Facile Preparation of 6-Chloro-9-amino-2-hydroxyacridine, a Urinary Metabolite of Quinacrine and Quinacrine Mustard

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Abstract □ 6-Chloro-9-amino-2-hydroxyacridine was found to be a metabolite of both quinacrine and the antimalarial alkylating agent quinacrine mustard. Its structure was confirmed by a one-step reaction of quinacrine with 48% hydrobromic acid. The presence of this compound as a metabolite of quinacrine mustard suggests a possible *in vivo* activation mechanism for its antitumor activity and a pharmacological basis for its toxicity to the liver. *In vitro* experiments showed that this new compound does react with chromosomes and, therefore, can be both a useful chromosome stain and an intercalating agent.

Keyphrases □ 6-Chloro-9-amino-2-hydroxyacridine—synthesis from quinacrine □ Quinacrine and quinacrine mustard metabolite—synthesis of 6-chloro-9-amino-2-hydroxyacridine from quinacrine

For the investigation of *in vivo* alkylation mechanisms related to cancer chemotherapy, the use of bisbenzimidazole fluorescent alkylating agents was studied (1, 2). Since quinacrine mustard (I*b*) is a known alkylating agent possessing both fluorescence and antitumor activity (2), it was of interest to study its metabolism. Although the design of I*b* was based on the activity of quinacrine (I*a*) in mouse tumors (3), no fluorescence uptake was observed in tumors tested with I*b* or I*a*. In rats treated with I*b*, there was extensive liver damage, resulting in death. Urine samples collected from these rats contained the same fluorescent metabolite, 6-chloro-9-amino-2-hydroxyacridine (II).

TLC and other data supported the identity of the metabolite found in quinacrine-treated rats, and it is now confirmed by a facile synthesis from quinacrine. Its presence was previously suggested (4) in a reaction of I*a* with concentrated hydrochloric acid[1]. Although several *in vivo* studies[2] (5–7) also suggested that this compound could be a metabolite of quinacrine, the antimalarial properties of II are not known.

EXPERIMENTAL

Animal Study—Compound I or II was dissolved in the medium and fed intravenously to Lewis–Wistar rats; urine samples were collected for 24 hr. The urine samples were freeze dried and analyzed by TLC on a silica gel plate [chloroform–methanol (1:1)]. In both samples, a fluorescent green-yellow (R_f 0.58) spot could be seen in addition to other tailing products. Other solvent systems were unsatisfactory. Two groups of experiments were run in triplicate, and no I (R_f 0.45) or II (R_f 0.74) was seen. An authentic synthetic sample produced a spot with the same R_f value.

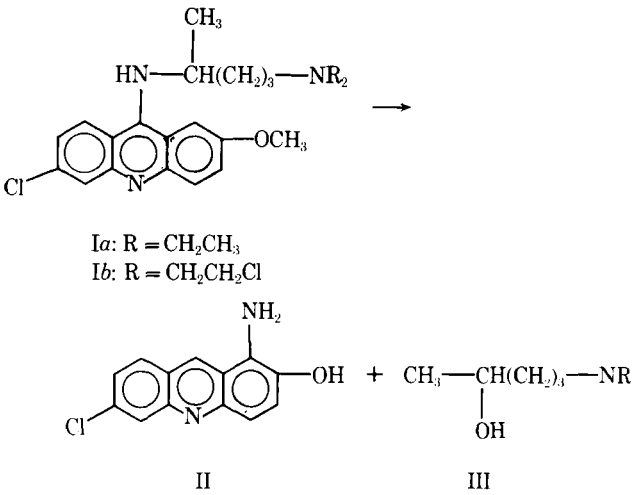
Preparation of 6-Chloro-9-amino-2-hydroxyacridine—A mixture of 5.0 g of quinacrine hydrochloride and 150 ml of 48% hydrobromic acid was refluxed overnight. Upon cooling, the yellowish-orange precipitate was collected and allowed to dry for 3 hr, yielding 3.2 g. Recrystallization from alcohol gave pure II as a light-yellow powder, 2.8 g (88%). The NMR spectra obtained in D_2O showed the loss of the alkyl, methylene, and methoxy protons of I. The IR spectra also showed the loss of methylene protons. The UV spectra showed an absorption at 272 nm, and the fluorescence emission was at 485 when excited at 435 nm.

Reaction of II with Chromosome Preparation—This new derivative was tested in a standard procedure of chromosome staining to compare it with quinacrine or quinacrine mustard. The resulting chromosomes were stained uniformly with no banding. The details will be reported elsewhere (8).

RESULTS AND DISCUSSION

The finding that II is a metabolite of both I*a* and I*b* demonstrated that both I*a* and I*b* could have been degraded *in vivo* to this compound. Therefore, both compounds could probably be biochemically converted *in vivo* by a hydroxylase-mediated mechanism, as shown in Scheme I.

There are ample examples in the literature to support the demethylation of the phenolic compound, even though the degradation of a long chain from an aromatic substituted amine is not known. Therefore, the final support for this mechanism will have to await the isolation of 4-hydroxypentyl nitrogen mustard (III) or another related product. The present data illustrate that the study of alkylation at the biological level is important and that such in-



Scheme I

[1] Reference 4 reported that treatment of atabrine with concentrated hydrochloric acid at 120–125° gave a mixture of 9-amino-6-chloro-2-methoxyacridine and 9-amino-6-chloro-2-hydroxyacridine. However, no proof was given and no attempt was made to separate the mixture.

[2] Based on polarographic evidence, 9-amino-6-chloro-2-hydroxyacridine and methoxy were suggested to be present in urine.

formation can be obtained with fluorescent alkylating agents (1). In addition, the *in vivo* activation mechanism for I*b* may account for the divergent physiological results of numerous other analogs (10).

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Interactions of Acetylcholine Mustard with Acetylcholinesterase

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Abstract □ The hydrolysis of acetylcholine and acetylcholine mustard by acetylcholinesterase was compared over a substrate concentration range of 1–10 m*M*. Reactions were allowed to proceed for 2 min at 25°. Results of these experiments reveal that the substrates have similar affinities for the enzyme, whereas the maximum velocity for the hydrolysis of acetylcholine mustard was significantly lower than for acetylcholine. These findings suggest that acetylcholine mustard has the ability to inactivate acetylcholinesterase.

Keyphrases □ Acetylcholine and acetylcholine mustard—hydrolysis by eel electroplax acetylcholinesterase □ Acetylcholinesterase—hydrolysis of acetylcholine and acetylcholine mustard

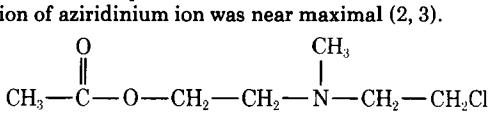
The synthesis of acetylcholine mustard [2-(chloroethylmethylamino)ethyl acetate] (I) was first reported by Hanby and Rydon (1). It was demonstrated that acetylcholine mustard can cyclize in buffered aqueous solutions to form an aziridinium ion (II) with alkylating ability (2, 3). This aziridinium ion is a close structural analog of acetylcholine.

In isolated muscle systems, it was found that acetylcholine mustard had about one-fifth the agonist potency of acetylcholine on the muscarinic receptors of the rat jejunum preparation or the nicotinic receptors of the frog rectus abdominis preparation (3). During a 1- or 2-hr exposure of the jejunum segments to either acetylcholine (1.0×10^{-4} *M*) or acetylcholine mustard (1.0×10^{-4} *M*), the contractions slowly declined; after washing, response to freshly applied acetylcholine was inhibited compared to control values. The decline in response was greater in the case of acetylcholine mustard, and the tissue did not fully recover even upon prolonged washing. Although the observed inhibition of response by acetylcholine and some of the effect of acetylcholine mustard could be accounted for by desensitization of receptors, the long-acting inhibition of the mustard was attributed to an irreversible inhibition brought about by alkylation of the receptors by the aziridinium ion (3).

A comparable agonist effect of acetylcholine mustard on guinea pig ileum was found, but no irreversible inhibition was observed (2). An increase in the resting tonus of the muscle after exposure to the mustard was also observed and might have been due to an inhibition of acetylcholinesterase (2). Therefore, this study was undertaken to examine the interaction between acetylcholine mustard and acetylcholinesterase.

EXPERIMENTAL

In all experiments, acetylcholine mustard was dissolved in buffer solution and allowed to stand for 1 hr at room temperature before use. Previous experiments indicated that at this time the concentration of aziridinium ion was near maximal (2, 3).



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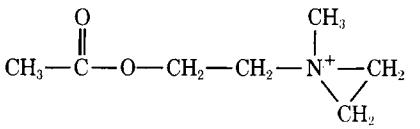

II