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Chemical and Pharmacological Investigations of Constituents of *Eleutherine bulbosa* (Miller) Urb. (Iridaceae)

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Abstract □ Eleutherin and eleutherol extracted from bulbs of *Eleutherine bulbosa* (Miller) Urb. (Iridaceae), collected in the Amazonian jungle and grown in Italy, were tested for biological properties. The extraction procedure and the results of antibacterial, cytotoxicity, and pharmacological assays are reported. Eleutherin has a weak and transient effect of decreasing the prothrombin time (*in vivo* in rats) and a weak antibacterial activity on *Bacillus subtilis (in vitro)*.

Keyphrases □ *Eleutherine bulbosa* (Iridaceae)—extraction of eleutherin and eleutherol, pharmacological screening □ Eleutherol and eleutherin—extracted from *Eleutherine bulbosa*, pharmacological screening □ Medicinal plants—extraction of eleutherin and eleutherol from *Eleutherine bulbosa*, pharmacological screening

In 1959, unknown bulbs, collected in the Peruvian part of the Amazonian jungle along the Ucayali River near Pucallpa[1], were sent to Europe[2]. Some bulbs, which arrived presumably a few months after collection, were planted in northern Italy near Milan at about 220 m above sea level, while others were subjected to biological screening.

Following the procedure described in this paper, a crystalline yellow substance was extracted from the dry bulbs. The substance proved to be a mixture of eleutherol and eleutherin[3]. The two compounds were separated, crystallized, and biologically assayed. Through specimens of bulbs and flowers obtained from the plant grown in Italy, the plant was identified[4] as *Eleutherine bulbosa* (Miller) Urb. [*E. plicata* (Ser.) Herb.].

Both eleutherol and eleutherin were described and chemically characterized earlier (1–3) by extracting the compounds from bulbs of *E. bulbosa* (Miller) Urb. (Iridaceae) collected in Java. The plant, cultivated in Java where it was used in a variety of illnesses by natives, originally came from equatorial America (1). A related plant, *E. plicata*, is said to be a popular medicine used by natives of north or northeast Brazil. Some studies on the chemical constituents of the plant have been performed (4).

While data on the chemical properties of eleutherol and eleutherin are abundant (1–3, 5–14), data on their biological properties are scarce (6). This paper reports the results of experiments performed on a sample of eleutherol and eleutherin extracted from bulbs of *E. bulbosa* grown in Italy.

EXPERIMENTAL[5]

Bulbs grown in Amazonia were used in the early phases of the research, while bulbs grown in Italy were used subsequently. The only apparent difference between them was that the yellow crystalline material on the outer scales of dry bulbs was more abundant on those of Amazonian origin than on those of Italian origin. The bulbs planted in Italy were allowed to grow for about 6–7 months; they then were harvested, separated from the aboveground parts, and air dried for about 3 months at room temperature.

Dry bulbs (1 kg) were sliced and left to macerate with 1 liter of 95% ethanol for 2 days; the procedure was repeated once. The filtered alcoholic extracts were combined and brought to dryness at 50° under reduced pressure. The residue was dissolved in warm acetone, and one volume of ether and six volumes of water were added to it. The aqueous phase was separated and extracted four times with a total amount of ether corresponding to 1.5–2 times the volume of the water. All ether extracts were combined, dried over anhydrous sodium sulfate, filtered, and brought to dryness under reduced pressure.

The residue was dissolved in a minimum amount of warm absolute ethanol. Refrigeration of the ethanol solution yielded a yellow crystalline material, which was recrystallized once from absolute

[1] For details about the geographical position of Pucallpa and for a view of the jungle along the Ucayali River, see *National Geographic*, Feb. 1964, and the companion map.
[2] People living in Pucallpa indicated that the aborigines call the plant "Piri-Piri" and use decoctions of the bulbs to stop postpartum hemorrhages.
[3] Identification was carried out by Professor A. Quilico, Istituto di Chimica Generale e Analitica, Politecnico di Milano, Milan, Italy.
[4] Identification was carried out by the late Professor F. Morton of Hallstatt. The specimen is deposited at the Botanischer Garten und Botanisches Museum, Berlin, Dahlem, Germany (herb. D. E. Meyer No. 2286).

[5] Melting points were taken on a Leitz hot-stage microscope and are uncorrected. Optical rotation measurements were determined on a Perkin-Elmer 141 polarimeter. UV spectra were determined in ethanol using a Beckman DU spectrophotometer. TLC was carried out on silica gel G (0.25 mm) plates using benzene–acetone (9:1) (Solvent A) and petroleum ether–ethyl acetate–chloroform (67:33:10) (Solvent B) as developing systems. Separated components on the plates were visualized by their appearance under 254-nm UV light. For TLC, 10 or 20 μg of the material under test was applied.

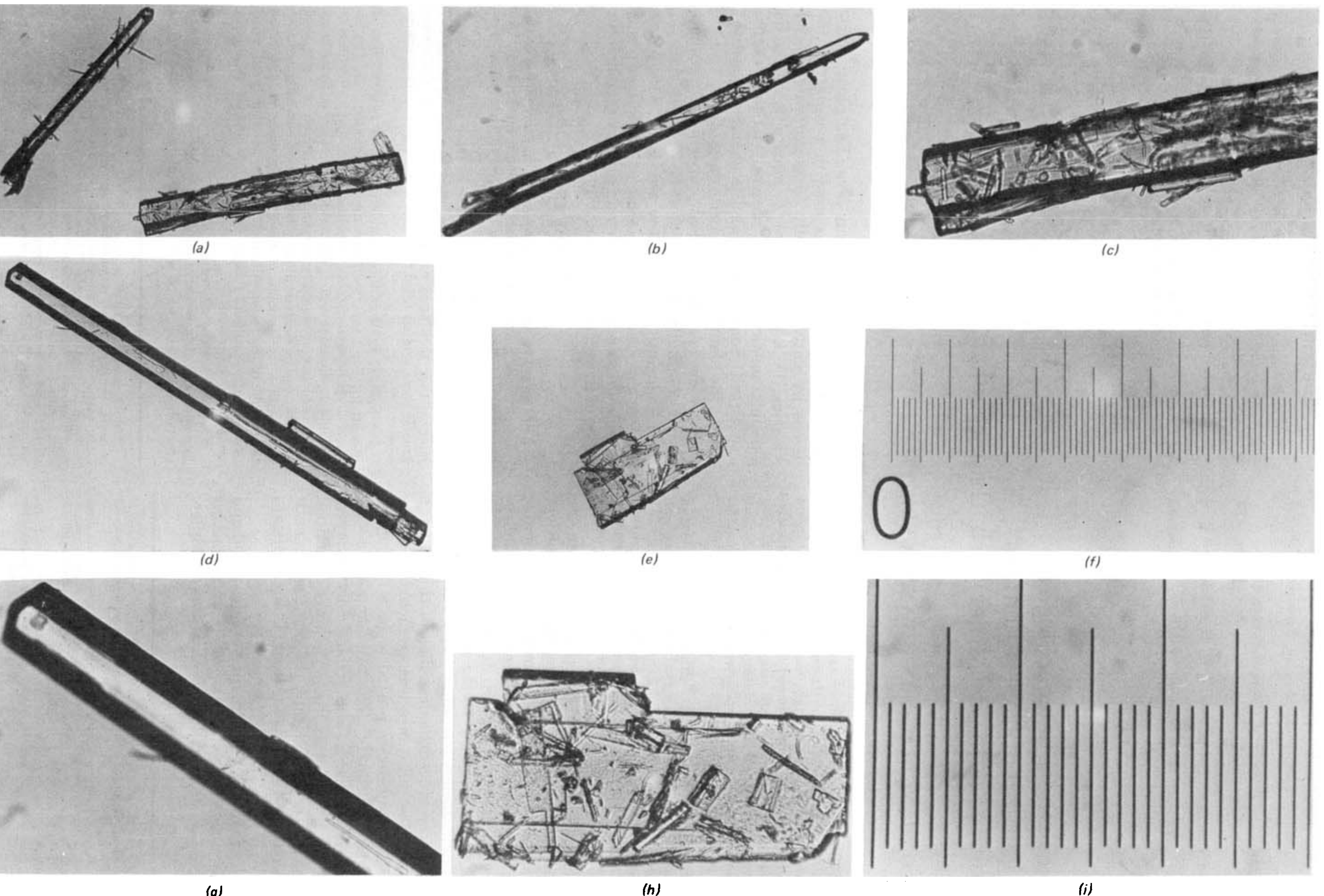
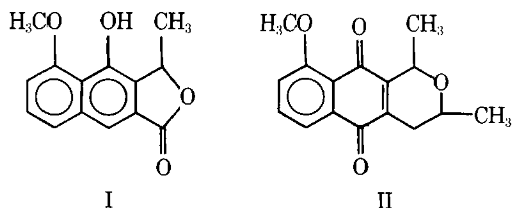


Figure 1—*Photomicrographs of crystals of eleutherol and eleutherin. Key:* (**a**) *crystals of eleutherol and eleutherin before fractionation, 40×;* (**b**) *crystal of eleutherol before fractionation, 100×;* (**c**) *crystal of eleutherin before fractionation, 100×;* (**d**) *crystal of eleutherol after fractionation, 40×;* (**e**) *crystal of eleutherin after fractionation, 40×;* (**f**) *scale 40× (1/10 of a millimeter is subdivided in 10 parts);* (**g**) *crystal of eleutherol after fractionation, 100×;* (**h**) *crystal of eleutherin after fractionation, 100×; and* (**i**) *scale 100× (1/10 of a millimeter is subdivided in 10 parts).*



alcohol, once from acetone, and twice from *n*-butanol, yielding 500 mg, mp 145–147°; $[\alpha]_D^{20}$ + 221° (c. 0.1, chloroform). The material turned red when treated with concentrated sulfuric acid.

TLC showed the presence of two major blue spots, R_f 0.73 and 0.57 with Solvent A and R_f 0.51 and 0.40 with Solvent B. In addition, six minor spots and a tail were seen when Solvents A and B, respectively, were used.

Isolation of Eleutherol—The crystalline material was dissolved in warm benzene (500 mg in 30 ml at 50°). An equal amount of petroleum ether was added to the solution after the undissolved resins were separated by centrifugation. On refrigeration, white crystals precipitated from the solution. They were washed twice with benzene–petroleum ether (1:1) and crystallized twice from methanol, yielding white crystals (120 mg), mp 202–203°; $[\alpha]_D^{20}$ + 91.4° (c 0.1, chloroform). The material turned yellow when treated with concentrated sulfuric acid (1).

TLC showed the presence of a major spot at R_f 0.73 with Solvent A and at R_f 0.51 with Solvent B and only trace amounts of one undetermined substance. The UV spectrum and the analytical data corresponded to those published by Schmid *et al.* (1) for eleutherol (I), mp 202–203°; $[\alpha]_D^{20}$ + 90° in chloroform.

Isolation of Eleutherin—The benzene–petroleum mother liquors remaining from the initial crystallization of eleutherol were taken to dryness under reduced pressure. The residue was crystallized twice from *n*-butanol to give yellow crystals (280 mg), mp 165–170°; $[\alpha]_D^{20}$ + 280° (c 0.1, chloroform). These crystals turned red when treated with concentrated sulfuric acid (3). TLC showed the presence of one major spot at R_f 0.57 with Solvent A and at 0.40 with Solvent B, as well as trace amounts of I and three undetermined substances. The UV spectrum corresponded to that published by Schmid *et al.* (3) for eleutherin (II), except for the presence of a maximum at 313 nm, which indicates the presence of I.

The analytical data are compatible with those for II published by Schmid *et al.* (3), mp 175°; $[\alpha]_D^{15}$ + 346° in chloroform. This sample of eleutherin differed from that described previously (3) in that it had a lower melting point and optical rotation. Such differences may be related to the presence of traces of eleutherol and other unidentified products in the eleutherin sample.

Photomicrographs of the isolated materials are shown in Fig. 1.

Due to the preliminary scope of the biological investigations, the samples of I and II were screened without further purification.

Antibacterial and Cytotoxicity Testing—For testing antibacterial and cytotoxic properties *in vitro*, stock solutions of II were made up in methanol (10 mg in 1 ml of warm methanol diluted to 40 ml with distilled water). The antibiotic activity was tested on *Bacillus subtilis* (ATCC 9466) with the agar plate diffusion method, using medium 1 (15). The antidehydrogenase activity was tested according to Miyamura (16) on sarcoma 180 cells surviving in agar.

General Pharmacological Testing—For testing general pharmacological properties in mice and rats *in vivo*, II was finely ground, sieved through a 200-mesh stainless steel screen, suspended in an aqueous suspending medium [0.1% carboxymethylcellulose (w/v) and 1% polysorbate (v/v)] and given intraperitoneally in the constant volume of 0.2 ml/10 g (mice) or 0.5 ml/100 g (rats).

Mice—Experiments were performed on groups of albino male mice of 20–22 g (five to 10 animals/group).

The general activity was tested according to Irwin (17) on animals observed for 3 hr after treatment. Rectal temperature was measured with an electrothermometer[6] and a rectal probe (RM 6) for instant measuring (18). Motor coordination was controlled for 5 min on a rough-surfaced rod (22 mm diameter) rotating at 7.5 rpm (19, 20).

The response to a noxious stimulus was controlled with the tail pinch method of Bianchi and Franceschini (21) and the hot-plate method (22). The mydriatic effect was tested with the Pulewska method (23, 24), using a binocular microscope and controlled illumination. The diameter of the pupil was expressed in arbitrary units (20 units = 1 mm; basal value of 40 control mice = 11.2 units). The experiments were carried out 1 hr after dosing the animals.

Anticonvulsant properties were tested in animals convulsing after pentylenetetrazol (125 mg/kg sc), strychnine (3 mg/kg sc), or electric shock (15 mamp for 0.2 sec) (25, 26). Antitremor properties were tested in animals showing sustained tremors after oxotremorine (2.5 mg/kg sc) (24), antireserpine properties were tested in animals showing ptosis and hypothermia after reserpine (2 mg/kg sc) (26), and depressant properties were tested in aggregated animals showing an increased sensitivity toward the toxic (26) and motor stimulant (27) effects of amphetamine (25 mg/kg sc using 10 mice/group and 1 mg/kg ip using five mice/group, respectively) and in animals showing loss of the righting reflex following pentobarbital administration (40 mg/kg ip) (28).

The effects on the autonomic nervous system were tested in animals treated with lethal doses of epinephrine (2.5 mg/kg iv) (26) and with doses of oxotremorine (2.5 mg/kg sc) (24) and carbacholine (100 μg/kg sc) (24) provoking parasympathetic overstimulation (salivation, diarrhea, diuresis, and lacrimation). The effects on the intestinal motility were tested with the charcoal bolus method (29). An interval of 60 min was allowed between the administration of II and the tests.

An additional test for analgesia was carried out on animals showing abdominal writhings following intraperitoneal administration of phenylquinone [0.1 ml/10 g of a 0.02% (w/v) solution in 6% (v/v) ethanol] (30). In this test, II was given subcutaneously 1 hr before phenylquinone.

Rats—The anti-inflammatory and analgesic properties were tested in groups of albino male animals of 100–110 g (10 animals/group) showing edema and hyperalgesia in an hindfoot after a subplantar injection of carrageenan (0.1 ml/rat of a 2% solution) (31). The determinations were carried out before and at hourly intervals for 5 hr after the administration of carrageenan. Compound II was given orally 30 min before carrageenan.

The effect on blood pressure was determined in six unanesthetized male animals of 300–400 g. Before and at intervals after II was given intraperitoneally (15, 30, and 60 min), blood pressure was measured by means of a pneumatic cuff and a piezocrystal pulse pick-up applied to the root of the tail. An automatic blood pressure recorder[7] (32) was used.

The Quick prothrombin time (33) was determined on the plasma of male animals of 180–200 g (three or four animals/group) at intervals after II was given intraperitoneally and orally.

Rabbits—The effects on blood pressure and respiration were determined in three male rabbits of about 3 kg, anesthetized with urethan (1 g/kg iv) and with the vagal nerves severed. Blood pressure was recorded by means of a pressure transducer connected to a carotid artery; respiration was measured by means of an impedance pneumograph connected to needle electrodes placed on the chest. A physiograph[8] was used to record the variations in blood pressure and respiration. Epinephrine (3 μg/kg), acetylcholine (3 μg/kg), and carotid occlusion were the methods used to assess the reactivity of the blood pressure-regulating mechanisms. Compound II dissolved in ethanol and normal saline (10 mg in 3 ml of 95° ethanol diluted with saline to 10 ml) was given slowly (0.3–0.6 ml/kg iv).

Isolated Heart—Amplitude, rate of contractions, and coronary flow in Langendorff rabbit isolated heart were recorded in three preparations perfused with II. Isoproterenol (0.04 μg) was used to increase the rate and force of contractions of the heart (34).

Due to a shortage of material, I was tested only for antibiotic and cytotoxic properties *in vitro* and for the general activity in mice *in vivo*. The methods used for the preparation of the necessary solutions or suspensions and for the execution of the assays were as already described.

The solutions or suspensions of I and II were used as soon as they were prepared.

RESULTS

Antibacterial and Cytotoxicity Testing—Compounds I and II were assayed at concentrations ranging from 7.17 to 125 μg/ml.

[6] Ellab, Electrolaboratoriet, Copenhagen, Denmark.

[7] W & W Electronic, Basel, Switzerland.
[8] EMI.

Compound I failed to inhibit the development of *B. subtilis* (ATCC 9466) up to 125 μg/ml. Compound II was found to inhibit the development at various concentrations down to 62.5 μg/ml. Tetracycline hydrochloride was active at doses ranging from 0.3 to 0.5 μg/ml. Compounds I and II, up to 125 μg/ml, did not show any antidehydrogenase activity on sarcoma 180 cells.

General Pharmacological Testing—*Mice*—Compound I was given intraperitoneally to six groups of mice (five animals/group) at various doses (800, 400, 200, 100, 50, and 25 mg/kg) without provoking overt toxic effects within 3 hr of observation. It only provoked abdominal writhing at all doses and hypothermia (mean fall in rectal temperature about 2°) at 800 and 400 mg/kg. The central nervous system, the autonomic nervous system, and the muscular system were not affected by the compound as judged by a direct observation of the treated mice. As indicated under *Experimental*, the compound was not tested further due to the shortage of material.

Compound II was given intraperitoneally at various doses (400, 200, 100, 50, and 25 mg/kg) to groups of mice (five animals/group); the animals were observed for 3 hr after treatment. At 400 mg/kg, II provoked the death of one animal; reduced spontaneous motility and curiosity; did not provoke Straub tail, mydriasis, salivation, lacrimation, diuresis, diarrhea, and piloerection; and did provoke abdominal writhings, twitches, and convulsions. At the same dose, it provoked hypothermia (about 2°), motor incoordination, and analgesia (tail pinch method and hot-plate method). At 200 mg/kg and at lower doses down to 25 mg/kg, it produced abdominal writhings in all mice, with the effect lasting about 30 min.

Compound II, given intraperitoneally at 200 mg/kg to groups of mice (five to 10 animals/group), did not show anticonvulsant properties (pentylenetetrazol, strychnine, and electric seizures), antichemotremor properties (oxotremorine tremors), and antireserpine properties (reserpine ptosis and hypothermia). It did not modify the hypnotic effects of pentobarbital, the stimulant properties of amphetamine in aggregated mice (toxicity and hypermotility), the parasympathetic stimulant properties of oxotremorine and carbacholine, the lethal effects of epinephrine, and the intestinal motility. Compound II, 200 mg/kg sc, did not modify the writhing syndrome brought about by an intraperitoneal injection of phenylquinone.

Rats—Compound II, 100 mg/kg po given to groups of rats (10 animals/group), did not show anti-inflammatory (carrageenan foot edema) or analgesic effects in edematous hyperalgesia (Randall–Selitto method using carrageenan as hyperalgic compound). The anti-inflammatory and analgesic tests were performed following oral administration of II, because irritant drugs may simulate anti-inflammatory effects when administered parenterally (35). Compound II has irritant properties, as shown by the writhing syndrome brought about by the compound when administered intraperitoneally in mice.

Compound II, 200 mg/kg po and ip, shortened the Quick prothrombin time from the control value of 13.2 to 8.3 sec (po) and to 8.1 sec (ip). The effect was manifest 15 min after treatment but not 30 min and 1, 4, and 24 hr after treatment. Menadione sodium bisulfite, 200 mg/kg po and ip, was still effective 24 hr after treatment (prothrombin time 10.7 sec po and 10.5 sec ip).

Compound II, 100 mg/kg ip, increased blood pressure 15 and 30 min after dosing (initial blood pressure of 175 mm Hg; increase of 15–20 mm Hg); blood pressure returned to normal within 60 min. Abdominal writhings were present for about 20 min after dosing. Reserpine, 1 mg/kg ip, provoked a long-lasting fall in blood pressure (initial blood pressure of 165 mm Hg; fall of about 30 mm Hg).

Rabbits—Compound II, 300–600 μg/kg, provoked an occasional and modest fall in blood pressure which lasted a few seconds only, but it failed to modify blood pressure considerably and for long periods. It did not alter the responses of the animals to epinephrine, acetylcholine, and carotid occlusion. At higher doses (2 mg/kg), II provoked the arrest of respiration and death.

Isolated Heart—Compound II, perfused at the concentration of 1 μg/ml, did not alter the rate and amplitude of contractions and coronary flow of the Langerdorff rabbit isolated heart and did not modify the positive inotropic and chronotropic effects of isoproterenol.

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