SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF [3,5-DIBROMO(DICHLORO)-1-HYDROXY-4-OXOCYCLOHEXA-2,5-DIEN-1-YL]ACETIC ACIDS AND THEIR DERIVATIVES

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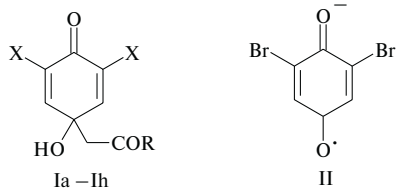
Amides Id (verongiaquinol) and Ih belonging to a group of compounds with the general formula I, occurring in nature in sponges of the *Verongidae (Aplysinidae)* family, were reported to exhibit a high cytostatic activity *in vitro* with respect to some tumor cells [1, 2] and a strong inhibiting effect with resect to Na^+, K^+, and Ca^{2+} ATPases [3, 4].



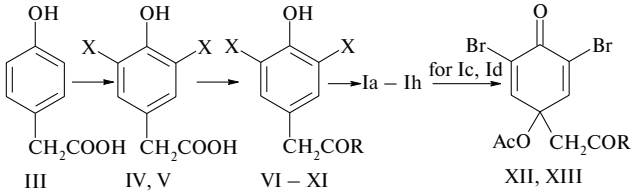
I: X = Br (a – d), Cl (e – h); R = OH (a, e), OMe (b, f); OEt (c, g), NH_2 (d, h).

It was also reported that amides Id and Ih have an inhibiting effect with respect to *St. aureus* and *E. coli* [1, 5]. However, the spectrum of antimicrobial activity of this class of compounds is still insufficiently studied.

The high biological activity of amides Id and Ih and the low content of these secondary metabolites in the aforementioned natural sources, which hinder their thorough pharmacological characterization, stimulated the development of various approaches to the synthesis of these substances. There are two effective pathways for the synthesis of amides Id and Ih. In the first scheme, the starting compound is *p*-hydroxyphenylacetic acid (III) and a key stage is the *ipso*-oxidation of the corresponding 2,6-dihalogen-substituted *p*-alkylphenol VIII or IX [6, 7]. In the second scheme, the starting compound is 2,6-dihalogen-substituted benzoquinone, which is converted into the target compounds under the action of low-available lithium reagents at low temperatures [8, 9].

The purpose of this study was to optimize the synthesis of natural amides Id and Ih proceeding from acid III. Another task was to obtain synthetic compounds Ia – Ic and Ie – Ig and study the spectrum of their antimicrobial activity.

In our experiments, commercial acid III was converted into 2,6-dibromoacid (IV) with a yield of 97% (against 64% reported in [6]). The transformation of acid IV into amide VIII was performed in two stages, IV → VI and VII → VIII, which allowed amide VIII to be obtained with a nearly quantitative yield. This procedure was more effective as compared to the previously described direct conversion of acid IV into amide VIII [7]. In the key stage of *ipso*-oxidation of the *p*-substituted phenols VI – XI, we tested various oxidizing agents including $PhI(OCOCF_3)_2$, $(NH_4)_2Ce(NO_3)_6$, $Ce(SO_4)_2$, RuO_4, $Tl(ClO_4)_3$, $Tl(OAc)_3$, $Pb(OAc)_4$, and HNO_3. The most effective oxidizer was 100% HNO_3 in glacial acetic acid solution.



X = Br (IV, VI – VIII), Cl (V, IX – XI); R = OMe (VI, IX), OEt (VII, X, XII), NH_2 (VIII, XI, XIII).

In this oxidation stage, somewhat better results were obtained using electrochemical oxidation on a Pt anode in aqueous $HClO_4$. Indeed, VIII oxidized by HNO_3 yields amide Id with a yield of 20%, while the electrochemical oxidation process increases this yield to 31% [10]. The total yields of *p*-quinols Ia – Id obtained from acid III were 30, 63, 64, and 19%, respectively (Table 1). For the chlorine-containing analogs Ie – Ih obtained by a similar method, the yields were 21, 65, 61, and 27%, respectively. Oxidized with lead triacetates, substrates VII and VIII yield, in addition to the target compounds Ic and Id, their acetates XII and XIII with a total yield of 22 and 34%, respectively.

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The *p*-quinols Ia – Ih synthesized as described above are colorless crystalline substances well soluble in EtOH, Me_2CO, DMSO, DMF, and AcOH, moderately soluble in aqueous ethanol (2 : 3 v/v), and weakly soluble in water on heating. The proposed structures were confirmed by the data of elemental analyses and by the results of IR and 1H NMR measurements (Table 1).

EXPERIMENTAL CHEMICAL PART

The IR spectra were recorded with a Specord 75IR spectrophotometer (Germany). The ^{1}H NMR spectra were measured on a Bruker WM-250 (250 MHz) spectrometer (Germany) using TMS as the internal standard. The melting temperatures were determined with the aid of a Boethius heating stage. The data of elemental analyses of the synthesized compounds agreed with the results of analytical calculations. The purity of the reaction products was checked by TLC on Silufol UV-254 plates eluted in a benzene – acetone (7 : 1 to 1 : 1) system. Individual compounds were isolated from the reaction mixtures by preparative column chromatography on a SiO_2 column (5 – 40 μm) with a sorbent – sorbate ratio of 40 : 1. The yields (recalculated for initial acid III) and some physicochemical characteristics of the synthesized compounds are presented in Table 1.

3,5-Dibromo-4-hydroxyphenylacetic acid (IV). To a solution of commercial acid III (1.52 g, 0.01 mole) in 50 ml of glacial acetic acid was added dropwise a solution of 3.30 g (0.021 mole) of Br_2 in 1 ml of the same solvent and the mixture was stirred for 96 h at 15°C. Then the reaction mixture was diluted with 100 ml of water and the precipitate was separated and crystallized from water to obtain 3.00 g (97%) of acid IV; m.p., 196 – 197°C (reported m.p., 195 – 196°C [6]); IR spectrum in KBr (ν_{max}, cm^{-1}): 1554, 1666, 1710, 2250 – 3100, 3404; ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 3.54 (s, 2H, CH_2), 7.43 (s, 2H arom), 8.76 (bs, 1H, OH), 9.93 (bs, 1H, COOH).

4-Hydroxy-3,5-dichlorophenylacetic acid (V). A mixture of commercial acid III (1.52 g, 0.01 mole) and 2.85 g (0.021 mole) of SO_2Cl_2 was kept for 1 h until gas evolution ceases; then 1.76 g (0.013 mole) of SO_2Cl_2 was added and the mixture was kept for another 1 h on a boiling water bath and allowed to stand for 16 h at room temperature. Finally, the excess SO_2Cl_2 was completely removed and the residue was crystallized from water to obtain 1.88 g (85%) of acid V; m.p., 177 – 179°C (reported m.p., 180 – 183°C [7]); IR spectrum in KBr (ν_{max}, cm^{-1}): 1570, 1660, 1712, 2260 – 3120, 3396; ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 3.62 (s, 2H, CH_2), 7.33 (s, 2H arom), 8.63 (bs, 1H, OH), 9.81 (bs, 1H, COOH).

TABLE 1. Yields and Physicochemical Characteristics of [3, 5-Dibromo(dichloro)-1-hydroxy-4-oxocyclohexa-2,5-dien-1-yl]acetic Acids and Their Derivatives (Ia – Ih, XII, and XIII)

Compound	Yield, %	M.p., °C	Empirical formula	IR spectrum *: ν, cm^{-1}				^{1}H NMR spectrum **: δ, ppm (J, Hz)		
				C = C	C = O	C(O)R	C – OH	CH_2(s)	2.6 -H	Other
Ia	30	195 – 196[a]	$C_8H_6Br_2O_4$	1594	1662	1700 (CO), 2605 – 3110 (OH)	3474	2.96	7.68	–
Ib	63	55 – 57	$C_9H_8Br_2O_4$	1602	1681	1715	3560	2.77	7.43	3.80 (s, 3H, $COOCH_3$)
Ic	64	124 – 125[b]	$C_{10}H_{10}Br_2O_4$	1600	1680	1714	3561	2.77	7.43	1.33 (t, 3H, J 7.1 Hz, $CH_2\underline{CH_3}$), 4.27 (q, 2H, J 7.1 Hz, $\underline{CH_2}CH_3$)
Id	19	189 – 191[c]	$C_8H_7Br_2NO_3$	1592	1662	1652 (CO), 3140 (NH_2)	3416	2.79	7.61	5.99 (bs, 1H, OH), 6.67 (bs, 1H, NH), 7.20 (bs, 1H, NH)
Ie	21	177 – 179	$C_8H_6Cl_2O_4$	1594	1672	1704 (CO), 2615 – 3115 (OH)	3480	2.92	7.38	
If	65	73 – 75	$C_9H_8Cl_2O_4$	1609	1696	1712	3566	2.80	7.18	3.80 (s, 3H, $COOCH_3$)
Ig	61	88 – 90	$C_{10}H_{10}Cl_2O_4$	1607	1698	1712	3563	2.80	7.21	1.29 (t, 3H, J 7.0 Hz, $CH_2\underline{CH_3}$), 4.24 (q, 2H, J 7.0 Hz, $\underline{CH_2}CH_3$)
Ih	27	160 – 163	$C_8H_7Cl_2NO_3$	1602	1668	1655 (CO), 3224 (NH_2)	3422	2.80	7.35	6.03 (bs, 1H, OH), 6.74 (bs, 1H, NH), 7.23 (bs, 1H, NH)
XII	22	86 – 89	$C_{12}H_{12}Br_2O_5$	1600	1688	1736	1755 (OAc)	2.89	7.54	1.27 (t, 3H, J 7.0 Hz, $CH_2\underline{CH_3}$), 2.09 (s, 3H, $OCOCH_3$), 4.18 (q, 2H, J 7.0 Hz, $\underline{CH_2}CH_3$)
XIII	34	119 – 122	$C_{10}H_9Br_2NO_4$	1602	1674	1689 (CO), 3190 (NH_2)	1754 (OAc)	2.93	7.78	2.10 (s, 3H, $OCOCH_3$), 6.57 (bs, 1H, NH), 7.12 (bs, 1H, NH)

* IR spectra of acids Ia, Ie and amides Id, Ih were recorded using samples pelletized with KBr; other samples were measured as solutions in $CHCl_3$;

** ^{1}H NMR spectra of acids Ia, Ie and amides Id, Ih were measured in d_6-acetone; other compounds were dissolved in $CDCl_3$; [a] reported m.p., 195 – 196°C [6]; [b] reported m.p., 121°C [6], 127 – 127.5°C [8]; [c] reported m.p., 195 – 196°C [6], 194 – 195°C [8].

TABLE 2. Antimicrobial Activity of compounds Ia – Ih, XII, and XIII

Compound	MIC 100, μg/ml					
	S. aureus	*E. faecium*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
Ia	6.25	> 100	> 100	> 100	> 100	> 100
Ib	> 100	> 100	50.0	> 100	-″-	-″-
Ic	> 100	> 100	100.0	> 100	-″-	-″-
Id	1.56	50.0	1.56	12.5	-″-	-″-
Ie	12.5	> 100	> 100	> 100	-″-	-″-
If	> 100	> 100	> 100	100.0	-″-	-″-
Ig	> 100	> 100	> 100	> 100	-″-	-″-
Ih	3.12	12.5	3.12	12.5	-″-	-″-
XII	> 100	> 100	25.0	> 100	-″-	-″-
XIII	> 100	> 100	1.56	50.0	-″-	-″-
Tetracycline	0.78	3.12	3.12	6.25	-″-	-″-
Streptomycin	3.12	0.39	0.78	3.12	-″-	-″-
Oleandomycin	0.39	0.195	0.195	12.5	-″-	-″-

3,5-Dibromo-4-hydroxyphenylacetic acid methyl ester (VI). A mixture of 620 mg (2.0 mmole) of acid IV, 15 ml of MeOH, 40 ml of C_6H_6, and 0.1 ml of concentrated H_2SO_4 was kept boiling in a setup equipped with a Dean – Stark attachment. Then the mixture was washed with of water (3 × 10 ml) and dried over anhydrous Na_2SO_4. Finally, the solvent is distilled off to obtain 642 mg (99%) of ester VI; m.p., 106 – 107°C (hexane); IR spectrum in $CHCl_3$ (ν_{max}, cm^{-1}): 1564, 1737, 3504; ^1H NMR spectrum in $CDCl_3$ (δ, ppm): 3.53 (s, 2H, CH_2), 3.73 (s, 3H, COOMe), 7.40 (s, 2H arom), 8.49 (bs, 1H, OH).

A similar procedure using acid V was used for the synthesis of 4-hydroxy-3,5-dichlorophenylacetic acid methyl ester (IX); yield, 98%; m.p., 83 – 85°C (hexane); IR spectrum in $CHCl_3$ (ν_{max}, cm^{-1}): 1578, 1736, 3524; ^1H NMR spectrum in $CDCl_3$ (δ, ppm): 3.52 (s, 2H, CH_2), 3.72 (s, 3H, COOMe), 7.20 (s, 2H arom), 8.36 (bs, 1H, OH).

A similar procedure using acid IV and EtOH instead of MeOH gives 3,5-dibromo-4-hydroxyphenylacetic acid ethyl ester (VII); yield, 98%); m.p., 105 – 106°C (hexane), reported m.p., 105°C [6]; IR spectrum in $CHCl_3$ (ν_{max}, cm^{-1}): 1566, 1730, 3506; ^1H NMR spectrum in $CDCl_3$ (δ, ppm): 1.26 (t, 3H, J 7.2 Hz, $CH_2\underline{CH_3}$), 3.52 (s, 2H, CH_2), 4.17 (q, 2H, J 7.2 Hz, $\underline{CH_2}CH_3$), 7.40 (s, 2H arom), 8.48 (bs, 1H, OH).

A similar procedure using acid V and EtOH yields 4-hydroxy-3,5-dichlorophenylacetic acid ethyl ester (X); yield, 97%; m.p., 75 – 76°C (hexane); IR spectrum in $CHCl_3$ (ν_{max}, cm^{-1}): 1587, 1728, 3527; ^1H NMR spectrum in $CDCl_3$ (δ, ppm): 1.27 (t, 3H, J 7.2 Hz, $CH_2\underline{CH_3}$), 3.51 (s, 2H, CH_2), 4.17 (q, 2H, J 7.2 Hz, CH_2CH_3), 7.22 (s, 2H arom), 8.35 (bs, 1H, OH).

3,5-Dibromo-4-hydroxyphenylacetamide (VIII). A mixture of 3.38 g (10 mmole) of ester VII, 49 mg (1 mmole) of NaCN, and 30 ml of a 9 M solution of gaseous NH_3 in anhydrous MeOH was heated in a stainless-steel hermetic ampoule at 45°C for 45 h. Then the solvent was distilled off, the residue was diluted with 50 ml of water, and the product was extracted with EtOAc (3 × 15 ml). The extract was dried over anhydrous Na_2SO_4, the solvent was distilled off, and the residue was chromatographed on a SiO_2 column eluted with a hexane – acetone (1 : 2) mixture to obtain 3.03 g (98%) of amide VIII; m.p., 182 – 184°C (reported m.p., 181 – 182°C [7]); IR spectrum in KBr (ν_{max}, cm^{-1}): 1548, 1650, 3166, 3208, 3401; ^1H NMR spectrum in $[(CD_3)_2CO]$ (δ, ppm): 3.45 (s, 2H, CH_2), 6.34 (bs, 1H, NH), 6.94 (bs, 1H, NH), 7.50 (s, 2H arom), 8.48 (bs, 1H, OH).

A similar procedure using ester X yields 4-hydroxy-3,5-dichlorophenylacetamide (XI); yield, 93%; m.p., 187 – 190°C; IR spectrum in KBr (ν_{max}, cm^{-1}): 1564, 1654, 3192, 3210, 3406; ^1H NMR spectrum in $[(CD_3)_2CO]$ (δ, ppm): 3.46 (s, 2H, CH_2), 6.36 (bs, 1H, NH), 6.97 (bs, 1H, NH), 7.32 (s, 2H arom), 8.80 (bs, 1H, OH).

Amide oxidation by 100% HNO_3. To a solution of 618 mg (2.0 mmole) of amide VIII in 3 ml of glacial acetic acid cooled to 10°C was added in one take 5.3 ml of a mixture of 100% HNO_3 and glacial acetic acid (1 : 9, v/v) cooled to 10°C, and the solution was stirred at 10 – 15°C for 4.5 h. Then 30 ml of water was added, the product was extracted with EtOAc (3 × 10 ml), and the extract was dried over anhydrous Na_2SO_4. Finally, the solvent was distilled off and the residue was chromatographed on a SiO_2 column eluted with a hexane – acetone (3 : 1) mixture to obtain 107 mg (20%) of 2,6-dibromo-4-hydroxy-4-carbamoylmethylcyclohexa-2,5-dien-1-one (Id) (Table 1).

Analogous procedures were used to obtain 2,6-dibromo-4-hydroxy-4-hydroxycarbonylmethylcyclohexa-2,5-dien-1-one (1a) from acid IV (yield, 31%); 2,6-dibromo-4-hydroxy-4-methoxycarbonylmethylcyclohexa-2,5-dien-1-one (1b) from ester VI (yield, 67%); 2,6-dibromo-4-hydroxy-4-ethoxycarbonylmethylcyclohexa-2,5-dien-1-one (1c) from ester VII (yield, 68%); 2,6-dichloro-4-hydroxy-4-hydroxycarbonylmethylcyclohexa-2,5-dien-1-one (1d) from acid V (yield, 25%); 2,6-dichloro-4-hydroxy-4-methoxycarbonylmethylcyclohexa-2,5-dien-1-one (1f) from ester IX (yield, 78%); 2,6-dichloro-4-hydroxy-4-ethoxycarbonylmethylcyclohexa-2,5-dien-1-one (1g) from ester X (yield, 74%); and 2,6-dichloro-4-hydroxy-4-carbamoylmethylcyclohexa-2,5-dien-1-one (1h) from amide XI (yield, 35%).

Amide oxidation by $Pb(OAc)_4$. Synthesis of compounds XII and XIII. A mixture of 224 mg (0.725 mmole) of amide VIII, 800 mg (1.8 mmole) of freshly prepared $Pb(OAc)_4$, and 2 ml of glacial acetic acid was stirred at room temperature for 5 h. Then 15 ml of water was added and the product was extracted with EtOAc (3 × 5 ml). The extract

was dried over anhydrous Na_2SO_4, the solvent was distilled off, and the residue was chromatographed on a SiO_2 column eluted with a hexane – acetone (3 : 5) mixture to obtain 96 mg (36%) of 4-acetoxy-2,6-dibromo-4-carbamoylmethylcyclohexa-2,5-dien-1-one (XIII) (Table 1). Then the column was eluted with a hexane – acetone (1 : 2) mixture to obtain 20 mg (9%) of amide Id analogous to that synthesized as described above.

A similar procedure using ester VII gives 4-acetoxy-2,6-dibromo-4-ethoxycarbonylmethylcyclohexa-2,5-dien-1-one (XII) with a yield of 23% and *p*-quinol Ic (yield, 13%) (Table 1)

EXPERIMENTAL BIOLOGICAL PART

The antimicrobial activity of compounds Ia – Ih, XII, and XIII was studied with respect to test cultures of Gram-positive (*Staphylococcus aureus* IFO 14462, *Enterococcus faecium* CIP 104105, *Bacillus subtilis* ATCC 6051 ^{T}) and Gram-negative (*Escherichia coli* IFO 15034, *Pseudomonas aeruginosa* KMM 433) bacteria and *Candida albicans* KMM 455 yeast cultures obtained from collections of the Pasteur Institute (CIP, France), Fermentation Institute (IFO, Japan), American Typical Culture Collection (ATCC, USA), and Sea Microorganism Culture Collection (Pacific-Ocean Institute of Bioorganic Chemistry, Vladivostok, Russia). The antimicrobial tests were performed as described previously [11] using a standard method of diffusion into agar in the media featuring optimum growth of the test microorganism cultures. The content of microbial cells per ml in the test suspensions was $(1.0 – 2.0) \times 10^8$ (OD_{660} = 0.13 – 0.20). Diameters of the zones of test culture growth inhibition were determined after incubation for 20 – 24 h in a thermostat at 37°C. The activity of compounds was evaluated as the minimum inhibiting concentration (MIC, μg/ml) completely suppressing the growth of test cultures. The reference drugs were tetracycline, streptomycin, and oleandomycin.

It was established that the synthesized compounds Ia – Ih are most active with respect to Gram-positive bacterial species, the activity level being significantly dependent on the type of radical R in the two-carbon side chain (Table 2). Only acids Ia and Ie and the corresponding amides Id and Ih inhibited the growth of *St. aureus*, whereas none of the esters Ib, Ic or If, Ig was effective. The growth of *B. subtilis* was significantly inhibited only by amides Id and Ih, but even these compounds could only moderately inhibit the growth of *E. faecium*.

It should be noted that acylation of the OH group in position 4 of amide Id (compound VIII) leads to a sharp drop in activity with respect to *St. aureus* and *E. faecium*, but not to *B. subtilis*. Moreover, acylation of this OH group in ester Ic even increases the activity with respect to *B. subtilis* (cf. data for compounds Ic and XII). As for the Gram-negative species of *E. coli*, only amides Id and Ih show a moderate activity with respect to these bacteria. Other Gram-negative species such as *P. aeruginosa*, as well as *C. albicans* yeasts, are insensitive to the synthesized compounds.

The results of our investigation indicate that some derivatives of (3,5-dihalogen-substituted 1-hydroxy-4-oxocyclohexa-2,5-dien-1-yl)acetic acids, such as amides Id and Ih, are of interest as potential antimicrobial agents.

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