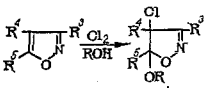
SYNTHESIS AND ANTIVIRAL ACTIVITY OF 4-CHLORO-5-HYDROXY-2-ISOXAZOLINE DERIVATIVES

S. D. Sokolov, S. M. Vinogradova, V. S. Ignatov, V. V. Paramonova, I. S. Nikolaeva, N. S. Bogdanova, and G. N. Pershin

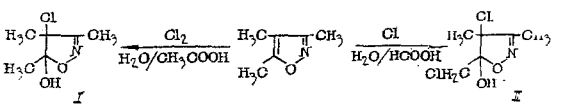
UDC 615.281.8:547.787].012.1

In 1964, the first short communication appeared on the addition of hypochlorous acid to the isoazole ring [1] with the formation of adducts, to which recently a strictly confirmed structure of 4-chloro-5-hydroxy-2-isoxazolines has been ascribed [2]. After biological activity had been discovered for the compounds obtained, we studied in detail the chlorination of various isoxazoles in hydroxyl-containing solvents. The reaction is extremely sensitive to the electronic effects of the substituents; acceptor groupings (NO_2, COOH, $H_3\overset{+}{N}$) not only in the heterocyclic, but also in the benzene ring of arylisoxazoles [3], and also in the side chain of methylisoxazoles, hinder addition to the $C_4=C_5$ bond. The reaction can be carried out with trisubstituted isoxazoles, with methyl or phenyl groups in any position of the ring (in the 4-position there can also be chlorine atom). 3,5-Disubstituted isoxazoles are first chlorinated at the C_4 atom and then enter the addition reaction. Data on the compounds obtained are listed in Table 1.



I — IX: R = H; X — XVII: R = CH_3, C_2H_5, C_3H_7; XVIII: R = CH_3CO.

5-Hydroxy-2-isoxazolines (I-IX) are obtained in aqueous solutions of formic or acetic acids in the presence of catalytic amounts of p-toluenesulfonic acid [4]; in formic acid the yields are generally higher. From 3,4,5-trimethylisoxazoles, different products can be obtained, depending on the solvent and reaction temperature. In acetic acid with heating, or in formic acid with cooling, and a strong chlorine current, 4-chloro-5-hydroxy-3,4,5-trimethyl-2-isoxazoline (I) is formed in a yield of 31 and 61%, respectively, and in formic acid with heating, additional chlorination of the methyl group in the 5-position takes place with the formation of compound II.



To avoid substitution in the side chain of 3,5-dimethyl-4-benzylisoxazole, this compound is also chlorinated rapidly in the cold. 3-Phenyl-4,4-dichloro-5-hydroxy-5-methyl-2-isoxazoline (VII) was found to be unstable in solution at high temperatures [3]; it can be isolated if the reaction is carried at 20°C.

The 4-chloro-5-hydroxy-Δ^2-isoxazoline structure of all the newly obtained compounds was confirmed by comparing their spectral properties with those of compounds V and VII, whose structure has been strictly proved in [2]. The presence of a chloromethyl group at position 5 is confirmed by comparing the PMR spectra of this compound and the isomer III, obtained from a known 3,5-dimethyl-4-chloromethylisoxazole, and also by the absence of chlorination of the side chain in 3,4-dimethyl-5-phenylisoxazole in the synthesis of adduct VIII.

We studied the chlorination of isoxazoles in alcohols also; the best results are attained in methanol with phenylisoxazoles; the yield of the addition products (XI-XV) was 70-90%. Other lower alcohols (ethanol and propanol) undergo vigorous side reactions of oxidation and chlorination, which considerably hinder the isolation of the end products. To decrease the side processes, a milder chlorinating medium is used, such as tert-butylhypochlorite with which, however, only the fairly nucleophilic 4-methylisoxazoles react. The addition products XVI and XVII had to be purified by chromatography.

S. Ordzhonikidze All-Union Scientific-Research Institute of Pharmaceutical Chemistry, Moscow. Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. No. 12, pp. 27-30, December, 1981. Original article submitted July 16, 1981.

0091-150X/81/1512-0854$07.50

TABLE 1. Derivatives of 4-Chloro-5-hydroxy-2-isoxazoline

Compound	Yield, %	mp, °C*	R	R^3	R^4	R^5	Found, %			Empirical formula	Calculated, %		
							C	H	Cl		C	H	Cl
I	61	81—82	H	CH_3	CH_3	CH_3	43,83	6,26	—	$C_6H_{10}ClNO_2$	44.05	6,16	—
II	28	90—91	H	CH_3	CH_3	$ClCH_2$	36,50	4,64	35,95	$C_6H_9Cl_2NO_2$	36,37	4,58	35,86
III	79	110—111	H	CH_3	$ClCH_2$	CH_3	36,34	4,60	—	$C_6H_9Cl_2NO_2$	36,37	4,58	—
IV	35	139—140	H	CH_3	$C_6H_5CH_2$	CH_3	60,25	5,65	14,84	$C_{12}H_{14}ClNO_2$	60,13	5,89	14,79
V	52	74—75†	H	CH_3	Cl	CH_3	—	—	—	—	—	—	—
VI	46	107,5—108,5	H	CH_3	Cl	tert-C_4H_9	42,68	5,86	—	$C_8H_{13}Cl_2NO_2$	42,50	5,75	—
VII	34	79,5—81	H	C_6H_5	Cl	CH_3	48,76	3,76	—	$C_{10}H_9Cl_2NO_2$	48,79	3,69	—
VIII	54	134—135,5	H	CH_3	CH_3	C_6H_5	58,25	5,34	16,27	$C_{11}H_{12}ClNO_2$	58,54	5,36	15,71
IX	74	144—145	H	C_6H_5	C_6H_5	C_6H_5	72,33	4,64	10,34	$C_{21}H_{16}ClNO_2$	72,10	4,61	10,13
X	63	—	CH_3	CH_3	CH_3	CH_3	47,45	6,80	—	$C_7H_{12}ClNO_2$	47,32	6,79	—
XI	70	52,5—54	CH_3	CH_3	Cl	C_6H_5	50,64	3,93	27,69	$C_{11}H_{11}Cl_2NO_2$	50,77	4,23	27,31
XII	73	—	CH_3	CH_6H_5	Cl	CH_3	50,53	4,05	27,20	$C_{11}H_{11}Cl_2NO_2$	50,77	4,23	27,31
XIII	78	124—125†	CH_3	C_6H_5	Cl	C_6H_5	—	—	—	—	—	—	—
XIV	73	116—117	CH_3	C_6H_5	CH_3	C_6H_5	67,55	5,44	11,88	$C_{17}H_{16}ClNO_2$	67,69	5,31	11,76
XV	90	157,5—158,5	CH_3	C_6H_5	C_6H_5	C_6H_5	72,46	5,26	9,87	$C_{22}H_{18}ClNO_2$	72,63	4,95	9,77
XVI	36	70—71	C_2H_5	C_6H_5	CH_3	C_6H_5	68,50	5,92	—	$C_{18}H_{18}ClNO_2$	68,46	5,74	—
XVII	72	—	C_3H_7	C_6H_5	CH_3	C_6H_5	69,23	6,60	—	$C_{19}H_{20}ClNO_2$	69,20	6,07	—
XVIII	71	131—133†	CH_3CO	C_6H_5	Cl	C_6H_5	—	—	—	—	—	—	—

* In sealed capillaries.

†Mixed probe with an authetic sample [1] does not depress the melting point.

TABLE 2. Antiviral Activity of 2-Hydroxy-3-chloro-4-isoxazolines toward Influenza Virus A/PR/8 (HO N1)

Compound	Concentration of compound, μg/ml	Number of neutralized EID_{100}
I	1000	1
	100	0
II	1000	1
	100	0
III	1000	0
V	1000	1
	100	1
	10	0
VI	1000	0
XX	1000	0
XXI	1000	1
	100	1
	10	1
	1	0

Chlorine acted on solutions of isoxazoles in formic acid to yield the readily hydrolyzable esters of 5-hydroxy-2-isoxazolines, and therefore only the hydroxy derivative could be isolated from the reaction mixture. It is probable that acetates are also formed when solutions of isoxazoles are treated in anhydrous acetic acid with tert-butylhypochlorite; in fact, acetate XVIII was obtained under the conditions in a 70% yield. The methyl derivative XIII and acetate XVIII are identical with compounds already synthesized from 3,5-diphenyl-4,4-dichloro-5-hydroxyisoxazoline (XIX) [1]. The properties of other esters X-XII, XIV-XVII are similar. The spectral characteristics of 5-hydroxy-2-isoxazoline derivatives will be published in another article.

The hydroxy derivatives I-III, V, VI, XIX, and 3-methyl-4,4-dichloro-5-hydroxy-5-phenyl-2-isoxazoline (XX) have been studied for antiviral activity toward influenza virus with a varying amount (1-100) · 100% of embryonal infection doses (EID_{100}). Table 2 shows that four out of seven compounds have a low antiviral activity, neutralizing 1 EID in concentrations of 1000–10 μg/ml. It was also found that compound V in doses of 2 and 1 mg per embryo exhibits a virus-inhibiting action on the influenza virus in ovo, suppressing its growth during infection of the embryos by 1 EID_{100}. Moreover, when introduced per os or intramuscularly in doses of 250 and 125 mg/kg, the compound has a low chemotherapeutic effect, ensuring survival of 80% of mice infected by 1 LD_{50} of influenza virus. Other compounds in this series (I-III, VI, XIX, XX) do not exhibit a virus-inhibiting action in ovo or therapeutic activity in vivo.

The activity of all the compounds studied did not exceed the activity of known antiviral agents.

EXPERIMENTAL (CHEMICAL)

The IR spectra were run in mineral oil on the Perkin Elmer 457 apparatus (Sweden). The PMR spectra were run on the JNM-4H-100 apparatus (Sweden) in deuterochloroform, using tetramethylsilane as internal standard.

3,5-Dimethyl-4,5-dichloro-5-hydroxy-2-isoxazoline (V). A current of chlorine gas was passed for 2 h at 70-80°C with vigorous stirring through a mixture of 10 ml (0.1 mole) of 3,5-dimethylisoxazole, 5 ml of 85% formic acid, and 1 ml of water containing catalytic amounts of p-toluenesulfonic acid. Excess chlorine was removed by blowing air, the mixture was cooled to 0°C, and the precipitate filtered. Yield, 9.94 g (52%), mp 72°C. The compound was purified by recrystallization from hexane.

Compounds II, XI, XIII-XV were obtained similarly in corresponding solvents. Compounds I, III, IV, VI-Ix, XII were synthesized at 20-30°C.

3-Methyl-4,4-dichloro-5-phenyl-5-hydroxy-2-isoxazoline (XX). The chlorination of 0.8 g (5 mmoles) of 3-methyl-5-phenylisoxazole in 1 ml of anhydrous formic acid was carried out similarly at 40-50°C. After the usual treatment, 1.03 g (84%) of compound XX, mp 112-113°C, was isolated; a mixed probe with an authentic sample [1] does not depress the melting point.

3,5-Diphenyl-4-methyl-4-chloro-5-ethoxy-2-isoxazoline (XVI). To a suspension of 0.24 g (1 mmole) of 3,5-diphenyl-4-methylisoxazole in 10 ml of absolute ethanol containing catalytic amounts of p-toluenesulfonic acid, 2.16 ml (18 mmoles) of tert-butylhypochlorite was added in portions, with vigorous stirring, while the temperature was maintained at 20-30°C. The reaction was carried out for 1 h until the initial isoxazole disappeared, as controlled on Silufol UV-254 plates in benzene. The solution was evaporated, the residue dissolved in benzene, the benzene solution was washed with water, and the product was purified on a column with silica gel, carrying out the elution with benzene. After evaporation of the solvent and thorough drying in vacuo, an analytically pure sample was obtained.

Compounds X, XVII and XIII were obtained similarly.

EXPERIMENTAL (CHEMOTHERAPEUTICAL)

The antiviral activity of the compounds was studied for influenza viruses type A: A/PR8 (H0 N1); A/Bethesda/63 (H2 N2); A/Hongkong/68 (H3 N2).

To determine the antiviral activity in vitro, equal volumes of different concentrations of aqueous solutions or suspensions of the compounds tested were mixed with a given amount (1-100) EID_{100} of influenze virus. The mixtures were held for 1 h at 14°C, and then were introduced in a volume of 0.2 ml into the allantoic pouch of 10-day-old chicken embryos.

To determine the inhibiting action of the compounds on the growth of the influenza virus in the shells of chicken embryos (in ovo), the compounds tested were introduced in maximally tolerated and smaller doses into the allantoic pouch of the embryos 1 h before they were infected with a virus. After 48 h of incubation at 37°C, the results of the action of the compounds were estimated from the hemagglutination reaction, and were expressed as the number of neutralized EID_{100} of the virus.

The chemothermapeutic activity of the compounds was studied on the model of influenza-induced pneumonia in mice. The compounds studied were administered to the mice per os or intramuscularly in maximally tolerated and smaller doses 1 h before intranasal infection of the animals with influenza virus. The animals received the compounds during the next 4 days also in the same doses, once daily. The chemotherapeutic activity was estimated by comparing the rates of survival of the experimental and control groups of mice.

LITERATURE CITED

1. S. D. Sokolov and N. K. Kochetkov, Dokl. Akad. Nauk SSSR, 156, 1391-1394 (1964).
2. S. D. Sokolov, V. F. Rudchenko, K. F. Turchin, et al., Dokl. Akad. Nauk SSSR, 258, 906-910 (1981).
3. S. D. Sokolov and I. M. Yudintseva, Khim. Geterotskil. Soed., 1325-1328 (1973).
4. S. D. Sokolov and V. V. Paramonova, Inventor's Certificate No. 551330 (USSR); Otkrytiya, No. 11, 82 (1977).