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SYNTHESIS AND BIOLOGICAL PROPERTIES OF S-DEOXO ANALOGS OF THE NATURAL ANTIBIOTIC SPARSOMYCIN

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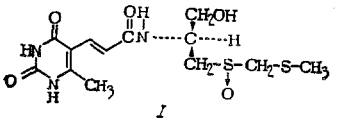
Among pyrimidine derivatives, the antibiotic sparsomycin, which is isolated from Streptomyces sparsogenes by fermentation of beef bouillon [5] and has structure I with an S configuration at the chiral carbon atom [17, 18], is attracting attention.



I

Sparsomycin has a broad spectrum of biological activity – antineoplastic [12], antibacterial [10, 12], fungicidal [12], and antiviral [14]; however, it has a toxic effect on the retina of the eye [7].

The ability of sparsomycin to inhibit the biosynthesis of protein [15] is possibly explained by interaction of the multiple bond with the SH groups of the ribosomal proteins via a reaction of the Michael type [13, 16].

In order to study the biological activity and toxicity of derivatives of S-deoxo sparsomycin we synthesized amides IIa-f.

Compounds IIa-c were obtained by amidation of acids VIa, b with D,L-S-methylthiomethylcysteine methyl ester (VIIa) [8] and D,L-β-phenyl-α-alanine methyl ester (VIIb) [6] by the method of mixed anhydrides with subsequent reduction to amino alcohols IId-f.

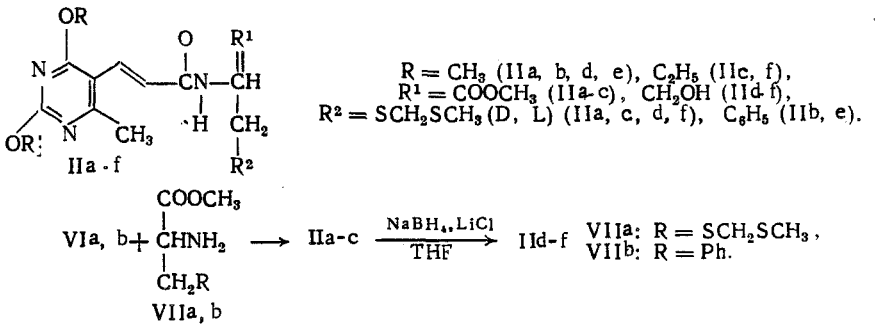


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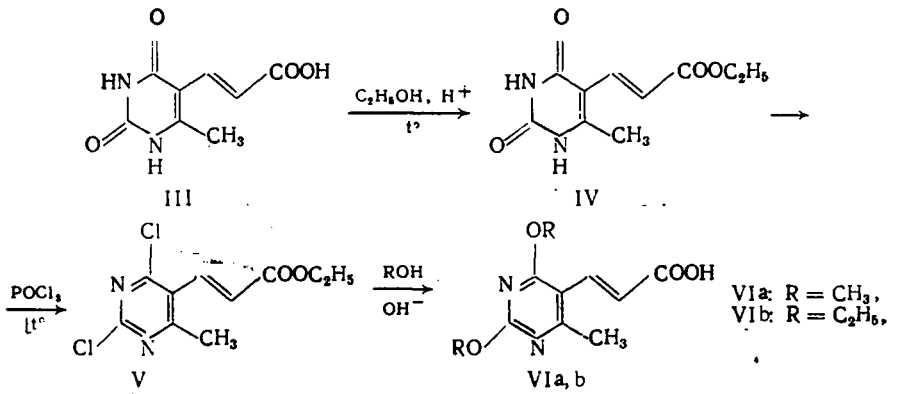
TABLE 1. Properties of IIa-f, V, and VIa, b

Compound	Yield, %	mp, °C	R_f	Found, %				Empirical formula	Calculated, %			
				C	H	N	S		C	H	N	S
IIa	66,0	124—5	0,60	47,35	6,10	10,12	16,06	$C_{16}H_{23}N_3O_5S$	47,86	5,77	10,47	15,97
IIb	68,0	158—9	0,55	61,85	5,85	11,40	—	$C_{0}H_{23}N_3O_5$	62,33	6,02	10,47	—
IIc	52,0	113—4	0,68	49,92	6,67	9,50	14,65	$C_{18}H_{27}R_3O_5S_2$	50,33	6,34	9,78	14.93
IId	66,7	102—3	0,38	48,75	5,91	11,78	17,43	$C_{15}H_{23}N_3O_4S$	48,24	6,21	11,25	17,17
IIe	64,7	160—1	0,25	63,26	6,89	11,50	—	$C_{19}H_{23}N_3O_4$	63,85	6,49	11,76	—
IIf	62,3	104—5	0,45	50,90	6,61	11,01	15,28	$C_{17}H_{27}N_3O_4S_2$	50,85	6,78	10,47	15,97
V*	83,0	48—50	0,86	45,51	4,21	10,90	—	$C_{10}H_{10}Cl_2N_2O_2$	46,0	3,86	10,73	—
VIa	74,4	214—5	0,58	53,95	5,34	12,00	—	$C_{10}H_{12}N_2O_4$	53,57	5,39	12,49	—
VIb	75,3	133—4	0,70	57,45	6,60	11,20	—	$C_1H_{16}N_2O_4$	57,14	6,35	11,11	

*Chlorine: found 26.83%; calculated 27.16%.

Acids VIa, b were obtained via the following scheme:



The synthesis of acid III described in the literature consists in the saponification of ethyl ester IV, obtained from methacil-5-aldehyde and carbethoxymethylenetriphenylphosphorane via the Wittig reaction [11]. In order to simplify the synthesis we, in analogy with the method described in the literature, initially obtained acid III from methacil-5-aldehyde and then esterified it [9]. By chlorination of ethyl ester IV with $POCl_3$–diethylanine we obtained dichloro derivative V, the reaction of which with alcohols in the presence of NaOH led to acids VIa, b.

The purity and individuality of the compounds obtained were proved by thin-layer chromatography and elementary analysis; the structures were confirmed by PMR spectroscopic data.

EXPERIMENTAL (CHEMICAL)

The PMR spectra of solutions in $CDCl_3$ were recorded with a Varian T-60 spectrometer (USA) with tetramethylsilane (TMS) as the standard. Chromatography was carried out on Silufol UV-254 plates in acetone–benzene (2:3). The spots were detected with a UI-1 ultrachemoscope.

Ethyl 2,4-Dichloro-6-methyl-5-pyrimidinylacrylate (V). A mixture of 22.4 g (0.1 mole) of IV, 300 ml of $POCl_3$, and 29.8 g (0.2 mole) of diethylaniline was stirred at 90°C for 20 h, after which the excess $POCl_3$ was removed by distillation, the residue was poured into ice water, and the aqueous mixture was extracted with benzene. The solvent was removed by distillation, and the oily V was crystallized from hexane (see Table 1). PMR spectrum, δ, ppm: 1.30 t (3H, CH_3CH_2O), 2.60 s (3H, 6-CH_3), 4.27 q (2H, CH_3CH_2O), 6.40 d and 7.60 d (2H, AB spectrum, J 16 Hz, trans-CH=CH).

2,4-Dialkoxy-6-methyl-5-pyrimidinylacrylic Acids VIa, b. A 15-ml portion of 5 N aqueous NaOH solution was added dropwise at 0°C to a solution of 2.61 g (0.01 mole) of V in 50 ml of alcohol, and the mixture was allowed to stand for 5 h. The alcohol was removed by distillation, the residue was dissolved in 50 ml of water, and the solution was acidified to pH 5.0 with CH_3COOH. The liberated oil was extracted with $CHCl_3$, and the extract was dried over Na_2SO_4. The solvent was removed by distillation, and the crystals were removed by filtration and washed with hexane (see Table 1). PMR spectrum, δ, ppm: VIa 2.43 s (3H, 6-CH_3), 3.87 s (3H, 4-OCH_3), 3.97 s (3H, 2-OCH_3), 6.40 d and 7.50 d (2H, AB spectrum, J 16 Hz, trans-CH=CH), 9.1 broad s (1H, COOH); VIb 1.33 t and 1.40 t (6H, 2- and 4-CH_3CH_2O), 2.50 s (3H, 6-CH_3), 4.33 q and 4.50 q (4H, 2- and 4-CH_3CH_2O), 6.63 d and 7.83 d (2H, AB spectrum, J 16 Hz, trans-CH=CH), 11.37 s (1H, COOH).

2,4-Dialkoxy-6-methyl-5-pyrimidinylacrylic Acid Amides IIa-c. A 1.37-g (0.01 mole) sample of isobutyl chlorocarbonate was added dropwise at −10°C to 0.01 mole of acid VI and 1.21 g (0.012 mole) of triethylamine in 50 ml of CH_3CN, the mixture was stirred for 10 min, a suspension of 0.012 mole of VIIa or VIIb and 1.21 g (0.012 mole) of triethylamine in 50 ml of CH_3CN was added, and the mixture was allowed to stand overnight at room temperature. The CH_3CN was removed by distillation, the residue was dissolved in CCl_4, and the solution was washed with 10% $NaHCO_3$ and 0.1 N HCl and dried over Na_2SO_4. The CCl_4 was removed, and the IIa-c were crystallized from ether (see Table 1). PMR spectra, δ, ppm: IIa 2.15 s (3H, SCH_3), 2.45 s (3H, 6-CH_3), 3.20 d (2H, CH_2S), 3.68 s (2H, SCH_2S), 3.75 s (3H, $COOCH_3$), 3.96 s (3H, 4-OCH_3), 4.00 s (3H, 2-OCH_3), 5.00 broad m (1H, CH), 6.80 d and 7.80 d (2H, AB spec-

trum, J 16 Hz, trans-CH=CH), 7.30 d (1H, NH); IIb 2.41 s (3H, 6-CH_3), 3.09 d (2H, CH_2), 3.69 s (3H, $COOCH_3$), 3.94 s (6H, 2- and 4-OCH_3), 4.99 broad m (1H, CH), 6.69 d and 7.69 d (2H, AB spectrum, J 16 Hz, trans-CH=CH), 6.72 d (1H, NH), 7.24 s (5H, C_6H_5); IIc 1.40 t and 1.42 t (6H, 2- and 4-CH_3CH_2O), 2.20 s (3H, SCH_3), 2.55 s (3H, 6-CH_3), 3.15 d (2H, CH_2S), 3.65 s (2H, SCH_2S), 3.80 s (3H, $COOCH_3$), 4.50 q (4H, 2- and 4-CH_3CH_2O), 5.00 broad m (1H, CH), 6.80 d and 7.80 d (2H, AB spectrum, J 16 Hz, trans-CH=CH), 7.00 d (1H, NH).

Amides of IId-f. A solution of 0.01 mole of amide IIa-c in 50 ml of THF was added dropwise at 0°C to a mixture of 1.14 g (0.03 mole) of $NaBH_4$ and 1.28 g (0.03 mole) of LiCl in 50 ml of THF, and the mixture was stirred for 15 h. The THF was removed by distillation, water was added to the residue, the aqueous mixture was extracted with CCl_4, and the extract was dried over Na_2SO_4. The CCl_4 was removed by distillation, and the IId-f were crystallized from hexane (see Table 1). PMR spectra, δ, ppm: IId 2.20 s (3H, SCH_3), 2.50 s (3H, 6-CH_3), 2.95 d (2H, CH_2S), 3.75 s (2H, SCH_2S), 3.80-4.40 broad m (10H, CH_2O, 2- and 4-OCH_3, OH, CH), 6.70 d and 7.70 d (2H, AB spectrum, J 16 Hz, trans-CH=CH), 7.00 broad hump (1H, NH); IIe 2.40 s (3H, 6-CH_3), 2.90 d (2H, $CH_2C_6H_5$), 3.65 m (2H, CH_2O), 3.90 s (6H, 2- and 4-OCH_3), 4.22 broad hump (2H, CH, OH), 6.60 d and 7.60 d (2H, AB spectrum, J 16 Hz, trans-CH=CH), 6.70 d (1H, NH), 7.20 s (5H, C_6H_5); IIf 1.30 t (6H, 2- and 4-CH_3CH_2O), 2.05 s (3H, SCH_3), 2.40 s (3H, 6-CH_3), 2.80 d (2H, CH_2S), 3.60 s (2H, SCH_2S), 3.70-4.60 broad m (4H, CH_2O, CH, OH), 4.30 q (4H, 2- and 4-CH_3CH_2O), 6.50 d and 7.50 d (2H, AB spectrum, J 16 Hz, trans-CH=CH), 6.80 d (1H, NH).

EXPERIMENTAL (BIOLOGICAL)

The antineoplastic, antibacterial, mutagenic, and antimutagenic activities of the synthesized compounds were studied.

The toxicities and antineoplastic activities of the compounds were determined by means of generally accepted methods [3, 4]. The toxicities of the substances were studied on white mongrel mice in the case of a single intraperitoneal administration; the absolutely lethal (LD_{100}) and maximally tolerable (MTD) doses were established for each compound. The antiblastic properties of the substances were studied on rats and mice of the $C57Bl_6$ strain with implanted tumors – sarcoma 45, Walker carcinosarcoma (KSU-256), and hemocytoblastosis La – in doses amounting to 1/12 and 1/20 of the LD_{100}. We used a total of 200 mice and 180 rats.

It was established that the LD_{100} values of most of the compounds ranged from 500 to 1000 mg/kg and that the toxicity decreased significantly when a phenylalanine residue was present in the molecule (LD_{100} for IIb was 2000 mg/kg, while LD_{100} for IId was 1000 mg/kg). It was shown that the investigated substances display weak antineoplastic activity with respect to sarcoma 45, decreasing the growth of the tumor by 30-49%. Only the compounds with methoxy radicals displayed similar activity with respect to Walker carcinosarcoma.

The chemotherapeutic activities of VIb and IIa-f were studied in the case of models of generalized staphylococcal and dysenteric infection of white mice [1]. The tolerance of the compounds in the case of a single internal administration was determined in experiments with healthy animals. The experiments were carried out on 160 white mongrel mice with masses of 17-19 g.

The infection was induced by intraperitoneal administration of cultures of staphylococcus aureus (Smith strain) and Flexner dysentery bacillus (strain 6858). Contaminating doses that induce the death of 90-100% of the control (untreated) animals were used. The compounds tested were administered internally in a dose of 1000 mg/kg simultaneously with the contamination.

The MTD of acid VIb for white mice was 2000 mg/kg, as compared with 3000 mg/kg in the case of IIa-f (1250 mg/kg for IIb, and 500 mg/kg for IId).

In the case of staphylococcal infection only IIf increased the total of days that the treated animals survived by 40% ($P < 0.001$).

In the model of dysenteric septicemia IIc and IIf increased the lifetime of the animals by 20-40% ($P < 0.01$). The other compounds were devoid of this activity.

The mutagenic and antimutagenic effects of VIb and IIa-f were studied by the dose method – the effect on the bacterial test systems Escherichia coli P-678 thr^- and Actinomyces rimosus 222 lys^- [2]. Only IIe had mutagenic activity, and only IIb and IId had antimutagenic activity (a decrease in the number of spontaneous mutations of 36% and 43%, respectively).

Thus, the substances obtained are relatively nontoxic and do not have mutagenic activity. Some of them have antibacterial and antimutagenic properties. These results, as well as the presence among them of IId with pronounced antineoplastic activity, indicate that it is expedient to search for more effective antiblastic substances in the series of sparsomycin derivatives.

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