	250	65	10,2±1,10	11,5±0,11	11,3	0,3	+0,9
III	700	600	500	70	3,1±1,14	45,0±0,98	22,3	0,4	—0,5
IV	1800	1600	1300	180	2,7±0,96	4,0±0,98	32,5	0,6	—0,5
V	350	250	150	35	12,5±1,2	22,2±2,88	43,6	0,99	+21,8
VI	300	200	120	30	12,3±0,49	22,2±2,88	44,6	0,99	12,4
VII	450	350	200	45	9,9±1,86	11,5±0,11	13,9	0,3	—8,3
VIII	2600	2400	2000	260	7,5±2,2	4,0±0,98	—87,5	0,1	—15,8

*Value significant.
†Growth coefficient.

TABLE 3. Antimutagenic Activity of Pyrimidines (II-VIII) in *Actinomyces rimosus* 222 lys⁻

Compound	Dose, mmole	Time, min	Effect on UV-induced mutations		
			% of control	number of revertants per 10^5 surviving spores	
				abs. (M±m)	% of control
II	10	10	80	7,2±0,46	120
III	10	10	100	6,0±0,56	100
IV	10	10	74	8,4±0,75	140
V	10	10	112	6,6±0,45	110
VI	10	10	185	4,5±0,38	75
VII	10	10	65	10,5±1,2	175
Control (UV-mutation)			100	6,0±0,55	100

EXPERIMENTAL CHEMISTRY

PMR spectra were obtained on a Varian T-60 instrument (USA) in deuterochloroform with tetramethylsilane as the standard. Mass spectra were recorded on an MX-1303 instrument with direct introduction of the samples into the ionization region at a temperature 40-50°C below their melting points; ionizing electron energy 30 eV. Chromatography was carried out on Silufol UV-254 plates in the system benzene—acetone (3:2 for (I), (III), (V), and (VII); 9:1 for (II), (IV), (VI), and (VIII)). The spots were visualized with a UI-1 ultrachemoscope and iodine vapor.

5-Allyl-2-methyl-4,6-dihydroxypyrimidine. Into a methanolic solution of sodium methoxide, obtained from 50 ml of methanol and 34.5 g (1.5 mole) of metallic sodium, were introduced 47.6 g (0.5 mole) of acetamidine hydrochloride and 100 g (0.5 mole) of diethyl allylmalonate [12], and the mixture was boiled for 8-10 h. When the reaction was complete, the methanol was evaporated to dryness, the residue dissolved in 500 ml of hot water, neutralized with acetic acid, and filtered hot. The solid was washed on the filter with a small amount of acetone, and dried in the drying cabinet. Yield, 60 g (72.3%), mp over 300°C. M^+ (mass spectrum), 166.

5-Allyl-2-methyl-4,6-dichloropyrimidine. In a mixture of 70 ml of phosphoryl chloride and 20 ml of dimethylaniline was dissolved 16.6 g (0.1 mole) of the dihydroxypyrimidine, and the mixture was heated on the water bath for 7 h. When the reaction was complete, the solvent was partially distilled off, and the residue poured on the finely-crushed ice and extracted

with chloroform. After drying over $MgSO_4$, the chloroform was distilled off, and the residue distilled *in vacuo*. Yield 12.3 g (60.6%), bp 90-91°C (4 mm).

Substituted 5-Allyl-4-amino-2-methyl-6-chloropyrimidines (I-IV). To 0.02 mole of 5-allyl-2-methyl-4,6-dichloropyrimidine in 30 ml of absolute ethanol was added an alcoholic solution of the appropriate amine (0.04 mole). The mixture was boiled on the water bath for 4-6 h (in the case of volatile amines, the reaction was carried out in a sealed ampul). The alcohol was distilled off, water added, and extracted with 50 ml of chloroform. On removal of the solvent, the residue crystallized, and was recrystallized from benzene (see Table 1).

Substituted 4-Amino-5-(2',3'-dibromo-1'-propyl)-2-methyl-6-chloropyrimidines (V-VIII). To 0.02 mole of the pyrimidine (I-IV) in 30 ml of chloroform was added dropwise a chloroform solution of 0.02 mole of bromine (3.2 g; 1 ml), and the mixture was boiled for 3 h. The solvent was distilled off, 50 ml of light petroleum added, and the mixture kept overnight. The crystals which separated were filtered off and recrystallized from ethanol (see Table 1).

EXPERIMENTAL BIOLOGY

The antitumor activities of the compounds were determined by a standard method [7, 8]. Toxicities were determined in mongrel male while mice by a single intraperitoneal dose.

The LD_{100} and LD_{50} values were determined together with the maximum tolerated dose (MTD), and the chemotherapeutic activity was determined in tumor-bearing rats (sarcoma 45). The results were treated statistically by the method of V. I. Romanovskii [9].

The results of the biological tests are given in Table 2. All the test compounds, with the exception of (VIII), possessed antitumor activity, the most active being 5-alkyl-4-amino-2-methyl-6-chloropyrimidine (inhibition index, 54.5). In contrast, 4-benzylaminopyrimidine (VIII) strongly stimulated the growth of the tumor.

Bromination of the double bond usually resulted in a reduction in activity [an exception being 4-methylaminopyrimidine (II)] and an increase in toxicity.

The genetic effects of (I-VIII) were examined on a biochemical mutant (*Actinomyces rimosus* 222 lys$^-$). Activity was measured by the frequency of occurrence of reverse mutations, from the auxotrophic to the prototrophic state at the locus responsible for lysine synthesis [10].

Most of the test compounds, tested in high molar concentrations over long periods, had slight mutagenic effects on the lysine locus of Actinomycetes, inducing mutations at a rate 2-5 times greater than in the controls.

Antimutagenic activity was determined in the same test organism by the method described in [11]. These studies showed that (Table 3) only (I), (VI), and (VIII) showed slight antimutagenic activity, reducing the number of UV-induced mutations by 15, 25, and 19%, respectively.

None of the test compounds displayed antibacterial activity against *Staphylococci* or *Shigella dysenteriae* Flexner *in vitro* when tested by serial dilution in a solid nutrient medium. Compounds (IV), (VI), and (VIII) were tested by internal administration in a dose of 1000 mg/kg to white mice with model generalized staphylococcal infection induced by intraperitoneal infection with *Staphylococcus* (Smith and 4-0 strains). These compounds had no therapeutic activity in this test.

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AMINOMETHYLACETOPHENONES AND THEIR BIOLOGICAL ACTIVITY

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We have previously synthesized [1, 2] some β-aminoketone derivatives of glycine and alanine. The glycine derivatives possessed high antiinflammatory and antipyretic activity.

In order to elucidate the relationship between structure and biological activity in β-aminoketone derivatives of amino acids, we have synthesized a number of N-[β-(p-substituted benzoyl)ethyl]tyrosines, phenylalanines, sarcosines, and glycines. Compounds (III-XVII) were synthesized by aminomethylating p-substituted acetophenones (I) and aminoacids (or their ethyl ester hydrochlorides) (II), as follows:

$$\underset{\text{I}}{p\text{-}RC_6H_4COCH_3} + \underset{}{CH_2O} + \underset{\text{II}}{HA} \xrightarrow{H^+} \underset{\text{III—XVI}}{p\text{-}RC_6H_4COCH_2CH_2A(HCl)}$$

III: R = H, A = HNCH(COOH)$CH_2C_6H_5$; IV: R = C_3H_7O, A = HNCH(COOH)$CH_2C_6H_5$; V: R = Br, A = HNCH(COOH)$CH_2C_6H_5$; VI: R = NO_2, A = HNCH(COOH)$CH_2C_6H_5$; VII: R = H, A = HNCH(COOH)$CH_2C_6H_4OH$-p; VIII: R = CH_3O, A = HNCH(COOH)$CH_2C_6H_4OH$-p; IX: R = C_2H_5O, A = HNCH(COOH)$CH_2C_6H_4OH$-p, X: R = Br, A = HNCH(COOH)$CH_2C_6H_4OH$-p; XI: R = NO_2, A = HNCH(COOH)$CH_2C_6H_4OH$-p; XII: R = H, A = $N(CH_3)CH_2COOH$; XIII: R = Br, A = $N(CH_3)CH_2COOH$; XIV: R = Cl, A = $N(CH_3)CH_2COOC_2H_5$; XV: R = C_6H_5, A = $N(CH_3)CH_2COOC_2H_5$; XVI: R = C_6H_5, A = $HNCH_2COOC_2H_5$.

The aminomethylation of (I) with these amino acids was studied under a variety of conditions. We have shown [1, 2] that glycine does not condense with paraformaldehyde and p-substituted acetophenones at pH 6.0-7.0, whereas alanine under the same conditions affords the aminoketones in yields of up to 12%. In acid solution, the yields reach 30%.

Further study of the Mannich reaction showed that in the case of glycine also, the pH had a considerable effect on the yield of the condensation product, and at pH 1.0-2.0 the reaction proceeded to the extent of 37%. Replacement of the paraformaldehyde by 10% formalin increased the yield to 42%.

We have found that when the reaction of (I) and (II) with paraformaldehyde is carried out at pH 6.0-7.0 (taking phenylalanine as an example), no reaction occurred, whereas at pH 1.0-2.0 the yields reached 20%. At these pH values, the use of 10% formalin resulted in increases in yield to 25.5 and 32%, respectively.

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