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SYNTHESIS AND BIOLOGICAL ACTIVITY OF ACYL DERIVATIVES OF 4-AMINO-2,3-DIMETHYL-1-PHENYLPYRAZOL-5-ONE

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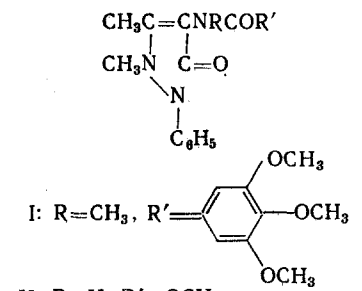
The high anti-inflammatory, antipyretic, and anesthetic activity of individual representatives of the 4-amino-2,3-dimethyl-1-phenylpyrazon-5-ones is well known. Such N,N-dialkyl derivatives as aminopyrene and dipyrone have been widely used as drug preparations for the last ten years.

Interest in N-acyl derivatives of 4-amino-2,3-dimethyl-1-phenylpyrazol-5-one and its homologs sharply increased in the 1960s, when their systematic synthesis and pharmacological uses were undertaken, resulting in the discovery of several compounds possessing high anti-inflammatory and antipyretic acitivty with much lower toxicity than that of aminopyrene and phenylbutazone [1-12].

The above work extended the spectrum of acyl derivatives of 4-amino-2,3-dimethyl-1-phenylpyrazol-5-ones in a search for new substances having antipyretic and anti-inflammatory activity.

The present work describes new acyl derivatives of 4-amino-2,3-dimethyl-1-phenylpyrazol-5-one of the general formula



I: R=CH3, R'=
II: R=H, R'=OCH3
III: R=CH3, R'=OCH3

and an investigation of their biological activity.

The synthesis of compound (I) was accomplished by the interaction of 4-methyl-aminopyrene or its hydrochloride with 3,4,5-trimethoxybenzoic acid or its functional derivatives, such as the acid chloride or anhydride, in the presence of a dehydration or dehydrohalogenation agent. Phosphorus oxychloride, thionyl chloride, phosphorus trichloride, phosphorus pentachloride, and the like can be used as dehydrating agents. As dehydrohalogenation agents, excess 4-methylaminoantipyrene, tertiary amines (triethylamine, pyridine) or in-

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TABLE 1. Influence of Compounds (I-III) and Aminopyrene on the Production of Agar Inflammation on Rat Paws

Experimental series	LD_{50}, mg/kg	Applied dose, mg/kg	Increase of volume at peak inflammation of rat paws, % of initial value
Control	—	—	112
I	912	90	59
II	58	12	80
III	902	90	64
Aminopyrene	344	35	116
Aminopyrene	344	100	97
Aminopyrene	344	120	80

TABLE 2. Influence of Compound (I) and Phenylbutazone on Formalin Inflamation of Rat Paws

Experimental series	LD_{50}, mg/kg	Applied dose, mg/kg	Increase of volume at peak inflammation of rat paws, % of initial value
Control	—	—	79
I	912	50	20
Phenylbutazone	215	50	44

organic bases (such as alkali metal carbonates and bicarbonates) have been used. The reaction may take place either in the absence or presence of inert solvents. If the use of a solvent seems expeditious, benzene or its methyl homologs, ethers, or excess tertiary amine (pyridine, for example) can be used [13].

Compounds (II) and (III) were obtained by the interaction of methyl chloroformate with the corresponding 4-amino (or methylamino)-2,3-dimethyl-1-phenylpyrazol-5-one in the presence of an aqueous soda solution.

The anti-inflammatory, antipyretic, and analgesic activity of compounds (I-III) were studied.

EXPERIMENTAL

The acute toxicity of compounds (I-III) and their use compared with phenylbutazone and aminopyrene was studied in experiments with white mice by intraperitoneal injection of the substance suspended in a constant volume of 1% starch. The observation time was 24 h. The LD_{50} data are given in Tables 1 and 2.

The anti-inflammatory activity of (I-III) and aminopyrene was studied in experiments on white rats under agar-induced inflammation, and that of (I) was studied with formalin-induced inflammation. The substances were injected intraperitoneally in 1% starch suspension 1 h before subplantar injection of 0.15 ml of 1% agar or 2% formalin. The controls were treated with a similar volume of starch suspension. The intensity of inflammation was judged by the change of volume of the inflamed paw, taking into account the dynamics of oncometrics.

Compounds (I-III) functioned as anti-inflammatory agents by agar-induced inflammation, as did compound (I) by formalin-induced inflammation (Tables 1 and 2).

Thus, with agar-induced inflammation, compounds (I) and (III) diminished the edema in the feet by 53-58% at a dose of 10% of their LD_{50}'s, for compound (II), the decrease was 32% by comparison with the control. Aminopyrene did not depress inflammation at a dosage of 10% (35 mg/kg) or 30% (100 mg/kg) of its LD_{50}, and at a dose of 35% (120 mg/kg) decreased foot inflammation by a total of 32%.

With formalin-induced inflammation, compound (I) and phenylbutazone were both introduced at the same dosage of 50 mg/kg. In spite of the fact that this dose constituted only 5.5% of the LD_{50} of compound (I), and 23.2% of the LD_{50} of phenylbutazone, compound (I) suppressed the formalin-induced edema significantly stronger than phenylbutazone.

The influence of compounds (I-III) on body temperature was studied in experiments on intact mice and on mice given 0.5 ml of a suspension of brewer's yeast subcutaneously 16 h before the experiment. The temperature was measured rectally by means of an electrothermometer.

Compound (I), used at a dose of 90 mg/kg (0.1 LD_{50}) decreased the body temperature of both groups of mice by 3.3-3.7°, the maximum lowering was recorded 1 h after the injection of (I). The hypothermic effects of compounds (II) and (III) approximated that of aminopyrene, i.e., 2.3°C.

Analgesic activity was evaluated by the "hot plate" method. The compounds were injected intraperitoneally in 2% starch suspension. The effects were evaluated after 30, 60, and 120 min, and showed that the test substances, as well as aminopyrene, did not possess anesthetic activity, even at a dose of 0.2 LD_{50}.

Compounds (I-III) are therefore biologically active substances, showing anti-inflammatory and antipyretic effects, the above-mentioned activity of compound (I) surpassing that of the known representative groups of anti-inflammatory and antipyretic substances of the pyrazolone series such as aminopyrene and phenylbutazone. The value of (I) lies in its low toxicity compared to aminopyrene and phenylbutazone.

EXPRIMENTAL (CHEMICAL)

UV spectra were obtained on a Perkin-Elmer 402 instrument (Swiss) in methanol, and mass spectra were recorded on an LKB-9000 instrument (Swiss) at ionization potentials of 70 and 14 eV, an emission current of 20 μA, and an ion source temperature of 250°C. The temperature of the substances admitted into the ion source was 50-70°C.

2,3-Dimethyl-4-(N-methyl-N-3',4',5'-trimethoxybenzoylamino)-1-phenylpyrazol-5-one (I) [13]. Method A. To a four-necked flask, fitted with a stirrer, thermometer, reflux condenser, and dropping funnel were added 23.07 g (0.1 mole) of 4-methylaminoantipyrene, 50 ml of absolute benzene, and 50 ml of absolute ether. A solution of 11.53 g (0.05 mole) of 3,4,5-trimethoxybenzoyl chloride was added dropwise with stirring and external cooling with cold water at a rate such that the temperature did not exceed 25-30°C. The reaction mixture was then heated and stirred under reflux for 4-5 h and filtered after cooling to room temperature. The precipitate of 4-methylaminoantipyrene hydrochloride was washed with absolute benzene and the filtrate was evaporated under vacuum. The caramel-like residue was triturated with petroleum ether and the precipitate was filtered. After recrystallization from a mixture of benzene and petroleum ether, 16.95 g (82.38%) of cream-colored crystals, mp 64-65°C, of (I) were obtained.

Found, %: C 64.39, H 5.70, N 10.34. $C_{22}H_{24}N_3O_5$
Calculated, %: 64.21, H 6.12, N 10.2.

The molecular weight was confirmed by mass spectroscopy [14].

UV spectrum, λ (log ε): 209 (4.516), 272 (4.072). Compound (I) was soluble in the usual organic solvents, either cold or heated. At 36-37,C, (I) dissolved in 30 parts of water.

Method B. To a flask fitted with a stirrer and a thermometer were added 10.61 g (0.05 mole) of 3,4,5-trimethoxybenzoic acid, 10.87 g (0.05 mole) of 4-methylaminoantipyrene, and 9.15 g of phosphorus pentachloride, and the mixture was heated gradually to 175-180,C. After stirring at this temperature for 35-40 min, the reaction mixture was cooled to room temperature, dissolved in 140-150 ml of water, and then made basic with a 10% soda solution (to pH 7.0-7.5). The solution was shaken with activated charcoal, filtered, and the filtrate was extracted 2-3 times with benzene. The benzene layer was separated, dried with calcined magnesium sulfate, filtered, and the filtrate was evaporated at room temperature to give a residue which gradually crystallized on standing to give 17.16 g (83.41 %), mp 63.5-64.5°C (from a mixture of benzene–petroleum ether).

Method C. To a three-necked flask fitted with a stirrer, thermometer, and reflux condenser were added 12.69 g (0.05 mole) of 4-methylaminoantipyrene hydrochloride, 10.61 g (0.05 mole) of 3,4,5-trimethoxybenzoic acid, 4.5 ml of phosphorus trichloride, and 60 ml of dry xylene. The mixture was stirred at 103-106°C for 5-6 h, cooled to room temperature, and dissolved in 100-110 ml of water. The solution was brought to pH 7.0-7.5 with 10% soda solution and extracted 2-3 times with benzene. The organic layer was separated, dried with calcined magnesium sulfate, filtered, and concentrated at room temperature to give a residue which crystallized on standing; yield 12.68 g (61.64%), mp 63.5-64.5°C (from benzene–petroleum ether).

4-Carbomethoxyamino-2,3-dimethyl-1-phenylpyrazol-5-one (II). To a flask fitted with a stirrer, thermometer, and dropping funnel were added 10.16 g (0.05 mole) of 4-aminoantipyrene and 30 ml of aqueous soda solution. Methylchloroformate (5.2 g, 0.055 mole) was added dropwise with external cooling at a rate sufficient to maintain the temperature at less than 40-42°C. After stirring for 45-50 min, the precipitate was filtered and dried to

give 13 g (98%) of slightly yellow crystals, mp 188-189°C (from a mixture of alcohol–ethyl acetate).

Found, %: C 59.89; H 5.83; N 16.23. $C_{13}H_{15}N_3O_3$.
Calculated, %: C 59.76; H, 5.79; N 16.08.

The molecular weight was verified by mass spectroscopy. UV spectrum, λ(ε): 209 (10049); 273 (10016).

Compound (II) was soluble in the usual organic solvents and in hot water.

2,3-Dimethyl-4-(N-methyl-N-carbomethoxyamino)-1-phenylpyrazol-5-one (III) Compound III was prepared analogously to compound (II) from 10.87 g (0.05 mole) of 4-methylaminoantipyrene and 5.2 g (0.055 mole) of methyl chlorocarbonate to give 9.8 g (71.2%), mp 82.5-83.5°C (from a mixture of ethyl acetate–petroleum ether).

Found, %: C 57.52; H 6.77; N 14.75. $C_{14}H_{17}N_3O_3 \cdot H_2O$.
Calculated, %: C 57.51; H 6.55; N 14.37.

The molecular weight was verified by mass spectroscopy.

UV spectrum, λ(ε): 209 (9812); 272 (9556).

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