Synthesis and Biological Activity of 9-Substituted Acridines

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Abstract □ Various 9-substituted acridine derivatives, *i.e.*, carbamic and thiocarbamic acid esters, urea, and thioureas, were synthesized and tested for potential biological activity. Some compounds were found active in antibacterial, metabolic, parasitologic, and GI screening procedures. The screening results indicate that acridine derivatives of these types possess biological activity similar to other known acridines, especially in the areas of antibacterial action and parasitology. The antisecretory action of one carbamate is a new potential use of acridines which has not been reported previously.

Keyphrases □ Acridines, 9-substituted—synthesis and biological screening □ Biological screening—9-substituted acridine derivatives

Interest in this laboratory in the use of acridines as potential medicinal agents resulted in the synthesis of some 9-substituted acridines, *i.e.*, carbamic acid esters (I), urea (II), thiourea (III), and thiocarbamic acid esters (IV). The antineoplastic activity of acridine derivatives of types I, II, and III was reported previously (1). A search of the scientific literature revealed that little or no information was available concerning additional biological activity of these types of structures. The carbamate, thiocarbamate, urea, and thiourea derivatives described are of special interest since some were found active in antibacterial, metabolic, parasitologic, and GI screening procedures. Compounds I*a*, I*c*, and II were also active in a preliminary virology screen involving a nonspecific nucleic acid polymerase, but the results are only qualitative at this time.

The syntheses of I*a*, I*b*, II, and III*a* were reported previously (1). The benzyl (I*c*) and 2-methylpropyl (I*d*) esters of 9-acridinecarbamic acid were prepared in one-step, high yield reactions starting with 9-aminoacridine. Compounds III*b* and IV also were not reported previously. Synthetic steps leading to these compounds are described in the *Experimental* section.

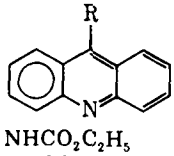
SCREENING RESULTS[1]

Compounds III*a* and III*b* were evaluated for antibacterial activity by a medicated agar dilution technique. In this test, the compounds are added to the agar and the test organisms are inoculated on the agar surface. The end-point is the level of compound that prevents emergence of visible growth. At 400-mcg./ml. levels, Compounds III*a* and III*b* were effective in inhibiting strains of *Staphylococcus aureus* (ATCC 6538), *Proteus vulgaris*[2], *Salmonella pullorum*[2], *Salmonella typhimurium*[2], *Escherichia coli*[2], *Klebsiella pneumoniae*[2], and *Diplococcus pneumoniae*[2]. Compound III*b* also inhibited *Proteus mirabilis*[2], *Streptococcus pyrogenes*[2], and *Pasteurella multocida*[2]. In comparison, 9-aminoacridine is effective at 40-mcg./ml. levels (2). Compound IV was also tested in an antibacterial screen (3) involving a variety of Gram-positive and Gram-negative bacteria and fungi of industrial importance. It was active against *Aerobacter aerogenes* (IPC 500)[3], *Bacillus mycoides* (IPC 509), and *S. aureus* (ATCC 6538) at 100-p.p.m. level, but it failed against these organisms at 5 p.p.m. 9-Aminoacridine is active against these organisms at 6–7-p.p.m. levels (4).

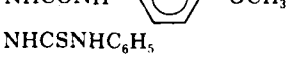
The activity of Compounds I*b* against certain other organisms was assessed by measuring inhibition of metabolic acitivity. One such test for anaerobic microorganisms is an assessment of reduction in formation from carbon dioxide and hydrogen by *Methanobacterium ruminantium*[2] at 39° for 2.5 hr. The level of methane is quantitated by passing a sample of the gas phase through a gas partitioner. Compound I*b* inhibited methane formation completely at 250 mcg./ml. and by 50% at 5 mcg./ml. However, under these same conditions, a 29.4-mcg./ml. level exhibited only a 20% inhibition after a 48-hr. incubation. This loss of effectiveness on continued incubation seems to be characteristic of a number of acridines[4].

Compound IV was active in an *in vitro* parasitology screen. Larvae or eggs of trichostrongyle nematodes were exposed to the test drug *in vitro*, initially at 100-mcg./ml. concentration. Efficacy was determined by direct microscopic inspection of the conditions of the parasites. Active compounds were retested in 10-fold serial dilutions to determine the end-point (5). The thiocarbamate (IV) was active against the eggs at 0.01 mg./ml., but it was inactive in the *in vivo* parasitology screen (6). In contrast, 9-aminoacridine has only shown effectiveness against parasites in *in vitro* testing at 2.5-mg./ml. levels (7). It is also inactive *in vivo*.

Compound I*a* was effective in inhibiting gastrin-stimulated acid secretion in an animal with a chronic gastric fistula. The carbamate (I*a*) in 50 ml. of aqueous methylcellulose (1%) was administered directly into the stomach of an unanesthetized chronic gastric fistula dog. One hour later, the gastric contents were drained and gastrin tetrapeptide (0:064 mg./kg. s.c.) was administered. Gastric secretions were collected over 2 hr., and the volume of secretions and total acid (meq./l.) were measured. The percent inhibition of volume and acid response were calculated using control values obtained in the same animal in an earlier experiment. Compound I*a*,



I*a*: R = $NHCO_2C_2H_5$
I*b*: R = $NHCO_2$-*n*-C_4H_9
I*c*: R = $NHCO_2CH_2C_6H_5$
I*d*: R = $NHCO_2CH_2CH(CH_3)CH_3$



II: R = NHCONH— —OCH_3

III*a*: R = $NHCSNHC_6H_5$
III*b*: R = $NHCSNHCH_2C_6H_5$
IV: R = $NHCSOC_2H_5$

[1] Tests for the various biological activities were carried out at Merck Sharp & Dohme Research Laboratories, Rahway, N. J. Only those acridine derivatives that showed activity in the testing are discussed.

[2] Identified by Dr. Eugene L. Dulaney, Merck Sharp & Dohme Research Laboratories, Rahway, N. J.

[3] IPC, Institute of Paper Chemistry, Appleton, Wis.

[4] H. B. Woodruff, Merck Sharp & Dohme Research Laboratories, personal communication, 1972.

at a dose of 16.1 mg./kg., reduced the volume of secretions and total acid content by approximately 35%.

The screening results indicate that acridine derivatives of the I, II, III, and/or IV types possess biological activity similar to 9-aminoacridine, especially in the areas of antibacterial action and parasitology. The antisecretory action of Compound Ia is a new potential use of acridine derivatives which has not been reported previously.

EXPERIMENTAL[5]

9-Acridinecarbamic Acid Esters (Ic and Id)—9-Aminoacridine (0.02 mole) and 0.02 mole of the necessary chloroformate ester were refluxed for 1 hr. in 300 ml. of acetone in the presence of 4 g. of sodium bicarbonate. The hot suspension was filtered, followed by evaporation of the acetone to yield a residue. The residue was recrystallized from ethanol or ethanol–water to give a solid, 80% yield.

The melting point for Ic was 210–213°.

Anal.—Calc. for $C_{21}H_{16}N_2O_2$: C, 76.81; H, 4.91; N, 8.53. Found: C, 76.91; H, 4.95; N, 8.46.

The melting point for Id was 152–154°.

Anal.—Calc. for $C_{18}H_{18}N_2O_2$: C, 73.44; H, 6.16; N, 9.51. Found: C, 73.29; H, 6.13; N, 9.59.

1-(9-Acridinyl)-3-benzyl-2-thiourea (IIIb)—*Method A*—9-Aminoacridine (0.02 mole) and 0.02 mole of benzyl isothiocyanate were refluxed for 1 hr. in 300 ml. of acetone.The acetone was evaporated, and the residue was recrystallized from ethanol–water to give a yellow solid, m.p. 178–180°, in 90% yield.

Method B—9-Isothiocyanatoacridine (0.02 mole) and 0.02 mole of benzylamine in 100 ml. of ethanol were heated on a water bath for 30 min. When the reaction mixture cooled to room temperature, the precipitate was collected and dried. Two recrystallizations from 25% ethanol–water gave a yellow powder, m.p. 178–180°, in 80% yield.

Anal.—Calc. for $C_{17}H_{17}N_3S$: C, 73.44; H, 4.99; N, 12.23. Found: C, 73.15; H, 5.15; N, 12.05.

Ethyl-N-(9-acridinyl)thiocarbamate (IV)—9-Isothiocyanatoacridine (0.02 mole) and 50 ml. of absolute ethanol were refluxed for 10 hr. The ethanol was evaporated to yield a residue, which was recrystallized from ethanol–water to give an orange solid, m.p. 160–162°, in 90% yield.

Anal.—Calc. for $C_{16}H_{14}N_2OS$: C, 68.06; H, 4.99; N, 9.92. Found: C, 68.16; H, 5.02; N, 10.04.

[5] Melting points were taken in capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were obtained from Atlantic Microlabs, Atlanta, Ga. IR spectra were recorded on a Perkin-Elmer model 237B spectrophotometer and were as expected. 9-Isothiocyanatoacridine was obtained from Eastman Organic Chemicals.

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Isolation of β-Amyrin and Ellagic Acid from *Couroupita amazonica*

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Abstract □ A phytochemical examination of *Couroupita amazonica* (Lecythidaceae) led to the isolation of β-amyrin and ellagic acid.

Keyphrases □ *Couroupita amazonica* (Lecythidaceae)—isolation, identification of β-amyrin and ellagic acid □ β-Amyrin—isolated and identified from *Couroupita amazonica* □ Ellagic acid—isolated and identified from *Couroupita amazonica*

The Amazonian plant *Couroupita amazonica* was selected for investigation because no phytochemical studies have as yet been reported on this genus of plants. These studies resulted in the isolation of β-amyrin and ellagic acid, which are commonly encountered plant principles.

EXPERIMENTAL

Plant Material[1]—The trunk bark of *Couroupita amazonica* Kunth. (Lecythidaceae) was collected during July 1969 in the vicinity of Iquitos, Perú.

Extraction—The coarsely milled plant material (1.1 kg.) was extracted continuously for 24 hr. in a soxhlet apparatus with petroleum ether (b.p. 30–60°). The petroleum ether extract yielded 6 g. of a semicrystalline residue. The defatted plant material was then air dried and extracted with 95% ethanol, which afforded 67 g. of residue following evaporation of the solvent. This residue was partitioned between equal volumes of chloroform and water, and the aqueous fraction was extracted four times with ethyl acetate.

[1] Voucher specimens (2331 and 2342) were identified by Dr. J. Wurdack, Smithsonian Institution, Washington, D. C., and are deposited at this address.