precipitate. When using this method, it was necessary first to determine the optimum dose of the compound which would be nontoxic for the LSC cells and normal fibroblasts *in vitro*, but would nevertheless have an inhibitory effect on the growth of the latter (as shown in Table 1). It was found that the optimum dose of (IV) was 5 μg/ml. After the LSC cells has been exposed to this dose of the compound for 18-24 h, the culture fluid was clarified at 6000g for 15 min, and the virus was then sedimented by ultracentrifugation at 100,000g for 1.5 h. The sediment was suspended in TPD buffer solution (Tris-HCl, potassium chloride, and dithiothreitol, pH 8.1), and the virus obtained was introduced into an exogenous synthesis system using poly pA—oligoαT as a synthetic matrix, and ^{3}H-tagged TTF. When the synthesis of the DNA product had proceeded for 1 h at 37°C, the product was precipitated with TCA, applied to millipore nitrocellulose filters, and the radioactivity estimated in a scintillation spectrometer.

This study of the effects of the complex of cadmium chloride with a copolymer of 1-vinylimidazole and 1-vinyl-2-pyrrolidone on various sarcoma strains (carcoma 37, Broker's sarcoma, and Pliss' lymphosarcoma) has shown it to possess nonspecific antitumor activity.

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SYNTHESIS AND CHOLAGOGIC ACTIVITY OF AMIDES, ARENESULFONAMIDES, ACYL-, AND ARENESULFONHYDRAZIDES OF 4-HYDROXYOXANILIC ACID

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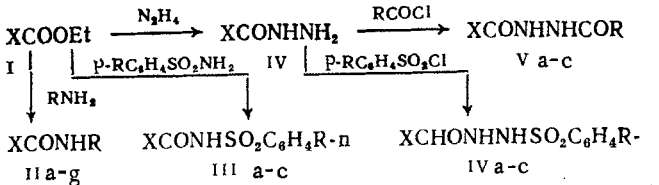
UDC 615.244:547.298.61.495].012.1

In a search for novel cholagogic agents, the substituted amides (IIa-g), arenesulfonamides (IIIa-c), and acyl- and arenesulfonhydrazides (Va-c and VIa-c) of 4-hydroxyoxanilic acid have been synthesized as follows:



IIa: R = H; IIb: R = $C_{10}H_{21}$; IIc: R = $CH_2CH{=}CH_2$; IId: R = CH_2Ph; IIe: R = = C_6H_4OH-o; IIf: R = C_6H_4OH-p; IIg: R = C_6H_3COOH-pOH-m; IIIa: R = H; IIIb: R = = OMe; IIIc: R = NH_2; Va: R = Me; Vb: R = Ph; Vc: R = $C_6H_4CH_3$-p; VIa: R = H; VIb: R = Me; VIc: R = OOCMe; X = $CONHC_6H_3OH$p-.

Amides (IIa-g) were obtained by aminating ethyl 4-hydroxyoxanilate (I) with aliphatic, araliphatic, or aromatic amines.

The acyl- and arenesulfonhydrazides (Va-c) and (VIa-c) were synthesized from 4-hydroxyoxanilic hydrazide (IV), obtained by the hydrazinolysis of the ester (I), and the appropriate acyl- or arenesulfonyl chormides.

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Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 18, No. 6, pp. 683-686, June, 1984. Original article submitted September 16, 1983.

0091-150X/84/1806-0394$08.50

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The structures of the compounds obtained were confirmed by their UV and IR spectra, and their elemental analyses.

The UV spectra of (II-VI) in ethanol displayed two maxima, at 205-215 nm (log ε = 4.08-4.11), and 280-300 nm (log ε = 3.89-4.03). Addition of sodium ethoxide resulted in a bathochromic shift to 236 and 320 nm, respectively. This is in accordance with the spectral properties of phenols [9].

The IR spectra of (II-VI) contained stretching frequencies for the OH (3640-3460 cm^{-1}), NH (3440-3100 cm^{-1}), and CO groups (1720-1660, 1630-1660 cm^{-1}), and those of (IIIa-c) and (VIa-c) for SO_2 (1360, 1180 cm^{-1}).

The ionization constants of some of the compounds were measured (Table 1). As would be expected in view of the low electronic conductivity of the oxamide group [6], substituents in the amide, sulfonamide, or hydrazide groups had little effect on the ionization constants of the hydroxyl group (pKa_1), whereas the acidities of the sulfonamide, sulfonhydrazide, and acylhydrazide groups were dependent on the polar effect of the substituent (pKa_2).

The pKa_2 values of (IIIa-c), (Va-c), and (VIa-c) are fairly high, and they form ammonium salts which are stable in aqueous solution and which form crystalline precipitates with the ions Ag^+, Fe^{2-}, Ni^{2+}, Co^{2+}, Cd^{2+}, Cu^{2+}, Fe^{3+}, and Bi^{3+}.

To determine the effect of the distance between the hydroxyl groups on the cholagogic activity, 4-p,p-dihydroxyadipic acid dianilide (VII) was prepared by acylating p-aminophenol with adipoyl chloride

$$(p\text{-}HOC_6H_4NHCOCH_2CH_2—)_2$$

VII

EXPERIMENTAL CHEMISTRY

UV spectra were obtained on a Specord UV-VIS spectrophotometer (East Germany) in ethanol and in a 0.1 M solution of sodium ethoxide, and IR spectra on a UR-20 instrument (East Germany) in KBr disks. Ionization constants were determined by potentiometric titration in 70% aqueous dioxane with an EV-74 pH meter.

Ethyl 4-Hydroxyoxanilate (I). This was obtained as described in [10].

4-Hydroxyoxanilic Acid Alkylamides (IIa-d). To a solution of 0.01 mole of the ester (I) in ethanol was added 0.011 mole of the amine, and the mixture was kept overnight. The solid which separated was filtered off, and crystallized from propanol.

4-Hydroxyoxanilic Acid Arylamides (IIe-g). A solution of 0.01 mole of the ester (I) and 0.011 mole of the arylamine in DMF was heated until a solid was formed which occupied the whole volume (∿1.5 h). The mixture was diluted with water, and the solid filtered off and crystallized from propanol.

4-Hydroxyoxanilic Acid Arenesulfonamides (IIIa-c). These were obtained as described in [5], and crystallized from propanol.

4-Hydroxyoxanilic Acid Hydrazide (IV). This was obtained as described in [4].

4-Hydroxyoxanilic Acid Acyl- and Arenesulfohydrazides (Va-c and VIa-c). The hydrazide (IV) (0.01 mole) was dissolved in dry pyridine, and 0.011 mole of the acid chloride added in portions with cooling. The mixture was then heated until the solid had dissolved, and left to stand until cold. It was then diluted with water and acidified with 1:1 hydrochloride acid to pH 2.0.

Adipic Acid p,p-Dihydroxyanilide (VII). Adipoyl chloride (0.01 mole) was added dropwise to a cooled solution of 0.02 mole of p-aminophenol in 10 ml of dry pyridine, the mixture diluted with three times its volume of water, and acidified with 1:1 hydrochloric acid to pH 2.0. The solid which separated was filtered off and crystallized, giving 91% of plates (from aqueous DMF), mp 260-262°C. Found, %: N 8.7. $C_{18}H_{20}N_2O_4$. Calculated, %: N 8.5.

EXPERIMENTAL PHARMACOLOGY

The cholagogic activity of (IIa, b, d) was determined by a standard method [2] in male white rats weighing 140-180 g. The test compound was administered in a single dose intra-

TABLE 1. Properties of (IIa-g), (IIIa-c), (Va-c), and (VIa-c)

Compound	Yield, %	mp, °C	Found, N, %	Empirical formula	Calculated, N, %	pKa_1	pKa_2
IIa	81	274—5	15,9	$C_8H_8N_2O_3$	15,6	11,80	—
IIb	81	195—7	8,6	$C_{18}H_{28}N_2O_3$	8,8	11,83	—
IIc	53	211—3	13,0	$C_{11}H_{12}N_2O_3$	12,7	...	—
IId	84	234—6	10,1	$C_{15}H_{14}N_2O_3$	10,4	11,82	—
IIe	62	280—2	10,4	$C_{14}H_{12}N_2O_4$	10,3	...	—
IIf	71	188—9	10,1	$C_{14}H_{12}N_2O_4$	10,3	...	—
IIg	69	270—2	9,2	$C_{15}H_{12}N_2O_6$	8,9	...	—
IIIa	72	206—8	9,0	$C_{14}H_{12}N_2O_5S$	8,7	11,81	5,75
IIIb	74	197—9	8,3	$C_{15}H_{14}N_2O_6S$	8,0	11,85	5,85
IIIc	57	237—9	8,6	$C_{14}H_{15}N_3O_5S$	8,7	11,81	5,90
Va	65	270—2	18,0	$C_{10}H_{11}N_3O_4$	17,7	...	...
Vb	53	265—7	13,8	$C_{15}H_{13}N_3O_4$	14,0	11,81	9,35
Vc	63	269—0	13,6	$C_{16}H_{15}N_3O_4$	13,4	11,80	9,32
VIa	60	255—7	12,3	$C_{14}H_{13}N_3O_5S$	12,5	11,79	9,28
VIb	71	262 (decomp.)	12,2	$C_{15}H_{15}N_3O_5S$	12,0	11,80	9,51
VIc	68	256—8	10,9	$C_{16}H_{15}N_3O_7S$	10,7	11,81	9,25

TABLE 2. Cholagogic Activity of (IIa-g), (IIIa-c), (Va-c), (VIa-c), (VII), and Oxaphenamide

Compound	Increase in rate of secretion of bile, %				Increase in concentration of cholates, %				Change in cholesterol concentration, %	Change in bilirubin concentration, %	Cholate–cholesterol coefficient
	1	2	3	4	1	2	3	4			
I	8	9	6	11	7	19	11	21	0	0	77
IIa	3	3	6	14	7	14	22	21	0	0	78
IIb	5	6	3	14	4	14	6	14	—33	0	110
IIc	0	3	0	7	—7	10	6	7	0	0	69
IId	8	9	16	21	0	10	11	14	0	0	73
IIe	16	14	13	7	0	5	0	—7	0	0	68
IIf	24	23	22	36	7	5	0	0	0	0	70
IIg	18	23	25	29	7	24	22	21	—33	50	95
IIIa	26	20	19	7	14	29	28	7	0	0	81
IIIb	0	—6	—9	—14	—4	0	—5	—7	0	0	71
IIIc	24	14	0	—7	18	25	11	—7	33	0	71
IV	8	6	6	11	0	5	0	7	0	50	69
Va	32	20	6	4	21	14	6	0	0	50	76
Vb	16	31	13	7	14	19	22	29	0	0	74
Vc	29	14	6	0	7	14	11	14	0	0	75
VIa	13	14	19	21	0	14	6	7	0	0	78
VIb	21	14	13	11	25	14	6	7	0	0	78
VIc	24	23	19	14	11	24	22	7	0	50	78
VII	26	20	19	11	11	14	11	0	—33	0	89
Oxaphenamide	32	57	38	43	0	0	0	0	0	0	73

duodenally in aqueous solution, in a dose of 30 mg/100 g. Cholagogic activity was assessed by the effect on the rate of secretion of bile, and changes in the factors characterizing the synthesis of bile acids, cholesterol, and bilirubin. Cholate concentration was measured as described in [3], bilirubin as in [8], and cholesterol as in [2].

Changes in the stabilizing properties of the bile were assessed from changes in the ratio of the concentrations of cholate and cholesterol. The results are shown in Table 2.

These studies of cholagogic activity showed that all the compounds except for (IId) and (IIIb, c) increased the rate of bile secretion.

The highest activity was shown by the 4-substituted amide (IIf), the activity of which was stable throughout the study. Moving one of the hydroxyl groups to the o-position (IIe), or increasing its distance substantially, (VII), reduced the activity. The introduction of a carboxyl group into the molecule increased the activity somewhat (cf. IIe and IIg). These findings are in accordance with those obtained in [7].

Compounds (IIe) and (IIIb) had an inhibitory effect on the synthesis of bile acids, but the other compounds had no significant effects on bile acid synthesis.

Treatment with (IIIc) increased the secretion of cholesterol in the bile, whereas (IIb, c) and (VII) suppressed it. The other compounds had no effect on cholesterol secretion.

The cholate-cholesterol ratio, which is a measure of the colloid state of the bile, shows that these compounds increase the stability of the bile as a colloidal system, compounds (IIb, g), (IIa), and (VII) being superior to oxaphenamide in this respect.

Of the compounds tested, only (IIg), (IV), (Va), and (VIc) increased the secretion of bilirubin in the bile.

Acute toxicities were determined in white mice weighing 18-20 g by the intraperitoneal route, the calculations being carried out as described in [1]. The LD_{50} values of the test compounds were $\geqslant 5$ g/kg, and they may be regarded as relatively safe compounds (the LD_{50} of oxaphenamide is 1500 mg/kg).

The search for cholagogic compounds of low toxicity in the 4-hydroxyoxanilic acid series may therefore be regarded as promising.

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ADDITION OF ISOQUINOLINES TO ACTIVATED C=C DOUBLE BONDS, AND THE BIOLOGICAL PROPERTIES OF THE PRODUCTS

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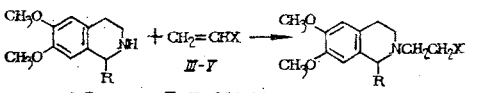
UDC 615.214-012.1.076.9

Esters of N-substituted β-alanines and β-aminoketones have marked effects of the central nervous system [2, 3, 7, 9]. In a search for more active compounds of this type, we have prepared some β-amino-derivatives of methyl propionate and propionitrile (VIa, c) and (VIIa), together with the 2-butanones (VIb) and (VIIb), in which the heterocyclic 1,2,3,4-tetrahydroquinoline system serves as the amine component.

The required compounds were obtained by the addition of substituted 1,2,3,4-tetrahydroquinolines (I) and (II) to methyl acrylate (III), methyl vinyl ketone (IV), and acrylonitrile (V).



I, II; I: R=H; II: R=CH₃

III: X=COOCH₃; IV: X=COCH₃; V: X=C≡N

VI, VII; VIa: R=H, X=COOCH₃; VIb: R=H, X=COCH₃; VIc: R=H, X=CN; VIIa: R=CH₃, X=COOCH₃; VIIb: R=CH₃, X=COCH₃

The catalytic effects of the hydrochlorides of organic bases on the addition of amines to the C=C bond have also been studied. It was found that when (I) and (II) were added to (IV) in benzene solution in the presence of diethylamine hydrochloride (cf.[2]), the yields

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Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 18, No. 6, pp. 686-689, June, 1984. Original article submitted August 9, 1983.

0091-150X/84/1806-0397$08.50

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