STRUCTURE, SYNTHESIS, AND PHARMACOLOGICAL ACTIVITY OF A METABOLITE OF ETIMIZOLE

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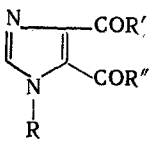
Investigations in [10] of the pharmacokinetics and metabolism of etimizole (I) showed that the first stage of its metabolic degradation includes fission of a methyl group from one of the methylcarbamoyl groups. The product of metabolism of (I) was assigned the structure of 1-ethyl-4-carbamoyl-5-methylcarbamoylimidazole (II) in [12] according to data of ^{13}C NMR.

The problem of the present work was the chemical synthesis of metabolite (II), the proof of its structure by chemical methods, and the investigation of the main pharmacological characteristics in comparison with (I).



I — VI

I: R = Et, R′ = R″ = NHMe; II: R = Et, R′ = NH_2, R″ = NHMe; III: R = H, R′ = OMe, R″ = NHMe; IV: R = Et, R′ = OMe, R″ = NHMe; V: R = Et, R′ = NHMe, R″ = OMe; VI: R = Et, R′ = NHMe, R″ = NH_2; VII: R = H, R′ = NH_2, R″ = NHMe.

As starting material 4(5)-methylcarbamoyl-5(4)-methoxycarbonylimidazole (III) [2] was taken for the synthesis of (II). Alkylation of it with ethyl iodide in the presence of MeONa gave a mixture of two isomers 1-ethyl-4-methoxycarbonyl-5-methylcarbamoylimidazole (IV) and 1-ethyl-4-methylcarbamoyl-5-methoxycarbonylimidazole (V). Resolution of this mixture was carried out by preparative TLC. The individual compounds (IV) and (V) were converted by treatment with aqueous ammonia into (II) and 1-ethyl-4-methylcarbamoyl-5-carbamoylimidazole (VI), respectively. An alternative route of synthesis of these compounds by alkylation of 4(5)-carbamoyl-5(4)-methylcarbamoylimidazole (VII) proved to be unacceptable due to the difficulty of a preparative separation of (II) and (VI).

According to data of HPLC the retention time of (II) was 10.2 min which corresponds to the metabolite of etimizole isolated from plasma in [10], while (VI) had a retention time of 9.2 min and, according to the data of [12], was not detected in biological specimens. The mass spectrum and ^{13}C NMR spectrum of compound (II) proved to be identical with the spectrum of the metabolite isolated from plasma in [12].

To establish the structure of (II) a route was selected for converting it into a cyclic derivative by the Hofmann reaction. It is known from [5] that under these conditions diamides of imidazole-4,5-dicarboxylic acids give xanthines. A product was obtained from compound (II) which coincided in melting point with 1-methyl-7-ethylxanthine (VIII) and differed from the isomeric 1-methyl-9-ethylxanthine (X) (see Table 1). The UV spectra of compound (VIII) also coincided with those described for 1-methyl-7-ethylxanthine (see Table 1).

Additional confirmation of the structure of (VIII) was that it was converted by the action of diazomethane into 1,3-dimethyl-7-ethylxanthine (IX) described in [9]. The isomeric 1,3-dimethyl-9-ethylxanthine (XI) is clearly distinguished by melting point. (Formula, top of following page.)

Thus the synthesis of the etimizole metabolite carried out by us and the confirmation of its structure by conversion into a compound of known structure made it possible to affirm

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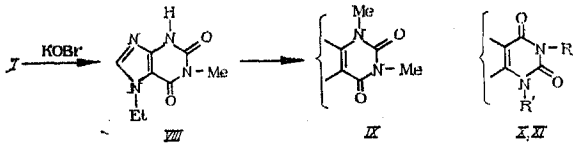

X: R=Me, R¹=H; XI: R=R¹=Me

that the etimizole molecule loses a methyl group from the nitrogen atom of the methylcarbamoyl group at position 4 of the imidazole in the first stage of metabolic degradation.

Etimizole is used clinically as a respiratory analeptic and also for the stimulation of the pituitary–adrenal system in the treatment of inflammatory disease of allergic etiology [3]. Consequently, we carried out a comparison of the pharmacological influence of etimizole and its metabolite (II) on the functional activity of the brain and respiration, the pituitary–adrenocortical system, and their antiinflammatory properties were also studied.

EXPERIMENTAL (CHEMICAL)

HPLC was carried out by the procedure of [11] on a Spectra-Physics SP 8000 instrument with a UV detector (model SP-770). The ^{13}C NMR spectra were taken on a Jeol FX-100 instrument (Japan) at 25 MHz in $CDCl_3$. Mass spectra were obtained on a Jeol JMS-D 100 instrument (Japan) by direct insertion, ionization energy 13 eV, emission current 300 μA, temperature 185°C. UV spectra were taken on a Unicam SP 8000 instrument (England).

1-Ethyl-4-methoxycarbonyl-5-methylcarbamoylimidazole (IV) and 1-Ethyl-4-methylcarbamoyl-5-methoxycarbonylimidazole (V). Compound (III) [2] (4.2 g: 0.021 mole) was dissolved by heating in abs. MeOH (400 ml) and to the cooled solution was added a solution of MeONa, prepared from sodium (0.7 g: 0.03 mole) in MeOH (50 ml), and an excess of EtBr (10 ml). The mixture was heated at 50-70°C for 7 h, further EtBr (10 ml) added, and the mixture heated for 4 h. The MeOH was distilled off, the residue extracted with hot $CHCl_3$ (3 × 100 ml), the cooled extracts filtered, evaporated to dryness, and the residue extracted with petroleum ether (70-100°C) with heating. On cooling a mixture (1.8 g: 43%) of (IV) and (V) of mp 108-115°C was precipitated. Resolution of the mixture was carried out by preparative TLC (Al_2O_3, $CHCl_3$). Extraction of (IV) (R_f 0.66) and (V) (R_f 0.11) was effected with hot $CHCl_3$. The yield of (IV) was 0.5 g (27.7% calculated on the mixture of isomers), mp 120-125°C (petroleum ether). Found, %: C 51.25, H 6.19; N 20.52. $C_9H_{13}N_3O_3$. Calculated, %: C 51.18; H 6.20; N 19.89. The yield of (V) was 0.45 g (25%), mp 113-115°C (petroleum ether). Found, %: C 51.00; H 5.97; N 20.00. The low yield on chromatographic separation is seemingly explained by hydrolysis of the esters by Al_2O_3.

1-Ethyl-4-carbamoyl-5-methylcarbamoylimidazole (II). Compound (IV) (0.11 g) was dissolved in MeOH (2 ml), aqueous ammonia (1 ml) added, and the mixture left overnight at ~20°C. The solution was evaporated to dryness and (II) (0.1 g: 100%) of mp 111-113°C (petroleum ether) was obtained in the residue. Found, %: C 48.90; H 6.26; N 28.19. $C_8H_{12}N_4O_2$. Calculated, %: C 48.96; H 6.61; N 28.56. Mass spectrum m/z (1%): 196 (100), 181 (31), 167 (48), 166 (30), 151 (36), 139 (40), 122 (51), 111 (54). ^{13}C NMR spectrum (d-chloroform), ppm: 138.2 (2-C), 127.8 (4-C), 134.4 (5-C), 166.3 (4-CO), 25.9 (5-CH_3), 159.7 (5-CO), 43.7 (CH_2), 17.0 (CH_3).

1-Ethyl-4-methylcarbamoyl-5-carbamoylimidazole (VI) was obtained similarly to (V). The yield was quantitative, mp 127-132°C (petroleum ether). Found, %: C 48.80; H 6.31; N 28.75. Mass spectrum m/z (1%): 196 (100), 181 (47), 166 (45), 151 (30), 139 (37), 122 (30), 120 (32), 111 (25). ^{13}C NMR spectrum (d-chloroform), ppm: 138.4 (2-C), 125.9 (4-C), 136.0 (5-C), 26.1 (4-CH_3), 164.1 (4-CO), 161.2 (5-CO), 43.5 (CH_2), 16.9 (CH_3).

1-Methyl-7-ethylxanthine (VIII). Compound (II) (0.2 g: 1 mmole) was added to a cooled (0-5°C) solution of KOBr prepared from KOH (0.6 g: 10 mmole), bromine (0.1 ml: 2 mmole), and water (6 ml). The resulting solution was kept at this temperature for 1.5 h, then heated for 15 min on a boiling water bath, cooled to ~20°C, and neutralized with AcOH. The solid precipitated on standing was filtered off, yield was 0.1 g (50%), mp 227-229°C (ethanol–water). According to literature data of [8], mp 225-226°C. Found, %: C 47.63; H 5.38; N 27.40. $C_8H_{10}N_4O_2 \cdot 0.5H_2O$. Calculated, %: C 47.28; H 5.49; N 27.47.

1,3-Dimethyl-7-ethylxanthine (IX). An excess of ethereal CH_2N_2 solution was added to compound (VIII) (0.05 g) in ethanol (1 ml), the reaction mixture was left in the refrigerator

TABLE 1. Melting Points and UV Spectra of Xanthine Derivatives (VIII)-(X)

Compound	Mp, °C	UV spectra, λ_{max}, nm			Source
		HCl (0.01 mole)	H_2O	NaOH (0.01 mole)	
VIII	225—226	267	268	291	[8]
IX	219—225	268	268	289	
X	335	262	265	278	[6,7]

for a day, then evaporated to dryness, and the residue washed with hexane. The yield of (IX) was 0.02 g (40%), mp 148-151°C (ethanol). According to literature data of [9] mp 154°C.

4(5)-Carbamoyl-5(4)-methylcarbamoylimidazole (VII). Aqueous ammonia solution (22 ml) was added to (III) [2] (1.5 g), the reaction mixture was left for three days at ~20°C, and the precipitated solid filtered off. Yield was 1.2 g (80%), mp 232-234°C (ethanol—water). Found, %: C 38.39; H 5.48; N 29.77. $C_6H_8N_4O_2 \cdot H_2O$. Calculated, %: C 38.67; H 5.40; N 30.10.

Alkylation of compound (VIII) with ethyl bromide gave a mixture of (II) and (VI) in 41% yield, mp 103-105°C.

EXPERIMENTAL (PHARMACOLOGIC)

Acute and chronic investigations of pharmacologic activity were carried out on male white mice (18-20 g), male white rats (180-200 g), and male rabbits (3-3.5 kg), obtained from the Rappolovo nursery. Respiratory movements and EEG were recorded for rabbits simultaneously on an Orion electroencephalograph with electrodes previously implanted in the sensomotor region of the cerebral cortex, the dorsal hippocampus, and the reticular formation of the mesencephalon. The excitability of structures of the brain was assessed by the threshold and duration of subsequent discharges (SD) caused by electrostimulation of the dorsal hippocampus (frequency 50 Hz, impulse duration 1 msec, stimulation time 5 sec).

The functional state of the pituitary—adrenal cortex system in rats was judged by the content of 11-hydroxycorticosteroids (11-HCS) in peripheral blood plasma determined by the fluorometric method of [4]. After chronic (two weeks) administration of preparations the mass of adrenal tissue was also assessed. The antiinflammatory properties of compounds were studied by an oncometric method in the aseptic inflammation model of rat paw, caused by subplantar injection of a 1% solution (0.1 ml) of carrageenan, three days after administration of the inflammatory agent. The obtained results were subjected to mathematical treatment by the variation statistics of [1].

Acute toxicity was investigated in mice on intraperitoneal administration of preparations. Calculation of LD_{50} was carried out by the method of Kerber from [1].

Influence on Excitability of Brain Structures and Respiratory Function. Compounds (I) and (II) proved to have a marked influence on the duration of SD. Thus in comparison with initial values the duration of SD after administration of (II) at doses of 2 and 5 mg/kg grew by two and three times, respectively. At lower doses preparation (II) gave no effect. At the same time etimizole caused an increase in SD duration of 2.5 times even on administering it at a dose of 0.5 mg/kg. The actual threshold of SD in response to stimulation of the dorsal hippocampus was unchanged or reduced insignificantly (by 9-10%) on administration of both etimizole and (II).

The initial frequency of respiration in rabbits was 50-60 movements per minute. Administration (intravenously) of both compounds led to an increase in the frequency and amplitude of respiratory movements. Under the influence of (II) at a dose of 1 mg/kg respiration became more frequent at 75 per minute. The effect grew on increasing the dose. The frequency of respiratory movements was 100 and 130 per minute at doses of 2 and 5 mg/kg, respectively. Under the influence of etimizole an increase in respiratory frequency to 80 per minute was observed on administering it at a dose of 0.5 mg/kg and at doses of 1 and 2 mg/kg the frequency of respiratory movements grew to 100 and 140 per minute, respectively.

Influence on the Pituitary—Adrenal Cortical System. As is seen from Fig. 1 the level of 11-HCS in plasma exceeded by 3 times their initial concentration 1 h after administration of preparations at a dose of 20 mg/kg. On administration of preparations at doses of 5 and 10

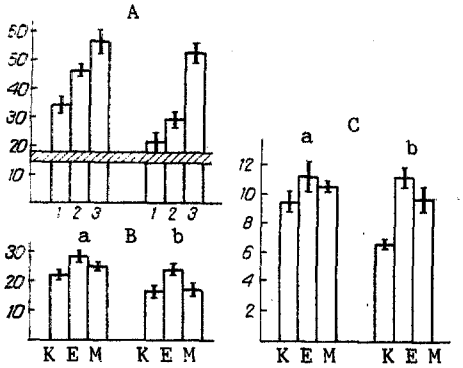

Fig. 1. Influence of etimizole and its metabolite (II) on the functional state of the pituitary—adrenal system of rats. A) Concentration of corticosteroids in peripheral blood plasma (μg %) 1 h after administration of etimizole or compound (II) at doses of: 1) 5 mg/kg, 2) 10 mg/kg, 3) 20 mg/kg. Etimizole is on the left and compound (II) on the right. The crosshatched band is the level of corticosteroids in control animals within confidence limits. B) Content of 11-HCS in plasma 2 weeks after a single administration of substance for: *a*) intact animals, b) administration of dexamethasone, and K) physiological solution (control), E) etimizole (10 mg/kg twice daily), M) compound (II) (10 mg/kg twice daily). C) Change in mass of adrenal tissue on chronic administration of preparations (mg per 100 g animal body weight) for: *a*) intact animals, and b) on administration of dexamethasone.

mg/kg the concentration of 11-HCS was 23 and 27 μg%, respectively, after administration of compound (II) and 37 and 45 μg% after administration of etimizole.

On chronic administration of preparations (at 10 mg/kg intraperitoneally twice daily) the concentration of 11-HCS in the blood of rats receiving test substances proved to be at a high level in comparison with that of control animals (see Fig. 1B). On combined administration with dexamethasone etimizole prevented the suppressing action of the latter, increasing 11-HCS concentration in blood to control values. Such a property was not observed for compound (II).

In experiments with a two week administration of dexamethasone a reduction of 28.5% was observed in adrenal weight. On combined administration of dexamethasone and preparations no atrophy of adrenal tissue was observed.

Investigation of Antiinflammatory Properties. Etimizole or (II) at doses of 10 mg/kg were injected 30 min before administration of carrageenan under the plantar aponeurosis of rat feet and then administered twice daily. Control animals received an injection of physiological solution at a volume of 0.5 ml. The extent of the inflammatory process was judged by the expression of edema of the extremity. Etimizole prevented the development of edema beginning in the first hours and paw volume was practically normalized by the second day. Administration of compound (II) was accompanied by a small drop in edema only in the middle of the first day which indicates its weak antiinflammatory activity in comparison with etimizole.

Toxicity of Preparations. The LD_{50} of etimizole and (II) were 220 mg/kg and 230 mg/kg, respectively, for male mice on intraperitoneal administration.

Consequently, the overall results of the investigations carried out in a comparative manner of the pharmacological activity of etimizole and its metabolite (II) may indicate that the latter retains a common directed action with etimizole according to the studied parameters. However, at the same toxicity it was surpassed by etimizole by 2-4 or more times in its activity.

LITERATURE CITED

1. M. L. Belen'kii, Elements of the Quantitative Assessment of the Pharmacologic Effect, 2nd edn. [in Russian], Leningrad (1963).

2. N. B. Vinogradova and N. V. Khromov-Borisov, Zh. Obshch. Khim., 35, 178-180 (1965).
3. M. D. Mashkovskii, Drugs, 8th edn. [in Russian], Moscow (1977), Vol. 1, p. 121.
4. I. Ya. Usvatova and Yu. A. Pankov, in: Modern Methods of Determining Steroid Hormones in Biological Fluids [in Russian], Moscow (1968), pp. 38-48.
5. R. A. Baxter and F. S. Spring, J. Chem. Soc., 232-236 (1945).
6. H. Biltz and K. Strufe, Liebigs Ann. Chem., 423, 200-226 (1921).
7. D. Lichtenberg, F. Bergmann, and Z. Neiman, J. Chem. Soc. (C), 1676-1682 (1971).
8. F. G. Mann and J. W. Porter, ibid., 751-760 (1945).
9. W. Schwabe, Jr., Arch. Pharm. (Weinheim), 245, 312-325 (1907).
10. L. Soltes, S. Bezek, T. Trnovec, et al., Pharmacology, 26, 198-204 (1983).
11. L. Soltes, Z. Kallay, T. Trnovec, et al., J. Chromatog., 273, 213-216 (1983).
12. L. Soltes, V. Mlyarik, and V. Mihalov, Xenobiotica, 13, 683-687 (1983).

SYNTHESIS AND BIOLOGICAL PROPERTIES OF DERIVATIVES OF 4-HETERYLMERCAPTOQUINAZOLINE

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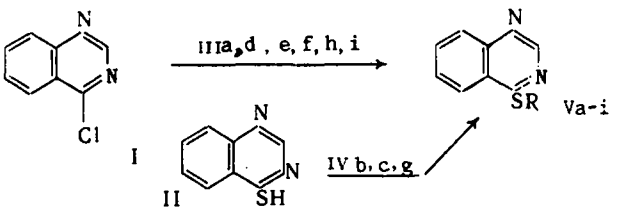
There is great interest in derivatives of quinazoline, among which are compounds having hypotensive, sedative, soporific, anticonvulsive, and antiinflammatory activities [2, 5, 7] and which have a broad spectrum of chemotherapeutic properties [3, 7]. All this attests to the obvious interest in the synthesis and investigation of the pharmacological properties of new quinazoline derivatives, for example those in the structure of which are introduced other heterocyclic radicals that are linked with the quinazoline nucleus through a sulfur atom in the 4-position.

For this purpose we reacted 4-chloroquinazoline (I) with 2-mercaptoquinoline (IIIa), 2-methoxy-9-mercaptoacridine (IIId), 2-ethoxy-9-mercaptoacridine (IIIe), 2-chloro-9-mercaptoacridine (IIIf), 2-methoxy-6-nitro-9-mercaptoacridine (IIIh), and 4-methoxy-6-nitro-9-mercaptoacridine (IIIi), or we reacted 4-mercaptoquinazoline (II) with 4-methoxy-9-chloroacridine (IVg), 5-nitro-8-chloroquinoline (IVb), and 2,6-dichloro-7-methylpurine (IVc) to obtain 4-heteryl-mercaptoquinazolines (Va-i, Table 1).



Va: R = quinolyl-2; Vb: R = 5-nitroquinolyl-8; Vc: R = 2-chloro-7-methylpurinyl-6; Vd: R = 2-methoxyacridinyl-0; Ve: R = 2-ethoxyacridinyl-9; Vf: R = 2-chloroacridnyl0; Vg: R = 4-methoxyacridinyl-9; Vh: R = 2-methoxy-6-nitroacridinyl-9; Vi: R = 4-methoxy-6-nitroacridinyl-9.

The synthesized compounds Va-i were isolated as the free bases and are yellow (Va, c, f), yellowish green (Ve), orange (Vb, g), red (Vd), or red-brown (Vh, i) crystalline compounds, insoluble in water and soluble in organic solvents. The infrared spectra of these compounds show vibration bands that are characteristic for the C—S—C bond in the region 770-750 cm^{-1}, for the C—N bond in the region 1610-1590 cm^{-1}, and for the C—O—C bond in the region 1110-1100 cm^{-1} (Vd-i). In the region 3430-3000 cm^{-1} some absorption bands related to stretching vibrations of the N—H bond (Vc) are present.

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