RESEARCHES ON HETEROCYCLIC RINGS CONTAINING NITROGEN AND SULFUR

II. Orotylamino Acids, their Esters, and Salts of Orotic Acid with Amines*

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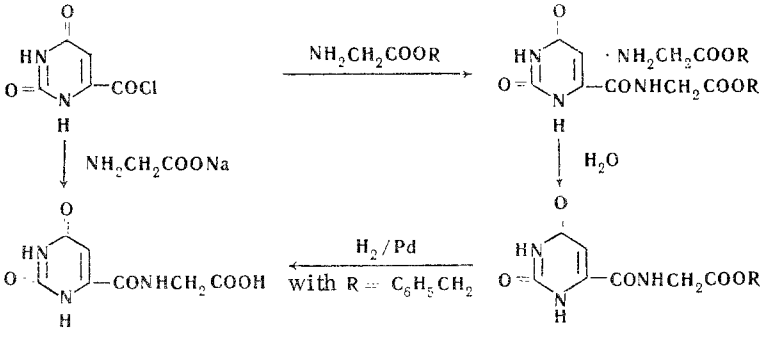
Esters of orotylamino acid are synthesized by reacting orotyl chloride with glycine and α- and β-alanine esters. Orotylamino acids are prepared by reducing the benzyl ester of orotylglycine, or by reacting orotyl chloride with sodium salts of amino acids. A series of salts of orotic acid are prepared by reacting orotic acid with amines.

Recently the synthesis of various derivatives of orotic acid [1, 2], among them orotylamino acids and their esters [3], has been undertaken. In developing previous work [4, 5] with a view to discovering compounds with antitumor activity, it was of interest to prepare orotylamino acids, their esters, and salts of orotic acid with esters of amino acids and other amines.

A number of methods were explored for preparing orotylamino acids (I–V, Table 1). Reaction of orotic acid with glycine methyl ester in the presence of N, N'-dicyclohexylcarbodiimide, as well as the method of mixed anhydrides led to the isolation of an orotic acid salt of glycine methyl ester (IX, Table 2) instead of the expected orotylglycine ester (I). Reaction of methyl orotate [1] with glycine ethyl ester gives orotylglycine ethyl ester in very low yield (about 2%). Esters of orotylamino acids I–V were obtained in better yields (53–67%) by reacting orotyl chloride with esters of amino acids in dry chloroform. Orotyl chloride [1, 3] is a rather unstable compound; it hydrolyzes rapidly. Consequently it was reacted in unpurified form with esters of amino acids. It was previously shown that technical orotyl chloride is of high quality, and that treatment of it with dry methanol gives methyl orotate (mp 241–242°) in almost quantitative yield (97–98%). When orotyl chloride is reacted with esters of amino acids, salts of esters of orotylamino acids are formed along with hydrochlorides of the starting amino acid esters. The former salts are unstable, and readily hydrolyzed to esters of orotylamino acids. Only in the preparation of II was the salt of orotylglycine ethyl ester isolated along with glycine ethyl ester.



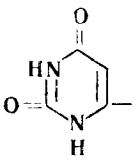
Orotylamino acids are synthesized by two methods. Reduction of the benzyl ester of orotylglycine (III) in the presence of highly active palladium black gave a 99% yield of orotylglycine (VI). The same compound, and orotyl-α- and -β-alanines (VII and VIII) are obtained by reacting orotyl chloride with the corresponding amino acids at a yield of 42–75%.

Salts from orotic acid and esters of amino acids IX–XVI (Table 2) are obtained in almost quantitative yield by reacting orotic acid with the appropriate amino acid esters in dry chloroform. These salts were quite stable, being unchanged on boiling in water. Orotic acid salts of some primary, secondary, and tertiary amines of the aliphatic, aromatic and alicyclic series (XVII–XXII, Table 2) are obtained similarly.

Compounds I, III, IV, VII, and VIII (Table 1), and IX–XII, XIV, XVI (Table 2) were tested biologically. Their one-shot toxicities for mice, and their antitumor activities with mice with intertwined sarcoma-180, LIO-1 lymphosarcoma, AK sarcoma, and with rats with intertwined sarcoma-45 and Jensen's sarcoma, were determined. The compounds were all injected intraperitoneally in the form of aqueous solutions (IV, VII, IX, X, XI, XIV), either as sus-

*For Part I see [8].

Table 1



Orotylamino Acids and their Esters

Compound no.	R	Mp, °C	Formula	Found, %			Calculated, %			Yield, %
				C	H	N	C	H	N	
I	CH_2COOCH_3	232–234	$C_8H_9N_3O_5$	42.40	4.13	18.56	42.29	3.99	18.50	53.7
II	$CH_2COOC_2H_5$	227—228**	$C_9H_{11}N_3O_5$	44.88	4.62	17.54	44.80	4.60	17.43	52.5
III	$CH_2COOCH_2C_6H_5$	232—233.5	$C_{14}H_{13}N_3O_5$	55.69	4.28	13.78	55.44	4.32	13.86	52.8
IV	$CH(CH_3)COOCH_3$	258—259	$C_9H_{11}N_3O_5$	44.65	4.62	17.28	44.80	4.60	17.43	63.7
V	$CH_2CH_2COOCH_3$	279—280	$C_9H_{11}N_3O_5$	44.58	4.68	17.11	44.80	4.60	17.43	66.8
VI	CH_2COOH	269—271***	$C_7H_7N_3O_5$	39.60	3.20	19.68	39.44	3.31	19.71	42.3
VII	$CH(CH_3)COOH$	274	$C_8H_9N_3O_5$	42.45	4.08	18.60	42.29	3.99	18.50	59.7
VIII	CH_2CH_2COOH	283	$C_8H_9N_3O_5$	41.93	4.10	18.32	42.29	3.99	18.50	76.8

*The melting points of the compounds were determined with a Kofler block, heating rate 4°/min; for analysis I was recrystallized from MeOH, while II–VIII were recrystallized ex EtOH. All the compounds could be purified by recrystallizing from water.

**[3] gives mp 247°–249°.

***Mp 269°–270° [3].

Table 2

Amine Salts of Orotic Acid

Compound no.	Amine	Mp (decomp) °C	Formula	Found, %			Calculated, %		
				C	H	N	C	H	N
IX	Methyl glycine ester	242	$C_5H_4N_2O_4 \cdot C_3H_7NO_2$	39.25	4.55	17.14	39.18	4.48	17.16
X	Ethyl glycine ester	233—234	$C_5H_4N_2O_4 \cdot C_4H_9NO_2$	41.67	5.04	16.53	41.70	5.05	16.21
XI	Methyl α-alanine ester	227—228	$C_5H_4N_2O_4 \cdot C_4H_9NO_2$	41.67	4.93	15.98	41.70	5.05	16.21
XII	Methyl β-alanine ester	215—216	$C_5H_4N_2O_4 \cdot C_4H_9NO_2$	41.40	5.05	15.96	41.70	5.05	16.21
XIII	Methyl glutamate	154—155	$C_5H_4N_2O_4 \cdot C_7H_{13}NO_4$	43.72	5.27	12.51	43.50	5.17	12.69
XIV	Ethyl glutamate	145	$C_5H_4N_2O_4 \cdot C_9H_{17}NO_4$	46.72	5.90	12.14	46.66	5.89	11.94
XV	Methyl valine ester	219—220	$C_5H_4N_2O_4 \cdot C_6H_{13}NO_2$	46.30	5.97	14.67	45.99	5.96	14.63
XVI	Methyl β-phenylalanine ester	211	$C_5H_4N_2O_4 \cdot C_{10}H_{13}NO_2$	53.69	5.32	12.70	53.73	5.11	12.53
XVII	Aniline	323	$C_5H_4N_2O_4 \cdot C_6H_7N$	52.81	4.56	16.55	52.80	4.83	16.80
XVIII	p-Toluidine	305	$C_5H_4N_2O_4 \cdot C_7H_9N$	54.55	4.93	15.83	54.74	4.98	15.97
XIX	Benzylamine	268	$C_5H_4N_2O_4 \cdot C_7H_9N$	54.47	5.01	15.98	54.74	4.98	15.97
XX	Cyclohexylamine	316	$C_5H_4N_2O_4 \cdot C_6H_{13}N$	51.64	6.87	16.42	51.76	6.70	6.47
XXI	Dicyclohexylamine	260—261	$C_5H_4N_2O_4 \cdot C_{12}H_{23}N$	60.57	7.98	12.63	60.51	8.07	12.45
XXII	Triethylamine	198	$C_5H_4N_2O_4 \cdot C_6H_{15}N$	51.19	7.33	16.28	51.35	7.45	16.38

*For analysis IX was recrystallized from aqueous EtOH (1:3), X from PrOH, XI and XII ex BuOH, XIII–XXII ex EtOH.

pensions in water (III, VIII, XVI), or as actual solutions in water containing 10% ethanol (I, XII). The preparations were injected once daily, the mice 6 times in all, the rats 8.

The tests showed the compounds to have low toxicities. Some of them (III, VII, X, XI, XII, XIV, XVI) restrain tumor growth. Esters of orotylamino acids (e. g. III), and the orotylamino acid VII were slightly active in isolated experiments with AK sarcoma (28–40% inhibition of tumor growth). Orotic acid salts of esters of amino acids X, XI, XII, XIV, XVI exhibit somewhat greater inhibition of tumor growth. For example, compound XVI gives a 55–56% inhibition of growth of sarcoma-180, compound XI 67% inhibition of sarcoma-45 and 40% inhibition of Jensen's sarcoma, while compound XIV inhibits sarcoma-45 39–45% and sarcoma-180 by 28–37%.

The tumor growth inhibition found does not give any grounds for considering these compounds to have any specific action on tumors. As orotic acid derivatives the compounds exhibit a general effect on exchange of materials in the organism which shows up with tumor growth.

EXPERIMENTAL

Orotyl chloride was prepared as described in [3], dried in a vacuum-desiccator, and reacted without further purification. Benzyl glycine ester was prepared as described in [6], bp 91–95° (8 mm); [7] gives 93–95° (8–11 mm).

Ethyl orotyllysine ester (II). A solution of 6.2 g (0.05 mole) ethyl glycine ester in 20 ml $CHCl_3$ was added to a suspension of 1.74 g (0.01 mole) orotyl chloride in 10 ml dry $CHCl_3$ which was vigorously stirred at 40°. After stirring for 2 hr, the reaction products were allowed to stand overnight, the solid filtered off, boiled with 85 ml water, the solution cooled, the precipitate filtered off, boiled with 85 ml water, the solution cooled, the precipitate filtered off and dried, yield of II 2.53 g, mp 223–224°.

Compounds I, III–V (Table I) were prepared similarly. They were colorless crystalline compounds, soluble on heating with water or the lower alcohols, insoluble in $CHCl_3$ and benzene.

Ethyl glycine ester salt of ethyl orotylglycine ester. Prepared similarly to II, but the reaction product from orotyl chloride and ethyl glycine ester was not heated with water, but directly recrystallized from BuOH. Colorless crystals, mp 117–119°, soluble in the lower alcohols and in water. Found: C 45.43; H 5.64; N 16.50%. Calculated for $C_9H_{11}N_3O_5 \cdot C_4H_9NO_2$: C 45.34; H 5.86; N 16.28%.

Orotylglycine (VI). a) A mixture of 1 g (0.003 mole) benzyl orotyl glycine ester III and 0.07 g palladium black in 100 ml dry MeOH, was hydrogenated at atmospheric pressure and room temperature. The catalyst was filtered off, the solution vacuum evaporated to small volume, and cooled, the precipitate filtered off, and dried. Yield of VI 0.78 g (99%), mp 268–269°.

b) 3.48 g (0.02 mole) orotyl chloride was added in small portions to a solution of 1.58 g (0.021 mole) glycine in 20 ml 4% NaOH. After adding each portion of orotyl chloride, a 10–15% NaOH solution was added, in such a way as to keep the medium alkaline. After all the chloride had been added, the mixture was stirred for 30 min at 18–20°, made acid with HCl, the solid filtered off, washed with water till neutral, and dried in a vacuum-desiccator over P_2O_5. Yield of VI 1.81 g (42.3%), mp 265–267°. Undepressed mixed mp with a specimen prepared by method (a). Orotyl-α- and -β-alanines (VII, VIII) were prepared similarly. The orotylamino acids VI–VIII were colorless crystalline compounds, soluble on heating with water and the lower alcohols.

Methylglycine ester of orotic acid (IX). A mixture of 1.74 g (0.01 mole) orotic acid monohydrate and 0.89 g (0.01 mole) methyl glycine ester in 20 ml dry $CHCl_3$, and dried. Yield of IX 2.41 g (98.2%). Salts X–XII were obtained similarly: they formed colorless crystals, which were readily soluble in water and lower alcohols.

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