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**Short communication**

# Synthesis and pharmacological study of ethyl 1-methyl-5-[2-substituted-4-oxo-3(4*H*)-quinazolinyl]-1*H*-pyrazole-4-acetates

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**Summary** — A number of new ethyl 1-methyl-5-[4-oxo-3(4*H*)-quinazolinyl]-1*H*-pyrazole-4-acetates substituted at the 2 position of the quinazolinone ring were prepared. The compounds were tested for analgesic and antiinflammatory activities, as well as for their acute toxicity and ulcerogenic effect. The 2-methyl, 2-ethyl and 2-phenyl derivatives proved to be more active than acetylsalicylic acid and phenylbutazone in the phenylbenzoquinone writhing test. The 2-methyl derivative was also as active as acetylsalicylic acid in the carrageenin paw oedema test. All the compounds showed very reduced ulcerogenic effects and systemic toxicity.

**5-[4-Oxo-3-(4*H*)-quinazolinyl]-1*H*-pyrazole-4-acetate / antiinflammatory activity / analgesic activity**

## Introduction

The 4-(3*H*)-quinazolinones bearing a heterocyclic nucleus at N-3 are associated with a range of pharmacological properties such as analgesic, antiinflammatory, antipyretic [1, 2], antimicrobial [3], anticonvulsant [4], antiparkinson [5], antidepressant and other central nervous system (CNS) activities [6]. Our research group has long been interested in the 3-pyrazolyl- and 3-isoxazolyl-substituted quinazolinones, which are characterized by good analgesic and antiinflammatory activities and, at the same time, very low ulcerogenicity [7, 8]. Many pyrazolyl derivatives bear an alkoxycarbonyl group at the C-4 position.

In order to improve the above pharmacological properties and gain more insight in the structure–activity relationships of 3-pyrazolylquinazolinones we performed the synthesis of new derivatives **8** whose pyrazole nucleus is substituted at the C-4 position by an ethoxycarbonylmethyl group.

The pyrazole moiety of these compounds presents the structural feature of the heteroarylalkanoic esters, which are claimed to be potent antiinflammatory agents in animals [9]. Moreover, Lonazolac-Ca, the active principle of the antiinflammatory agent Irritren, is a 1*H*-pyrazole-4-acetic acid derivative [10, 11].

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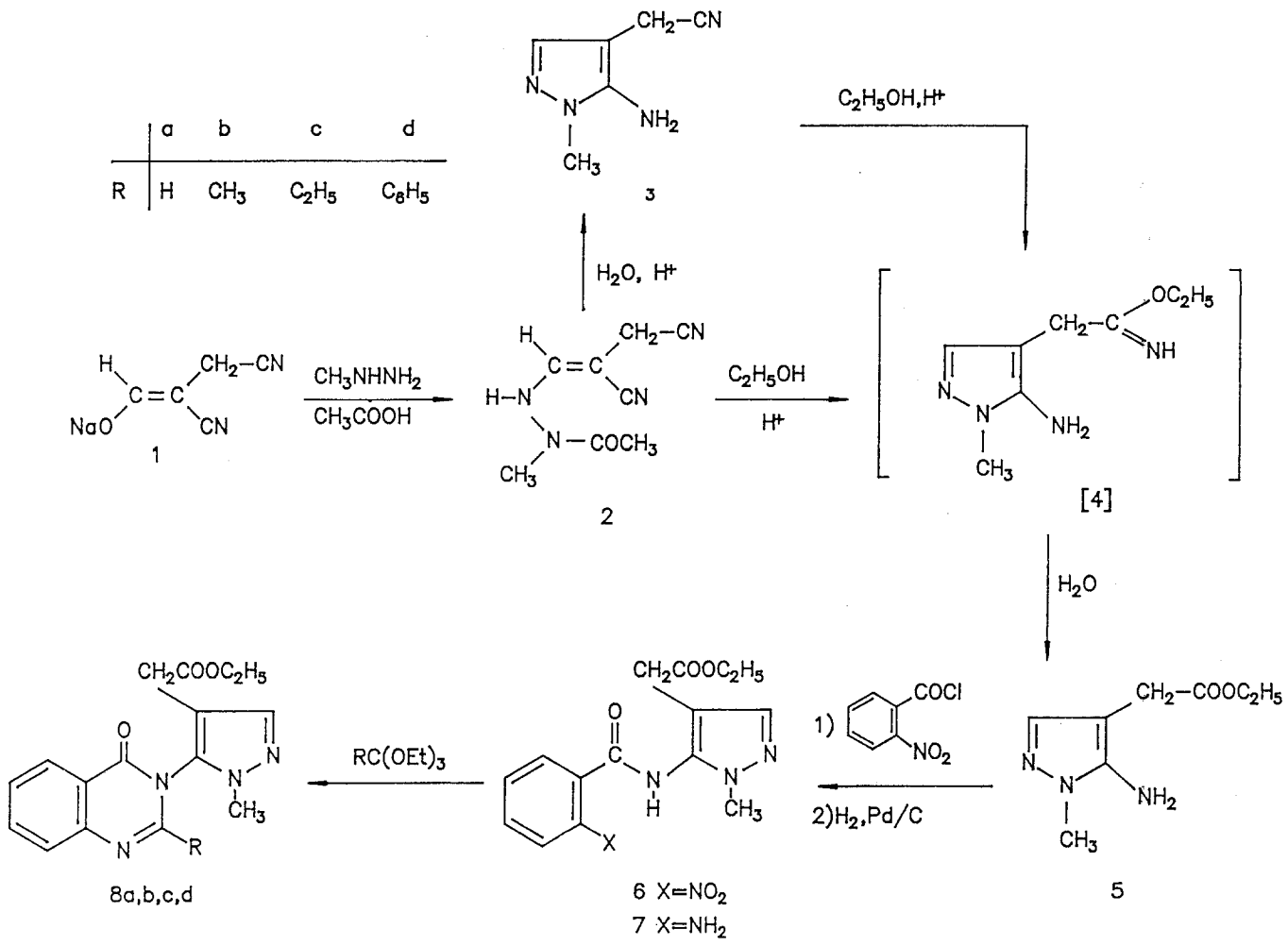
## Chemistry

The synthesis of the quinazolinones **8** was carried out according to scheme 1. The starting compound ethyl 1-methyl-5-amino-1*H*-pyrazole-4-acetate **5** was obtained as follows: the sodium formylsuccinonitrile **1** was reacted with methylhydrazine in acetic acid to give *N*-methyl-*N*-acetyl-*N'*-(2,3-dicyano-1-propen-1-yl)-hydrazine **2** which, in turn, was transformed into the pyrazole derivative **5** by 2 different routes. The first route involves deacetylation of the acetohydrazide **2** followed by ring closure in aqueous hydrochloric acid affording the pyrazole derivative **3**. Ethanolysis of the cyano group of **3** was accomplished with absolute ethanol containing dry hydrochloric acid. The intermediate iminoderivative [4] was transformed into **5** *in situ* by adding water. The second synthetic route involves simultaneously *N*-deacetylation, ring closure and ethanolysis of the cyanomethyl group by reaction of **2** with dry hydrochloric acid in absolute ethanol. As in the previous route, the intermediate [4] was transformed *in situ* with water.

The second route was more advantageous than the first being faster and giving higher yields of compound **5**.

At this point, the ethyl 1-methyl-5-amino-1*H*-pyrazole-4-acetate **5** was reacted with 2-nitrobenzoyl chloride in dry chloroform to give the ethyl 1-methyl-5-(2-nitrobenzamido)-1*H*-pyrazole-4-acetate **6**, which, in




**Scheme 1.**

turn, was reduced with hydrogen and palladium on activated charcoal to give the corresponding 2-amino-derivative **7**. Finally, when the latter compound, used as crude product, was reacted with the appropriate orthoesters, the desired pyrazolylquinazolinones **7** were formed.

The structures of the new compounds were assigned on the basis of analytical and spectroscopic data. The IR spectra of the aminopyrazole **5** showed a broad intense band at 1725 $cm^{-1}$ for the ester carbonyl group and bands at 3220 and 3380 $cm^{-1}$ for the amino group. Moreover, the $^1$H-NMR spectrum exhibited, among other signals, a broad signal for the amino group at 3.82 δ, exchangeable with $D_2O$, a sharp singlet at 3.36 δ for the methylene group, and a singlet at 7.06 δ for the C-3 methine proton of the pyrazole nucleus.

The IR spectra of quinazolinones **8** exhibited carbonyl stretching at 1740 and 1690 $cm^{-1}$, attributable to the ester and amidic groups, respectively. Furthermore, $^1$H-NMR of the ethyl 1-methyl-5-[oxo-3(4*H*)-quinazolinyl]-1*H*-pyrazole-4-acetates **8b–d** in deuterochloroform solution exhibited AB quartets in the 3.19–3.45 δ region, arising from methylene protons linked to the pyrazolic C-4, indicating hindered internal rotation about the pyrazolic $C_5$-quinazolic $N_3$ bond.

## Pharmacology

All the new quinazolinones **8a–d** were investigated in order to ascertain their analgesic, antiinflammatory

activities, behavioral effect, acute toxicity and, finally, ulcerogenic potential. Acetylsalicylic acid (ASA) and phenylbutazone (PBZ) were included in all tests as reference standards.

## Results and discussion

Pharmacological results are summarized in table I.

*Behavioral effects and acute toxicity in mice*

The test compounds did not show any significant overall behavioral and toxicological effects at doses up to 750 mg/kg po and 500 mg/kg ip in mice. At dose levels higher than 1000 mg/kg os and 750 mg/kg ip all drugs produced a marked sedation, about 10 min after administration. This effect lasted for a period of 1–3 h. At such a dose level, death generally occurred at 1–3 h postdrug in 40–60% of animals. The surviving mice appeared normal and remained so throughout the 7-day observation period.

*Analgesic activity*

In a phenylbenzoquinone-induced writhing test in mice, quinazolinones **8b–d** exhibited a remarkable analgesic action at 10 mg/kg po and are much more active than the reference drugs. This activity dramatically fell for the 2-unsubstituted quinazolinone **8a**.

*Antiinflammatory activity*

In the acetic acid peritonitis test, the quinazolinones **8a–d** showed an antiexudative activity comparable to that of PBZ but less than ASA. The substitution on the 2-position of quinazolinone ring does not affect this activity.

Compounds **8a** and **8b** were more active than **8c** and **8d** in the rat paw oedema test. Compound **8b** was about as active as ASA but less active than PBZ. It should be stressed that the most active compounds, **8a** and **8b**, bear a hydrogen and a methyl substituent, respectively. The less active quinazolinones, **8c** and **8d**, bear the more bulky ethyl and phenyl groups, respectively.

*Ulcerogenic activity*

No compound showed any harmful effects on the stomach at the dose of 300 mg/kg po, when administered twice at a 2 h interval in fasted rats, whereas ASA and PBZ caused serious gastric ulcers at lower doses in all animals.

## Conclusion

Pharmacological assays show that in the set of compounds tested, quinazolinones **8b–d** showed remarkable analgesic activity associated with appreciable antiexudative properties and very reduced ulcerogenic effects and systemic toxicity. Fair antiinflammatory activity was exhibited only by quinazolinones **8a** and **8b**; **8b** was as active as ASA.

**Table I.** Pharmacological data.

| *Compound* | *Approximate $LD_{50}$ in mice (mg/kg)* | | *Phenylbenzoquinone writhing test % inhibition (10 mg/kg po)* | *Acetic acid peritonitis % inhibition (100 mg/kg po)* | *Carrageenin paw oedema % inhibition (100 mg/kg po)* | *Gastric ulcer score in rats*[a] |
|---|---|---|---|---|---|---|
| | *po* | *ip* | | | | |
| **8a** | > 1000 | ~ 750 | 0 | 22* | 28* | 0.25 |
| **8b** | > 1000 | > 750 | 48* | 24* | 33* | 0.25 |
| **8c** | > 1000 | > 750 | 44* | 22* | 18 | 0.25 |
| **8d** | ~ 1000 | > 500 | 65* | 18 | 19 | 0 15 |
| ASA | ~ 1000 | ~ 500 | 20 | 60* | 39* | 2.25 |
| PBZ | ~ 750 | ~ 300 | 25* | 24* | 56* | 2.50 |

[a]Dose levels po: test compounds (300 mg/kg), ASA (200 mg/kg x 2) and PBZ (100 mg/kg x 2). *$P < 0.05$ Student's *t*-test *versus* controls.

## Experimental protocols

*Chemistry*

All melting points were taken on a Büchi–Tottoli capillary melting-point apparatus and are uncorrected. IR spectra were recorded on a Jasco IR-810 spectrophotometer in nujol (except compound **7**, which was used neat). $^1$H-NMR spectra were obtained on a Brüker AC-E 250 MHz spectrometer using tetramethylsilane as internal reference.

Microanalyses were performed in the laboratories of the Institut de Chimie Pharmaceutique, Université de Genève, Switzerland and C, H, N values were within ± 0.4% of theoretical values.

*N-Methyl-N-acetyl-N'-(2,3-dicyano-1-propen-1-yl)hydrazine* **2**
Methylhydrazine (0.1 mol, 5.25 ml) was added in portions to a cooled (water-ice bath), stirred suspension of powered sodium formylsuccinonitrile [12] (0.1 mol, 13 g) in 39 ml acetic acid. After addition of methylhydrazine, cooling was stopped and the reaction mixture was stirred for a further 10 min and then refluxed for 8 h. Ethanol (40 ml) was then added to the solution. After cooling to 20°C, crystallization of the reaction product was induced by scratching. The mixture was allowed to stand overnight and the crystalline product was filtered off. The product was air-dried and extracted with chloroform (2 x 150 ml). The combined extracts were evaporated under vacuum to yield a product, which was recrystallized from ethanol (95% v/v) to give compound **2**, mp 170–172°C; yield 20%. IR (cm$^{-1}$): 3300–3000 (NH); 2240 (C≡N); 1635, 1650 (CO). $^1$H-NMR (DMSO-$d_6$) (δ): 1.99 (s, 3H, $CH_3$); 3.62 (s, 2H, $CH_2$); 3.73 (s, 3H, $CH_3$); 7.62 (s, 1H, =CH); 9.98 (s, 1H, exchangeable with $D_2O$, NH).

*1-Methyl-4-cyanomethyl-5-aminopyrazole* **3**
*N*-Methyl-*N*-acetyl-*N'*-(2,3-dicyano-1-propen-1-yl)hydrazine **2** (5.5 g) refluxed with 11 ml of an aqueous 6 N hydrochloric acid solution for 5 min. After cooling, the solution was basified with 50% aqueous potassium hydroxide solution and extracted with chloroform (4 x 30 ml). Drying (sodium sulfate) and evaporation under reduced pressure left a crude product which was recrystallized from ethyl acetate/petroleum ether to give compound **3**, mp 219–221°C; yield 60%. IR (cm$^{-1}$): 3420–3160 ($NH_2$); 2250 (C≡N). $^1$H-NMR (DMSO-$d_6$) (δ): 3.43 (s, 2H, $CH_2$); 3.60 (s, 2H, exchangeable with $D_2O$, $NH_2$); 3.70 (s, 3H, $CH_3$); 7.20 (s, 1H, pyrazole H-3).

*Ethyl 1-methyl-5-amino-1H-pyrazole-4-acetate* **5**

*Route A.* Crude compound **3** (3 g) was refluxed in a solution of anhydrous hydrochloric acid (2.5 g) in absolute ethanol (10 ml) for 4 h. Water was added (0.36 ml) and reflux was continued for 30 min. The mixture was concentrated to half its original volume, water was added (9 ml) and the pH was adjusted to 8 with solid sodium carbonate. The solution was extracted with chloroform (4 x 30 ml), dried (sodium sulfate) and evaporated under reduced pressure. The residue was recrystallized from diethyl ether to give compound **5**, mp 58–60°C; yield 64%. IR (cm$^{-1}$): 3400–3060 ($NH_2$); 1725 (CO). $^1$H-NMR ($CDCl_3$) (δ): 1.27 (t, 3H, $J$ = 7 Hz, $CH_3$); 3.35 (s, 2H, $CH_2$); 3.67 (s, 3H, $CH_3$); 3.82 (br s, 2H, exchangeable with $D_2O$, $NH_2$); 4.15 (q, 2H, $J$ = 7 Hz, $CH_2$); 7.05 (s, 1H, pyrazole H-3).

*Route B.* Compound **2** (2 g) was refluxed in an anhydrous hydrochloric acid solution (2.5 g) in absolute ethanol (10 ml), following the procedure of *Route A*. The compound obtained was identical in all respects with compound **5** prepared by *Route A*; yield 82%.

*Ethyl 1-methyl-5-(2-nitrobenzamido)-1-pyrazole-4-acetate* **6**
Equimolar amounts of compounds **5** (10.9 mmol) and 2-nitrobenzoylchloride in dry chloroform (50 ml) were refluxed for 5 h. After the first hour of reflux, triethylamine (1.5 ml) was added in 4 aliquots (0.75, 0.38, 2 x 0.19 ml, respectively), with an interval of 1 h between each addition. The solution was evaporated under reduced pressure and the oily residue was washed with 2 x 10 ml of water and then crystallized from 95% (v/v) ethanol to give compound **6**. The product was purified for analysis by flash silica-gel chromatography [13] using ethyl acetate as eluent; mp 98–100°C (ethanol); yield 65%. IR (cm$^{-1}$): 3300–3120 (amidic NH); 1735 (esteric CO); 1700–1630 (multiple bands, amidic CO). $^1$H-NMR ($CDCl_3$) (δ): 1.26 (t, 3H, $J$ = 7 Hz, $CH_3$); 3.62–3.66 (superimposed signals, 5H, $CH_2$ and $CH_3$); 4.15 (q, 2H, $J$ = 7 Hz, $CH_2$); 7.27–8.12 (a set of signals, 5H, $C_6H_4$ and pyrazole H-3); 9.08 (br s, 1H, exchangeable with $D_2O$, NH).

*Ethyl 1-methyl-5-(2-aminobenzamido)-1H-pyrazole-4-acetate* **7**
Compound **6** (7.5 g) in ethanol (250 ml) and 10% palladium on activated charcoal as a catalyst (750 mg) was hydrogenated in a Parr apparatus at 45–50 psi for 8 h. The reaction mixture was heated to boiling and filtered. The solution obtained was evaporated under vacuum to give an oily residue. A chromatographic procedure of purification of the residue was carried out to give a sample that was not sufficiently pure for the elemental analysis but gave satisfactory IR and $^1$H-NMR spectra. IR (cm$^{-1}$): 3500–3100 (multiple bands, amino and amidic groups); 1730 (esteric CO), 1650 (amidic CO). $^1$H-NMR ($CDCl_3$) (δ): 1.17 (t, 3H, $J$ = 5.8 Hz, $CH_3$); 3.48 (s, 2H, $CH_2$); 3.62 (s, 3H, $CH_3$); 4.07 (q, 2H, $J$ = 5.8 Hz, $CH_2$); 5.62 (br s, 2H, exchangeable with $D_2O$, $NH_2$); 6.52–7.53 (m, 5H, $C_6H_4$ and pyrazole H-3); 9.18 (br s, 1H, exchangeable with $D_2O$, NH).

*Ethyl 1-methyl-5-[4-oxo-3(4H)-quinazolinyl]-1H-pyrazole-4-acetates* ***8a–d***
The crude amino derivative (2 g) and the appropriate ethyl orthoester (3.5 ml) were refluxed for 5 h. After cooling at room temperature, the crystalline solid that separated out was filtered off and recrystallized; yields 48–75%.

**8a** (R = H): IR (cm$^{-1}$): 1740 (esteric CO); 1680 (amidic CO). $^1$H-NMR ($CDCl_3$) (δ): 1.12 (t, 3H, $J$ = 5.70 Hz, $CH_3$); 3.50 (s, 2H, $CH_2$); 3.93 (s, 3H, $CH_3$); 4.03 (q, 2H, $J$ = 5.70 Hz, $CH_2$); 7.52–8.37 (a set of signals, 6H, $C_6H_4$ pyrazole H-3 and quinazolinone H-2).

**8b** (R = Me): IR (cm$^{-1}$): 1735 (esteric CO); 1685 (amidic CO). $^1$H-NMR ($CDCl_3$) (δ): 1.35 (t, 3H, $J$ = 5.70 Hz, $CH_3$); 2.36 (s, 3H, $CH_3$); 3 29 (d, 1H, geminal CH, $J$ = 13.68 Hz); 3.45 (d, 1H, geminal CH, $J$ = 13.68 Hz); 3.93 (s, 3H, $CH_3$); 4.05 (multiplet, 2H, $CH_2$); 7.46–8.28 (a set of signals, 5H, $C_6H_4$ and pyrazole H-3).

**8c** (R = Et): IR (cm$^{-1}$): 1740 (esteric CO); 1690 (amidic CO). $^1$H-NMR ($CDCl_3$) (δ): 1.14 (t, 3H, $J$ = 5.70 Hz, $CH_3$); 1.26 (t, 3H, $CH_3$); 2.44–2.64 (multiplets, 2H, $CH_2$); 3.25 (d, 1H, geminal CH, $J$ = 13.55 Hz); 3.43 (d, 1H, geminal CH, $J$ = 13.55 Hz); 3.94 (s, 3H, $CH_3$); 4.03–4.08 (multiplet, 2H, $CH_2$); 7.45–8.27 (a set of signals, 5H, $C_6H_4$ and pyrazole H-3).

**8d** (R = Ph): IR (cm$^{-1}$): 1740 (esteric CO); 1690 (amidic CO). $^1$H-NMR ($CDCl_3$) (δ): 1.16 (t, 3H, $J$ = 5.65 Hz, $CH_3$); 3.19 (d, 1H, geminal CH, $J$ = 14 Hz); 3.33 (d, 1H, geminal CH, $J$ = 14 Hz); 3.77 (s, 3H, $CH_3$); 4.04 (q, 2H, $CH_2$); 7.28–7.83 (a set of signals, 10H, $C_6H_5$, $C_6H_4$ and pyrazole H-3).

*Pharmacology*

Experiments were carried out on male albino Swiss mice (24–26 g) and Sprague–Dawley rats (140–160 g). Test compounds were administered orally or ip in 0.5% methylcellulose suspension.

Statistical analysis was made using the Student's *t*-test *versus* controls. The level of significance was set at $P < 0.05$.

*Behavioral effects and acute toxicity in mice*
Irwin's screening evaluative procedure [14] was used on groups of 6 mice. Compounds were administered at 3 dose levels orally (500, 750, 1000 mg/kg) and intraperitoneally (250, 500, 750 mg/kg). Mice were kept under observation for 6 h and symptoms were checked again 24 h later. The approximate $LD_{50}$ was obtained from mortality observed 7 days later.

*Analgesic activity*
For the phenylbenzoquinone writhing test [15], groups of 6 mice were injected with a 0.02% hydroalcoholic solution of phenylbenzoquinone (10 ml/kg) 1 h after oral administration of test compounds. Writhing movements of each animal were counted for 5 min (between the 5th and the 10th min after injection of the irritant). The analgesic effect of test compounds administered orally was expressed as percentage of protection as compared with the control group.

*Antiinflammatory activity*

*Acetic acid peritonitis [16].* Groups of 4 rats received intraperitoneally 10 ml/kg of a 0.5% acetic acid solution 1 h after drug administration. They were killed 30 min later by ether inhalation. Peritoneal exudate was collected and measured. Antiexudative response was expressed as the percent decrease of exudate volume compared with the controls.

*Carrageenin-induced rat paw oedema [17].* Groups of 6 rats were given the test compounds orally. After 60 min, 0.1% carrageenin solution was injected into the plantar surface of the right hind paw of each rat. Paw volume was measured using a mercury plethysmometer before injection of carrageenin and 3 h later. The percentage inhibition of oedema in treated rats as compared with controls was calculated.

*Ulcerogenic activity [18]*
Groups of 5 rats fasted for 24 h were used. Drugs were given orally and treatment was repeated after 2 h. Rats were killed by ether inhalation 6 h after the first dose. Their stomachs were removed, opened along the greater curvature and examined (using a dissecting microscope) for the presence of gastric ulcers. The extent of lesions, their number and size were rated on a scale from 0 to 3. In order to take into account the percentage of rats having ulcers, an index of ulceration was calculated on the basis of the following formula:

$$\frac{\text{mean degree of ulcers} \times \text{No of animals with ulcers}}{\text{No of animals}} \times 100$$

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