BIOLOGICAL PROPERTIES OF 2,4-DIOXO-3H-QUINOLINE-3-CARBOXYLIC ACID AND ITS ETHYL ESTER

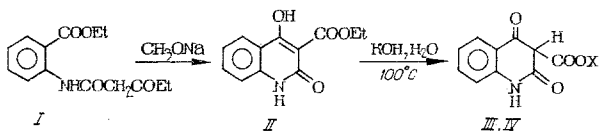
P. A. Bezuglyi, I. V. Ukrainets, V. I. Treskach, A. V. Turov, S. V. Gladchenko, Yu. F. Krylov, N. P. Moryakov, and G. M. Aleksandrova

UDC 615.012.1:001.8:547.831

Substances with high antibacterial [16], antitumor [8], analgesic [11], and other types of biological activity were previously found in the series of quinoline-3-carboxylic acid derivatives.

It was therefore interesting to carry out a comprehensive study of the biological properties of 2,4-dioxo-3H-quinoline-3-carboxylic acid and its ethyl ester, which, being direct analogs of the 3-substituted 4-hydroxyquinol-2-ones widely distributed in nature [6], have regrettably remained virtually unstudied from the pharmacological viewpoint up to the present time.

We obtained 3-carbethoxy-4-hydroxy-2-quinolone (II) by the intramolecular cyclization of ethyl 2-carbethoxymalonaniliate (I) using the Dieckmann reaction. The alkaline hydrolysis of the ester (II) leads to the formation of the salt (III); acidification of the latter with HCl led to the isolation of 2,4-dioxo-3H-quinoline-3-carboxylic acid (IV).



I II III, IV

X=K(III), H(IV)

The compounds obtained are white crystalline substances which are insoluble in water, and have good solubility in aqueous solutions of alkalis and the carbonates of alkali metals. The chemical structure was confirmed by the data of the elemental analysis and the PMR spectra.

EXPERIMENTAL (CHEMICAL)

The PMR spectra of the compounds synthesized were recorded on the "Bruker WP-100 SY" instrument (FRG); the working frequency was 100 MHz. The solvent was DMSO-d_6, and the chemical shifts were presented using the δ scale relative to TMS. The data of the elemental analysis satisfy the calculated values.

3-Carbethoxy-4-hydroxy-2-quinolone (II). To the solution of 2.79 g (0.01 mole) of the anilide (I) in 10 ml of absolute methanol is added the solution of 0.26 g (0.02 mole) of metallic sodium in 5 ml of methanol, and the mixture is left for 1 h. Water (50 ml) is added prior to the acidification with HCl to the pH 3-4. The residue is filtered off, washed with water, and dried. The yield was 2.19 g (94%). The mp was 203-204°C (ethanol). $C_{12}H_{11}NO_4$. The PMR spectrum was as follows: 13.43 (1H, s, OH), 11.53 (1H, s, NH), 7.95 (1H, dd, H-5), 7.63 (1H, td, H-7), 7.31 (1H, d, H-8), 7.22 (1H, td, H-6), 4.37 (2H, q, OCH_2), and 1.33 (3H, t, CH_3).

2,4-Dioxo-3H-quinoline-3-carboxylic Acid (IV). The mixture of 2.33 g (0.01 mole) of the ester (II) and 1.68 g (0.03 mole) of KOH in 20 ml of water is boiled using a reflux condenser for 10 h; the mixture is cooled and treated as in the first experiment. The yield was 1.96 g (96%). The mp was 320-323°C (DMF). $C_{10}H_7NO_4$. The PMR spectrum was as follows: 11.26 (1H, s, COOH), 11.17 (1H, s, NH), 7.81 (1H, dd, H-5), 7.50 (1H, td, H-7), 7.29 (1H, d, H-8), 7.15 (1H, td, H-6), and 5.76 (1H, s, H-3).

Khar'kov Pharmaceutical Institute. T. G. Shevchenko Kiev University. N. A. Semashko Moscow Medical Stomatology Institute. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 26, No. 2, pp. 33-35, February, 1992. Original article submitted April 27, 1990.

 0091-150X/92/2602-0138$12.50

TABLE 1. Acute Toxicity, Antiinflammatory Activity, and Analgesic Activity of the Compounds Synthesized

Compound	LD_{50}, mg/kg	Antiinflammatory activity		Analgesic activity	
		ED_{50}, mg/kg	relative activity	relative activity	relative activity
Phenylbutazone	430	56 (35—89)	1	120 (83—174)	1
Voltaren	120	8 (5—13)	7	5 (3—5)	24
II	163	14 (8—25)	4	26 (13—45)	4,6
IV	375	22 (14—34)	2,5	12 (9—18)	10

TABLE 2. Antinociceptive Action of the Ester (II), the Acid (IV), and Voltaren Using the Model of the FPR in Mice

Compound	Dose, mg/kg	First phase		Second phase	
		number of licks	effect, %	number of licks	effect, %
Control	—	12,8±1,5	—	7,4±0,85	—
II	26	7,2±0,86*	44	3,4±0,64*	54
IV	12	4,0±0,85*	69	0,8±0,21*	89
Voltaren	5	5,5±0,64*	57	1,6±0,43*	78

*$p < 0.05$ in relation to the control.

EXPERIMENTAL (PHARMACOLOGICAL)

The acute toxicity of the substances synthesized was studied by experiments using white male mice with the ip method of application. The LD_{50} values were calculated by the method of Kerber [4]. According to the classification of the toxicity of chemical substances [7], the compounds (II) and (IV) may pertain to the low-toxic type (Table 1).

The antiinflammatory (anti-exudative) action was studied by an oncometric method using the model of the acute carragenin inflammation (swelling) of the paw of white male rats of the mass 140-160 g [17]. The analgesic activity was studied with male rats of the mass 140-170 g using the models of writhing induced by acetic acid [12]. The study of the antinociceptive action was carried out using the model of the formalin pain reaction (FPR) in male mice of the mass 20-24 g, which received, sc into the back, 25 µl of a 0.5% solution of formalin [13]. The investigated substances were given in the form of a finely dispersed aqueous suspension stabilized with Tween-80, po, 1 h before the introduction of the carragenin or the application of the pain stimulation. The ED_{50} values were calculated by the method of B. M. Shtabskii and coauthors [10]. Preparations for comparison – voltaren and phenylbutazone – were utilized at the doses corresponding with the ED_{50} [9]. The results of all the experiments were treated statistically taking account of Student's criterion.

The evaluation of the results obtained shows that the greatest inhibition of the exudative reaction is induced by the ester (II), which surpasses phenylbutazone by fourfold in the extent of the antiinflammatory action. Both compounds investigated exhibit marked analgesic activity using the model of acetic writhing, the basic role in the genesis of which is played by endogenous kinins formed under the conditions of the decreased pH of the medium.

It is known that the endogenous opioid system, which changes the perception of pain, is activated in the formalin pain reaction [14]. The first phase of the FPR (the first 5 min) is associated with the direct stimulation of the nerve endings and determines the

TABLE 3. Diuretic and Neurotropic Activity of the Compounds Synthesized

Compound	Diuretic activity		Duration of narcotic sleep	
	ml in 4 h	% to control	min (M ± m)	% to control
Control	6,3±0,21	100	87,6±8,02	100
II	5,61±0,18	89	81,4±6,54	93
IV	2,84±0,19	45	61,3±6,06	70
Hypotiazid	9,58±0,24	152	—	—
Adiurekrin, 50 U/kg	2,39±0,16	38		
Aminazin	—	—	120,8±9,97	138

TABLE 4. Changes in the Indicators of Thromboelastography in Rabbits with the po Application of the Compounds Synthesized at the Dose of 10 mg/kg

Compound	Indicator of TEG	Value of indicators of thromboelastography (M±m)	
		before application	after application
II	R	330,0±32,0	586,0±65,9*
	K	150,8±14,6	243,6±28,2*
	Ma	60,8±2,7	62,3±8,4
IV	R	327,5±47,6	416,0±33,6*
	K	167,5±4,2	172,0±22,5
	Ma	67,5±3,4	63,6±2,3

Notes. The time of the beginning of the reaction is given by R (sec). The time of the formation of the clot is given by K (sec). The maximal amplitude is given by Ma (mm). The asterisk indicates that $p < 0.05$ relative to the control.

central component of the forming of the pain reaction. In the second phase (15-20 min), the sensitization and activation of the nociceptors by endogenous chemical products of inflammation such as histamine, serotonin, prostaglandins, and kinins are observed; this mediates the peripheral component [5, 15]. Both of the investigated compounds and voltaren possess antinociceptive action, and suppress both phases of the FPR (Table 2); this allows the proposition of central and peripheral mechanisms for the suppression of pain.

However, the analgesic effect in the phase II is more marked and is probably associated with the influence on the metabolism of the mediators of inflammation. Therefore, the compounds (II) and (IV) possess marked antiinflammatory and analgesic action. The compounds are somewhat inferior to voltaren, but surpass phenylbutazone significantly, in the given types of activity. The analgesic effect is realized on account of the influence on the central and, to a larger degree, the peripheral components of the pain reaction.

The antibacterial activity of the substances (II) and (IV) was studied by the method of twofold serial dilutions in beef–peptone broth; the solvent was DMF with the utilization of the test strains Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, and Bacillus subtilis ATCC 6633. The results obtained show that the activity of the compounds synthesized in regard to the indicated cultures is insignificant, and does not appear beyond the level of activity of the solvent.

The influence of the compounds (II) and (IV) on the urine-excreting function of the kidneys and the duration of narcotic sleep was studied by methods which we have described previously [2]. The data presented in Table 3 permit the high antidiuretic action of the acid (IV) to be noted.

It was established that the quinolones (II) and (IV) do not exhibit anticonvulsant properties. Likewise, they do not influence the systemic arterial pressure, the contractile

function of the myocardium, and the respiratory amplitude. The study was carried out by known methods [1, 3].

In the continuation of investigations, which we commenced previously, into the production of synthetic anticoagulants of indirect action [2], it was of interest to study the influence of the substances synthesized on the blood clotting system. The results obtained confirmed the high anticoagulant activity of the ester (II) and, particularly, the acid (IV) (Table 4), and showed that it is most rational to perform the search for new anticoagulants by the synthesis of substances having a structure close to those of the compounds (II) and (IV).

Therefore, the comprehensive study of the biological properties of 2,4-dioxo-3H-quinoline-3-carboxylic acid and its ethyl ester showed the necessity for a widened investigation of this group of compounds with the object of producing highly effective drugs with antinociceptive, antiinflammatory, analgesic, and anticoagulant action.

LITERATURE CITED

1. P. A. Bezuglyi, B. A. Samura, V. I. Treskach, et al., Khar'kov State Pharmaceutical Institute, Moscow (1987). Dep. Rukop. Khim.-farm. Zh. 26.05.87, No. 4 MP.
2. P. A. Bezuglyi, V. I. Treskach, I. V. Ukrainets, et al., Khim.-farm. Zh., 24, No. 4, 31-32 (1990).
3. P. O. Bezuglii, V. I. Trickach, I. V. Ukrainets, et al., Farm. Zh., No. 2, 37-40 (1988).
4. M. L. Belen'kii, Elements of the Quantitative Evaluation of the Pharmacological Effect [in Russian], Gos. Izd. Med. Lit., Leningrad (1963), p. 152.
5. Yu. P. Limanskii, The Physiology of Pain [in Russian], Zdorov'ya, Kiev (1986), pp. 25, 42.
6. M. Lukner, Secondary Metabolism in Microorganisms, Plants, and Animals [Russian translation], Mir, Moscow (1979), p. 548.
7. K. K. Sidorov, Toxicology of New Industrial Chemical Substances [in Russian], No. 13, Meditsina, Moscow (1973), pp. 47-51.
8. N. M. Sukhova, T. V. Lapina, and M. Yu. Lidak, Khim. Geterotsikl. Soedin., 11, 1521-1523 (1983).
9. G. Ya. Shvarts and R. D. Syubaev, Farmakol. Toksikol., No. 2, 46-49 (1982).
10. B. M. Shtabskii, M. I. Biegotskii, M. R. Biegotskii, et al., Sanit. Gigiena, No. 10, 49-51 (1980).
11. F. Clemence, O. Martret, F. Delevallce, et al., J. Med. Chem., 31, No. 7, 1453-1462 (1988).
12. H. O. J. Collier, L. C. Dineen, C. A. Johnson, and C. Schnieder, Br. J. Pharmacol., 32, 295-310 (1968).
13. S. Hunskaar, Pain, 25, No. 1, 125-132 (1986).
14. S. Hunskaar, O. B. Fasmet, and K. Hole, Neurosci. Meth., 14, No. 1, 69-76 (1985).
15. M. Shibata, T. Ohkabo, H. Takahashi, and T. Kudo, Folia Pharmacol. Jpn., 87, No. 4, 405-415 (1986).
16. M. Tsukamura, Microbiol. Immunol., 27, No. 12, 1129-1134 (1983).
17. C. A. Winter, E. A. Risley, and G. W. Nuss, Proc. Soc. Exp. Biol. Med., 111, 544-548 (1962).