Chlorpromazine Metabolism IV: Quaternization as a Key to Determination of Picomoles of Chlorpromazine and Other Tertiary Amine Drugs

ROLAND E. LEHR and PUSHKAR N. KAUL ˣ

Abstract □ Reaction of chlorpromazine with 9-bromomethylacridine under appropriate conditions yields a nonfluorescent quaternary ammonium derivative which, on subsequent photolysis, liberates fluorescence. The major component of this fluorescence is 9-methylacridine (86%), while two minor components are 9-acridinecarboxaldehyde (6%) and 9-acridinemethanol (8%). The mechanism of photolysis leading to formation of these products appears to involve homolytic as well as heterolytic cleavages of the quaternary salt. Both the quaternization and the photolysis are stoichiometric. Appropriate isolation of the fluorescence and its quantitative determination constitutes the basis of a new and highly sensitive assay applicable to chlorpromazine and other tertiary amine drugs.

Keyphrases □ Chlorpromazine—assay by quaternization and photolysis, picomole quantities □ Tertiary amine drugs—assay by quaternization and photolysis, picomole quantities □ Metabolism—chlorpromazine assayed by quaternization and photolysis, picomole quantities □ 9-Bromomethylacridine—quaternization reagent for picomole assay of chlorpromazine and tertiary amine drugs

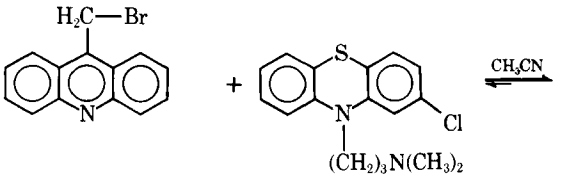
Currently increasing interest in generic bioequivalence of drugs and in drug dose–blood level–clinical response relationships of potent agents has created a general need for sensitive assay methods of high precision. Many tertiary amine drugs acting on the central nervous system exhibit a large volume of distribution and, therefore, yield low blood levels which are not amenable to available analytical methods. In some instances, GC or GLC alone or in conjunction with mass spectrometry has been successful in assaying levels less than 20–30 ng/ml. However, in the case of phenothiazine derivatives, one limitation of GC has been poor precision, and coupled GC–mass spectrometry is not easily available in most clinical laboratories. Therefore, there is a need for a sensitive assay applicable to potent tertiary amine drugs requiring routine monitoring in a clinical situation or warranting pharmacokinetic studies.

An approach of introducing a fluorescent tag into the tertiary amine molecule of chlorpromazine *via* a quaternization reaction was considered. On the bases of relatively high absorptivity and quantum yield of fluorescence of acridine, 9-bromomethylacridine was chosen for the purpose. However, the quaternary product obtained did not fluoresce as such, but on photolysis (Scheme I) it yielded fluorescence that could be determined fluorometrically. Preliminary attempts to apply this approach to assaying solutions and blood samples containing tertiary amine drugs were recently reported (1).

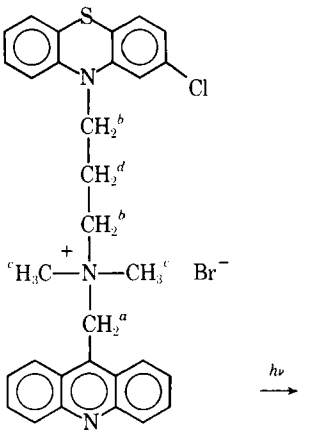
Other papers in this series have dealt with various analytical and pharmacological aspects of chlorpromazine metabolites (2–6). This paper describes the syntheses of various compounds involved in the quaternization of chlorpromazine and in confirming the nature of the fluorescent products resulting from the photolysis of the quaternary salt.

EXPERIMENTAL

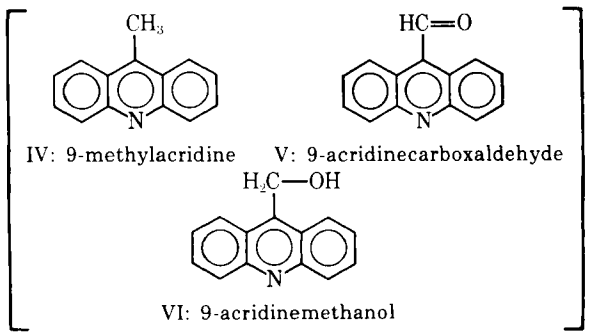
Synthesis of 9-Methylacridine—Two procedures, both based upon literature methods, were used. Method B has proven more successful than Method A for the preparation of large quantities of 9-methylacridine.



I: 9-bromomethylacridine II: chlorpromazine



III: quaternary salt of chlorpromazine



IV: 9-methylacridine V: 9-acridinecarboxaldehyde

VI: 9-acridinemethanol

Scheme I—Quaternization reaction of chlorpromazine and the fluorescent products resulting from the photolysis of the quaternary salt; a, b, c, *and* d *refer to the protons reflected in NMR spectrum*

Method A: From 9-Chloroacridine (7)—To 16.8 g (74.8 mmoles) of 9-chloroacridine in 48 ml of hot toluene was added ethyl sodiomalonate derived from the treatment of 18.7 g of diethyl malonate with 2.6 g of sodium in 48 ml of ethanol. The mixture was refluxed for 17.5 hr, and 7.0 g (48%) of 9-methylacridine was isolated according to the published procedure.

Method B: From Diphenylamine (8)—To 50 g (295 mmoles) of diphenylamine in a pressure bottle were added 30 ml of acetic acid and 85 g of anhydrous zinc chloride; the bottle then was heated at 220° for 14 hr. The crude product mixture was extracted with chloroform, and the chloroform layer was washed with water and evaporated under reduced pressure to yield the crude product. Although 9-methylacridine could be obtained by fractional crystallization from hexane, the remaining diphenylamine interfered with the crystallization. Thus, diphenylamine was separated from 9-methylacridine by column chromatography on silica gel[1], with toluene being used to elute the diphenylamine, which left 9-methylacridine on the column. Subsequent elution with acetone gave 17 g (30%) of 9-methylacridine, mp 113–115° [lit. (8) mp 115–116°].

Synthesis of 9-Bromomethylacridine—According to a modified literature procedure (7), 3.1 g (16.1 mmoles) of 9-methylacridine and 3.4 g (19.1 mmoles) of *N*-bromosuccinimide in carbon tetrachloride were mixed with a few milligrams of dibenzoyl peroxide. The suspension was refluxed to dissolve all components, and the appearance of 9-bromomethylacridine was monitored by TLC. Acetone–benzene (5:95) was used as the developing solvent in which 9-bromomethylacridine had a slightly higher R_f than that of 9-methylacridine. In some instances, refluxing did not initiate the reaction, but irradiation[2] of the mixture was successful. After 1 hr of reflux, the reaction mixture was filtered hot to remove succinimide. On cooling the filtrate, 9-bromomethylacridine crystallized and 3.4 g (12.5 mmoles, 78%) was collected by filtration. Recrystallization from carbon tetrachloride yielded 2.1 g (7.6 mmoles), mp 166–168° [lit. (9) mp 169–170° dec.].

Synthesis of 9-Acridinecarboxaldehyde—The selenium dioxide oxidation method (9) was followed, with 100 mg (0.51 mmole) of 9-methylacridine and 58 mg of selenium dioxide being added to 30 ml of xylene. After the mixture was refluxed for 1 hr, the organic phase was extracted repeatedly with 0.5 *N* HCl until the extracts appeared nearly colorless. The pooled aqueous phases were combined, made basic with sodium carbonate, and extracted with chloroform. The chloroform layer was dried over sodium sulfate. Filtration and removal of chloroform under reduced pressure afforded a crude product, from which 51 mg (48%) of 9-acridinecarboxaldehyde was obtained by preparative TLC[3], with acetone–benzene (5:95) being used as the developing solvent, mp 145–146° [lit. (10) mp 147°].

Synthesis of 9-Acridinemethanol—A new synthesis of the alcohol was devised. To 50 ml of methanol was added 455 mg (2.2 mmoles) of 9-acridinecarboxaldehyde. The solvent was heated to dissolve the aldehyde, and 100 mg of sodium borohydride was added. Some precipitate formed immediately. The reaction mixture was stirred for 30 min, treated with 10 ml of 2 *N* sodium hydroxide, and refluxed for 1 hr. The mixture, on cooling to room temperature, gave 280 mg (61%) of 9-acridinemethanol, mp 166–167.5° [lit. (7) 164–165°].

Quaternization Reaction—For quaternization on a preparative scale, 179 mg (0.56 mmole) of chlorpromazine in 5 ml of acetonitrile was added to a solution of 200 mg (0.73 mmole) of 9-bromomethylacridine in 45 ml of dry acetonitrile. The mixture was stirred at room temperature for 4 hr and concentrated under vacuum to 20 ml. On cooling in ice water, 198 mg (68%) of hygroscopic quaternary salt (III, Scheme I) was obtained, mp 178.5–179.5°; UV (ethanol): 254 (log ϵ 5.176) and 366 (3.987) nm; IR (KBr): major peaks at 1558, 1450, 1400, 1315, 1270, 1225, 1082, 1025, 920, 830, 801, 770, and 740 cm^{-1}. The NMR spectrum [$(CD_3)_2SO$] (Fig. 1) exhibited acridine aromatic protons at δ 8.52 (d, 2, J = 8 Hz), 7.98 (d, 2, J = 8 Hz), and 7.3–7.7 (m, 4); the chlorpromazine aromatic protons signaled at 6.6–7.1 (m, 7); other absorptions were at 5.57 (s, 2, H^a), 3.3–3.8 (m, 4, H^b), 2.8 (s, 6, H^c), and 1.9 (s, 2, H^d). The peak at δ 3.14 was found to be due to water, since it could be enhanced by addition of more water to the solution. The multiplet at δ 2.26 was inherent to dimethyl sulfoxide due to its incomplete deuteration.

Anal.—Calc. for $C_{31}H_{29}BrClN_3S\cdot0.5H_2O$: C, 62.05; H, 5.04; Br, 13.32; N, 7.00. Found: C, 61.83; H, 4.87; Br, 13.30; N, 6.95.

Photolysis of Quaternary Salt of Chlorpromazine—A solution of the quaternary salt (III) was spotted on a silica gel TLC plate, and the dried spot was exposed to UV light of 200–300-nm wavelength, with peak emission at 254 nm. The result of irradiation was the appearance of a fluorescent spot which, after elution with 0.01 *N* sulfuric acid containing 20% methanol, was subjected to spectrofluorometric determination.

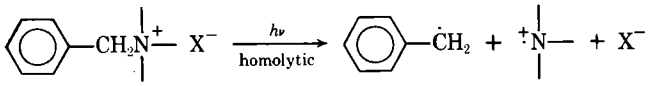
RESULTS AND DISCUSSION

The reaction of chlorpromazine with 9-bromomethylacridine under the conditions described to yield III appears to be stoichiometric. Assignment of the structure of the product as III is in accord with elemental analysis and NMR, UV, and IR spectra. Also consistent with the assignment of the product as a quaternary ammonium salt, as opposed to a sulfonium salt, is the reaction of promazine and chlorpromazine sulfoxide with 9-bromomethylacridine to form products with properties similar to those of III. Chlorpromazine *N*-oxide and chlorpromazine 5,*N*-dioxide do not react under the cited conditions to produce any quaternary products.

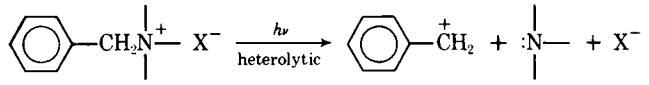
The quaternary product obtained is fairly stable as a solid or in organic solutions at temperatures up to 80°, but it is highly sensitive to light of a wide range of wavelengths. Effective photolysis occurs with a UV source emitting between 200 and 300 nm, with peak emission at 254 nm. With this source of light, the optimum photolysis of the quaternary salt occurred in 3 min, provided the distance between the source and the salt was maintained at 12 cm.

Photolysis of the quaternary salt III on a silica gel TLC plate produced a fluorescent spot exhibiting absorption and emission maxima at 350 and 474 nm, respectively. However, on development with methanol–benzene (1:9), this fluorescent spot separated into three fluorescent components. Of the total fluorescence, the major component contributed 86% while the minor spots were responsible for 6 and 8%, in order of their increasing R_f values, respectively. Structural assignments to these components were made by isolating sufficient quantities of the products following large-scale photolysis and comparing their physical, spectral, and TLC properties with those of the synthesized authentic samples. The major component was found to be 9-methylacridine, while the minor components were 9-acridinemethanol and 9-acridinecarboxaldehyde in order of their increasing R_f values. Based on calculations, the yields of the photolytic products obtained from the quaternary compound are as follows: 9-methylacridine, 26%; 9-acridinecarboxaldehyde, 1.0%; and 9-acridinemethanol, 0.6%.

Although a detailed mechanism of photolysis of III has not been studied, the products obtained are in line with the work of Ratcliff and Kochi (11), who presented evidence for competing solvolytic and radical processes in the photolysis of benzylammonium salts as shown in Schemes II and III.



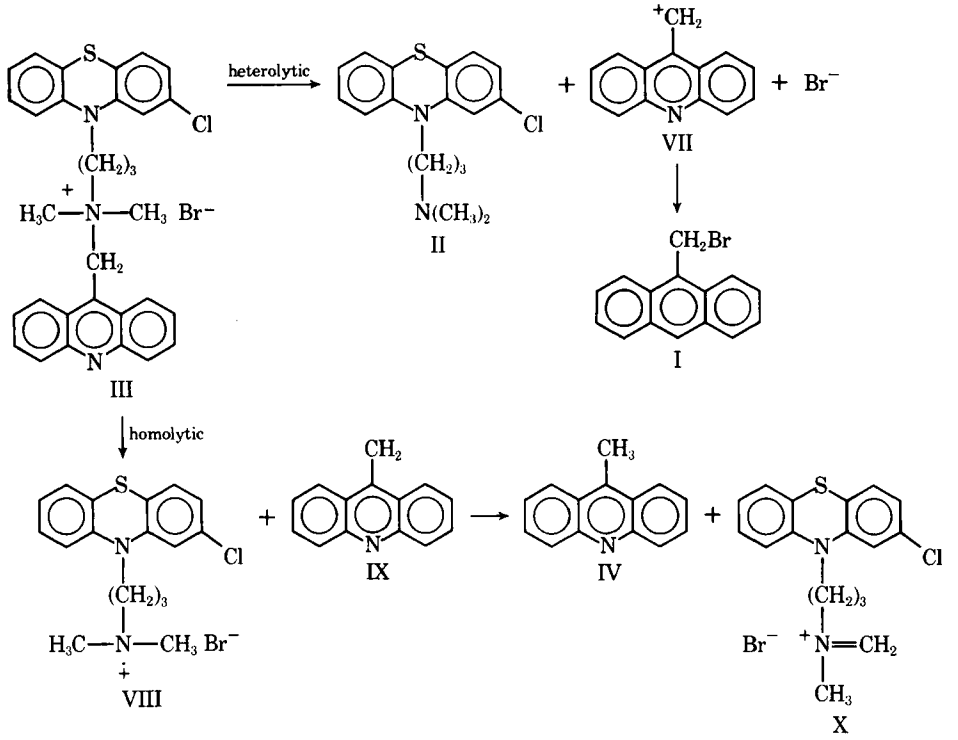
Scheme II



Scheme III

The homolytic pathway subsequently leads to formation of toluene by hydrogen abstraction from a N—CH_3 group by the benzyl radical. Scheme IV indicates an analogous manner in which the various products of photolysis of the chlorpromazine quaternary salt could arise. Thus, 9-methylacridine would reasonably arise by homolytic cleavage, followed by hydrogen abstraction by the 9-acridinyl radical (IX) from the chlorpromazine cation radical (VIII). One minor component, 9-acridinecarboxaldehyde, could result from a reaction of IX with oxygen, followed by decomposition. This latter pathway was not observed by Ratcliff and Kochi (11), but their experiments were carried out in solution whereas this photolysis was effected on silica gel. That 9-acridinecarboxal-

[1] Fisher Silicar CC-7.
[2] General Electric sunlamp.
[3] E. Merck silica gel $PF_{254+366}$.



Scheme IV—Postulated photolytic reaction mechanisms

dehyde is not a secondary photochemical product resulting from irradiation of 9-bromomethylacridine or 9-methylacridine was established by photolyzing pure samples of those two compounds adsorbed on silica gel. In neither instance was a detectable amount of 9-acridinecarboxaldehyde formed.

Heterolytic cleavage (Scheme II) of III should lead to formation of 9-bromomethylacridine (I), a product that is not observed under the reaction conditions. However, photolysis of I adsorbed on silica gel rapidly yields 9-acridinemethanol (VI), as shown by TLC comparison of the product with an authentic sample. This finding suggests that 9-bromomethylacridine is the precursor for 9-acridinemethanol observed in the photolysis of III and that both homolytic and heterolytic cleavages are occurring in the photolysis.

Although the structures of the phenothiazine nucleus-containing products have not been rigorously established, ferric chloride-active spots of R_f values [developing solvent of acetone–methanol–ethanolamine (100:30:1)] identical to those of chlorpromazine and

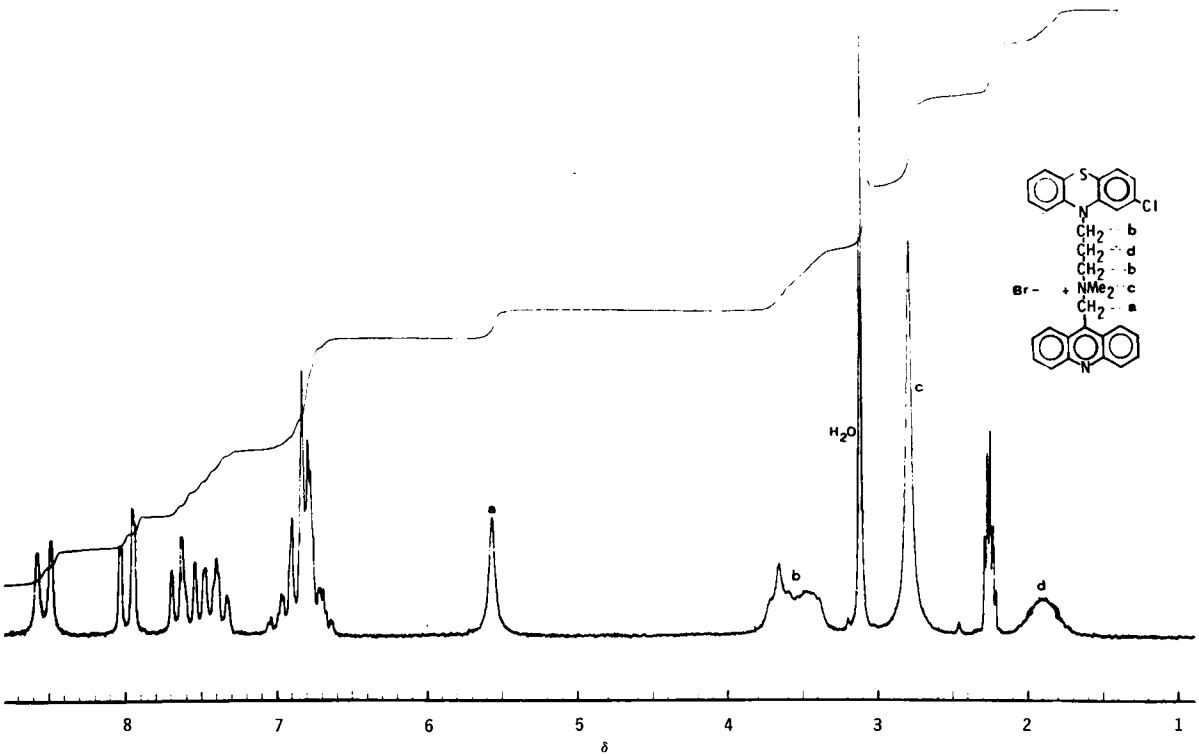
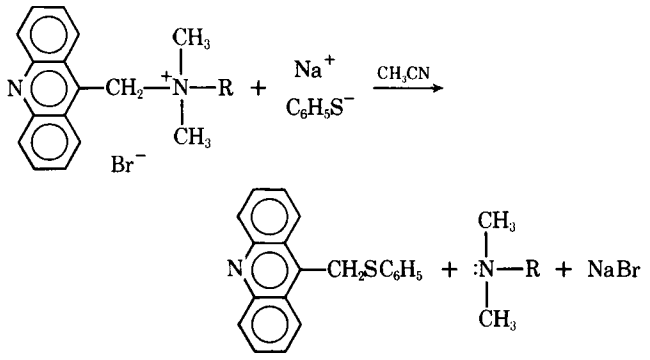


Figure 1—*NMR spectrum of 9-acridylmethyl quaternary salt of chlorpromazine in deuterated dimethyl sulfoxide;* a, b, c, *and* d *refer to the protons labeled in the structure of the quaternary salt shown.*



Scheme V

its monodemethylated derivative were observed. The latter should arise from hydrolysis of the chlorpromazine immonium salt (X) resulting from homolytic cleavage as shown in Scheme IV.

In applying the quaternization reaction to the assay of chlorpromazine (1), an aliquot of the reaction mixture after completion of the reaction is spotted on a silica gel TLC plate and developed first with methanol–methylal–benzene (2:2:1) and then with methanol–chloroform–benzene–ammonia (35:16:10:5). The first solvent system removes all nonquaternary products to the solvent front; the second system moves the quaternary product, which can now be photolyzed to yield determinable fluorescence. In the case of chlorpromazine, however, a minor secondary quaternary spot also arises with an R_f value slightly greater than that of the chlorpromazine quaternary. Although this minor product has not been rigorously identified, it is believed to be the quaternary salt resulting from the reaction of chlorpromazine sulfoxide with 9-bromomethylacridine on the basis of several pieces of indirect evidence. First, the spot also develops fluorescence upon photolysis. Second, the product has an R_f value identical to that of an independently prepared quaternary salt of chlorpromazine sulfoxide. Third, the quaternary salts of both chlorpromazine and its sulfoxide can be dequaternized with thiophenoxide according to Scheme V.

Pure, recrystallized III, upon dequaternization, yielded chlorpromazine as the only phenothiazine nucleus-containing product, as judged by TLC comparison with an authentic chlorpromazine sample. Similarly, upon dequaternization, the quaternary prepared from chlorpromazine sulfoxide yielded only chlorpromazine sulfoxide as the phenothiazine product. However, when chlorpromazine was reacted with 9-bromomethylacridine under the conditions of quaternization and an aliquot of the reaction mixture was treated with thiophenoxide for dequaternization, chlorpromazine as well as its sulfoxide was observed on TLC fractionation. Under identical conditions, but in the absence of 9-bromomethylacridine, chlorpromazine did not convert to any detectable amounts of its sulfoxide. This finding suggests that the chlorpromazine sulfoxide quaternary was produced under the reaction conditions of quaternization.

Furthermore, two-dimensional TLC has shown that the chlorpromazine quaternary can be converted to the sulfoxide product on the silica gel TLC plate, which is consistent with the reported (12) conversion of chlorpromazine to chlorpromazine sulfoxide on silica gel. On the whole, however, the generation of the sulfoxide product is generally limited to a total of <10%. In the actual assay, the calculations can be appropriately adjusted for this conversion.

Of significance are the facts that the quaternization reaction as well as the photolytically generated fluorescence is stoichiometric and that the fluorescing products can be successfully eluted from the TLC plate for fluorometric determination. This, in essence, constitutes the basis for the potential of the described quaternization reaction as a tool for a sensitive assay for chlorpromazine and other tertiary amine drugs. Several compounds other than chlorpromazine, including chlorpromazine sulfoxide, atropine, imipramine, chlorprothixene, codeine, morphine, and pilocarpine, were tested for reactivity with 9-bromomethylacridine; all yielded quaternary products and generated fluorescence on subsequent photolysis.

The fluorescence of the acridine moiety is totally quenched following introduction *via* quaternization into a tertiary amine molecule. Although no plausible explanation for this observation can be offered at present, a series of acridine derivatives is now being synthesized to determine whether or not the charge on the tertiary amine nitrogen is responsible for this total quenching of the acridine fluorescence or if there are other reasons for it.

The data presented and other preliminary experiments have confirmed that the overall approach of quaternization and photolysis is applicable to the determination of several tertiary amine drugs. In the case of chlorpromazine and its sulfoxide, for example, 50 ng and higher amounts have been quantitated (1). The amine concentration–fluorescence correlation is linear over a range of 0.05–1.00 μg. The detailed development and standardization of the specific assay for various tertiary amine drugs in aqueous solutions and human blood will be reported later.

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