1-ACYL-3-CYCLOOCTYLGUANIDINES

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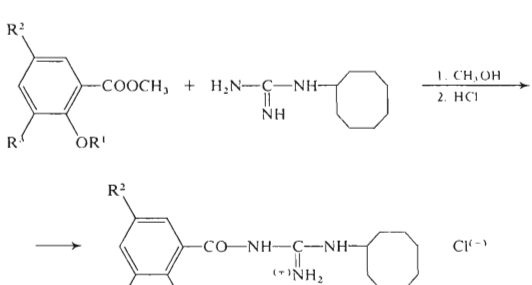
On reaction of cyclooctylamine with the sulfate of S-methylisothiourea cyclooctylguanidine was formed which was acylated with the methyl esters of 5-halogeno- and 3,5-dihalogeno-2-alkoxybenzoic acids. The 1-acyl-3-cyclooctylguanidine *I—XVII* formed were tested for their antiviral effect against the influenza virus A/NWS, A-PR8 and A_2 Singapore, and further against the viruses NDV, herpes 2, vaccinia and WEE. In the *in vivo* test against the influenza virus A_2 Singapore and herpes simplex 1-(5-bromo-2-dodecyloxybenzoyl)-3-cyclooctylguanidine is more active and less toxic than cyclooctylamine and 1-cyclooctylguanidine.

The work of various authors has demonstrated the antiviral activity of guanidine and some of its derivatives. Guanidine itself acts *in vitro* as an inhibitor of poliomyelitis virus, ECHO-virus and Coxsackie virus[1-3]. Among derivatives of guanidine 1-adamantylguanidine, effective against the virus of influenza[4] is especially important. In connection with this fact the substitution of guanidine with a cyclooctyl group was of interest, since cyclooctylamine is known from clinical experiments to possess a demonstrable effect against the influenza virus A_2 Hong-kong[5]. Therefore we decided to prepare cyclooctylguanidine and some of its acyl derivatives in order to investigate their possible antiviral effects.

The starting cyclooctylguanidine sulfate was prepared on reaction of S-methylisothiuronium sulfate with cyclooctylamine. The sulfate obtained was converted to its base and acylated with methyl esters of 5-chloro-, 3,5-dichloro- and 5-bromo-2-alkoxybenzoic acids. These acids were prepared by a method described earlier[6].

1-Acyl-3-cyclooctylguanidines *I—XVII* thus obtained were first tested *in vitro* on tissue cultures of chicken fibroblasts against the influenza virus A/NWS and the viruses NDV, herpes 2, vaccinia and WEE. On the basis of this screening, compound *XIV* was selected for the final evaluation. This compound was active against the influenza virus A/NWS and herpes 2 and at the same time it was practically non-toxic. For this testing 3 types of influenza virus (A/NWS, A-PR8 and A_2 Singapore) and one strain of herpes simplex type 2 were used. Substance *XIV* was inactive against the influenza virus A/NWS. Against the influenza virus A-PR8 it was slightly active and it prolonged the average survival time of mice infected with a 5 LD_{50} dose of the virus for 24% and with a 50 LD_{50} dose for 23%. In peroral application it was

inactive against the influenza virus A_2 Singapore, while on subcutaneous application it prolonged the survival by 23% (5 LD_{50}) and 59% (50 LD_{50}). The activity was confirmed in a repeated experiment. In the test against the herpes simplex type 2 virus substance *XIV* was inactive after application *per os*. After subcutaneous administration it delayed epidermal and encephalitic symptoms of the action of the



I–XVII

I, $R^1 = R^3 = H$, $R^2 = Cl$
II, $R^1 = CH_3$, $R^2 = Cl$, $R^3 = H$
III, $R^1 = C_2H_5$, $R^2 = Cl$, $R^3 = H$
IV, $R^1 = n\text{-}C_3H_7$, $R^2 = Cl$, $R^3 = H$
V, $R^1 = CH_3OCH_2CH_2$, $R^2 = Cl$, $R^3 = H$
VI, $R^1 = CH{\equiv}CCH_2$, $R^2 = Cl$, $R^3 = H$
VII, $R^1 = CH_3$, $R^2 = R^3 = Cl$
VIII, $R^1 = C_2H_5$, $R^2 = R^3 = Cl$
IX, $R^1 = R^3 = H$, $R^2 = Br$

X, $R^1 = CH_3$, $R^2 = Br$, $R^3 = H$
XI, $R^1 = C_2H_5$, $R^2 = Br$, $R^3 = H$
XII, $R^1 = n\text{-}C_3H_7$, $R^2 = Br$, $R^3 = H$
XIII, $R^1 = i\text{-}C_3H_7$, $R^2 = Br$, $R^3 = H$
XIV, $R^1 = n\text{-}C_{12}H_{25}$, $R^2 = Br$, $R^3 = H$
XV, $R^1 = CH_3OCH_2CH_2$, $R^2 = Br$, $R^3 = H$
XVI, $R^1 = CH_2{=}CHCH_2$, $R^2 = Br$, $R^3 = H$
XVII, $R^1 = CH{\equiv}CCH_2$, $R^2 = Br$, $R^3 = H$

herpetic virus (skin eczema and pareses of hind legs) for 24 h and prolonged the average survival time in days at a virus concentration of 10^{-2} by 20%, and at a 10^{-3} concentration by 8%. Cyclooctylamine and 1-cyclooctylguanidine when tested simultaneously *in vitro* on tissue cultures against the virus A_2 Hong-kong, vaccinia and herpes simplex are also inactive. In *in vitro* experiments the dose of both substances had to be decreased to one third owing to their excessive toxicity, *i.e.* from 3 mg to 1 mg/mouse/day; at this dose both substances were practically inactive.

EXPERIMENTAL

The melting points were determined on a Mettler FP 2 instrument.

Cyclooctylguanidine Sulfate

A mixture of S-methylisothiuronium sulfate (209 g, 1·5 mol) and cyclooctylamine (210 g, 1·65 mol) was heated on a boiling water bath for 10 h. The solidified melt was recrystallized from water (600 ml) under addition of charcoal. Yield, 167 g (51·0%), m.p. 287·1—288·5°C. For $C_{18}H_{40}N_6O_4S$ (436·7) calculated: 49·52% C, 9·23% H, 19·25% N, 7·34% S; found: 49·45% C, 9·27% H, 19·36% N, 7·53% S.

Methyl Esters of 5-Chloro-,3,5-dichloro- and 5-bromo-2-alkoxybenzoic Acids

A mixture of the appropriate acid (0·1 mol), methanol (30 ml) and conc. sulfuric acid (1 ml) was refluxed for 6 h. The excess of methanol was distilled off in a vacuum, the residue dissolved in ether, extracted with water, with saturated $NaHCO_3$ solution and again with water. After drying over magnesium sulfate the ether was distilled off and the crude ester used for acylation; in some instances methyl ester was purified by crystallization.

Methyl 5-chloro-2-ethoxybenzoate, yield 89·5%, m.p. 50·5—51·0°C (light petroleum). For $C_{10}H_{11}ClO_3$ (214·7) calculated: 55·95% C, 5·17% H, 16·52% Cl; found: 56·14% C, 5·24% H, 16·33% Cl.

Methyl 5-chloro-2-propoxybenzoate, yield 90·4% m.p. 25·5—27·0°C (light petroleum). For $C_{11}H_{13}ClO_3$ (228·7) calculated: 57·77% C, 5·73% H, 15·51% Cl; found: 57·76% C, 5·92% H, 15·40% Cl.

Methyl 5-chloro-2-(2-methoxyethoxy)benzoate, yield 86·0%, m.p. 39·6—40·3°C (light petroleum). For $C_{11}H_{13}ClO_4$ (244·7) calculated: 54·00% C, 5·35% H, 14·49% Cl; found: 54·50% C, 5·62% H, 14·44% Cl.

Methyl 3,5-dichloro-2-methoxybenzoate, yield 88·0%, m.p. 36·1—36·7°C (light petroleum). For $C_9H_8Cl_2O_3$ (235·1) calculated: 45·98% C, 3·43% H, 30·17% Cl; found: 45·87% C, 3·30% H, 30·07% Cl.

Methyl 5-bromo-2-(2-methoxyethoxy)benzoate, yield 84·5%, m.p. 41·3—42·0°C (light petroleum). For $C_{11}H_{13}BrO_4$ (289·1) calculated: 45·69% C, 4·53% H, 27·64% Br; found: 45·98% C, 4·34% H, 27·53% Br.

1-(2-Alkoxy-5-halogenobenzoyl)- and 1-(2-Alkoxy-3,5-dihalogenobenzoyl)-3-cyclooctylguanidine *I—XVII*

Cyclooctylguanidine sulfate (0·1 mol) was added under stirring to a solution of sodium (0·1 gat) in methanol (80 ml), followed by the methyl ester of corresponding acid (0·1 mol). The mixture was stirred at room temperature for 5 h (in the case of *I* and *IX* it was refluxed), methanol was distilled off in a vacuum and the residue triturated with acetone (200 ml). The undissolved residue was filtered off and the filtrate acidified with diluted hydrochloric acid to pH 1. Standing in a refrigerator overnight caused precipitation of the required product. In some instances it was necessary, however, to preparare first a crystalline inoculum by rubbing the product with a glass rod, necessary for the seeding of the main fraction. The precipitated product was filtered off, washed with acetone and crystallized from a suitable solvent. The yields, m.p. the solvents used and elemental analyses are given in Table I.

TABLE I

Hydrochlorides of 3-(2-Alkoxy-5-halogenobenzoyl)- and 3-(2-Alkoxy-3,5-dihalogenobenzoyl)--1-cyclooctylguanidines

Compound Yield, %	M.p., °C solvent	Formula (mol. mass)	Calculated/Found				
			% C	% H	% Br	% Cl	% N
I	212·8—214·1	$C_{16}H_{23}Cl_2N_3O_2$	53·34	6·43	—	19·68	11·66
71·5	ethanol	(360·3)	52·64	5·62	—	19·67	11·57
II	183·4—185·2	$C_{17}H_{15}Cl_2N_3O_2$	54·55	6·73	—	18·94	11·23
55·4	water	(374·3)	53·93	6·49	—	18·60	11·00
III	114·0—115·6	$C_{18}H_{27}Cl_2N_3O_2$	55·67	7·01	—	18·26	10·82
47·8	20% methanol	(388·3)	55·77	7·00	—	18·06	10·83
IV	170·4—171·6	$C_{19}H_{29}Cl_2N_3O_2$	56·71	7·27	—	17·62	10·44
52·2	95% acetone	(402·4)	56·68	7·48	—	17·63	10·56
V	125·1—126·7	$C_{19}H_{29}Cl_2N_3O_3$	54·55	6·99	—	16·95	10·04
41·8	98% acetone	(418·4)	54·93	7·37	—	17·03	10·62
VI	205·4—206·2	$C_{19}H_{25}Cl_2N_3O_2$	57·29	6·33	—	17·80	10·55
83·4	60% acetone	(398·3)	56·93	5·73	—	17·81	10·89
VII	152·1—152·8	$C_{17}H_{24}Cl_3N_3O_2$	49·95	5·92	—	26·02	10·28
50·4	50% methanol	(408·8)	49·83	6·12	—	26·03	10·52
VIII	142·7—143·8	$C_{18}H_{26}Cl_3N_3O_2$	51·13	6·20	—	25·16	9·94
37·9	50% methanol	(422·8)	51·06	6·27	—	24·99	9·74
IX	229·2—231·6	$C_{16}H_{23}BrClN_3O_2$	47·48	5·73	—	—	10·38
71·0	ethanol	(404·7)	47·54	5·81	—	—	10·23
X	132·1—134·8	$C_{17}H_{25}BrClN_3O_2$	48·76	6·02	19·08	8·47	10·03
26·8	40% methanol	(418·8)	48·14	6·29	18·66	8·28	9·75
XI	128·3—129·8	$C_{18}H_{27}BrClN_3O_2$	49·95	6·29	18·47	8·19	9·71
43·5	acetone	(432·8)	49·86	6·23	18·75	8·32	9·60
XII	191·5—192·9	$C_{19}H_{29}BrClN_3O_2$	51·08	6·54	17·89	7·94	9·40
26·0	80% acetone	(446·8)	51·00	6·56	18·04	8·00	9·33
XIII	165·0—166·6	$C_{19}H_{29}BrClN_3Oa$	51·07	6·54	17·89	7·94	9·40
53·8	acetone	(446·8)	51·17	6·49	18·00	7·96	9·30
XIV[a]	115·7—116·3	$C_{28}H_{46}BrN_3O_2$	62·67	8·64	14·89	—	7·83
23·0	ethyl ether	(536·6)	62·95	8·71	14·70	—	7·77

TABLE I
(*Continued*)

Compound Yield, %	M.p., °C solvent	Formula (mol. mass)	Calculated/Found % C	% H	% Br	% Cl	% N
XV 63·0	157·1—158·1 98% acetone	$C_{19}H_{29}BrClN_3O_3$ (462·8)	49·30 49·68	6·32 6·34	17·27 17·33	7·66 7·61	9·08 9·20
XVI 36·0	153·6—154·5 acetone	$C_{19}H_{27}BrClN_3O_2$ (444·8)	51·30 50·77	6·12 6·13	17·97 17·62	7·97 7·62	9·45 9·23
XVII 56·6	209·4—210·5 60% acetone	$C_{19}H_{25}BrClN_3O_2$ (442·8)	51·53 51·44	5·69 5·72	18·05 18·29	8·01 8·11	9·49 9·57

[a] Free base.

Testing Methods *in vitro* and *in vivo*

The antiviral activity was evaluated *in vitro* on tissue cultures of chicken fibroblasts, on the basis of the inhibition of the formation of plaques in agar diffusion test, by the modified Hsiung and Melnick method[7], and the methods by DeSomer and Prinzie[8] and Herrmann and coworkers[9]. A suspension of chicken embryonal cells was cultivated in lactalbumin hydrolysate medium VEL (USOL, Prague) with 10% of inactivated calf serum (USOL, Prague). A monolayer cell culture was infected with the virus and overlayered with Earle solution with growth proteins of calf serum and lactalbumin hydrolysate (USOL, Prague — Sevac IV) with 0·5% of Difco-agar, on to which discs of (Whatman No 1) filter paper of 10 mm diameter were placed after solidification of the agar. The discs were previously saturated with a 2—4% solution or suspension of the substance evaluated. After 48—72 h of cultivation at 37°C the monolayer of the cells was dyed with the second layer of the agar medium containing 0·1% of neutral red.

The viruses of the influenza A/NWS — D No 47 and herpes simplex type 2, batch 130175, were obtained from Dr Z. Janda from USOL, Prague. The strains NDV-Hertfordshire and WEE-USA were kindly supplied by Dr J. Závada, Virological Institute, Czechoslovak Academy of Sciences, Bratislava. The virus of vaccinia (Brussels, Prague strain) was used in the form of a dermovaccine (USOL, Prague). For the estimation of the *in vivo* activity female mice were used, weighting 11—12 g. The mice were infected using the tail-method of Yoshimura and coworkers[10] and treated by subcutaneous application of 0·2 ml of solution or suspension of the substances tested, dissolved in the physiological solution with Tween 80, in a 250 mg/kg dose, twice a day, for 5 days.

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