(100 ppm) favored the formation of the more energetic calcium oxalate dihydrate, apparently by lowering the energy needed for the formation of this form. It was found that calcium oxalate dihydrate formed in the presence of pyrophosphate is identical to the bipyramidal octahedral calcium oxalate dihydrate crystals obtained from the urine of chronic stone formers (12). The results obtained in silica gel are important for two reasons:

1. They emphasize the importance of environmental conditions on crystal morphology and growth rate of calcium oxalate crystals.
2. They show that it is possible to grow a single crystal of calcium oxalate dihydrate identical to those encountered *in vivo*.

In the gelatin gel system described, calcium oxalate grew in a completely different manner. Figure 3 shows the concretion found in the gelatin gel after 6 days and a surface profile illustrating the organization and orientation of the calcium oxalate crystallites in these aggregates. In this system (unlike silica gel), gelatin offered a suitably structured substrate on which calcium oxalate crystals nucleated and developed such an oriented pattern. The presence or absence of pyrophosphate or magnesium ions in the gel did not influence either the pattern of growth or the proportion of the calcium oxalate aggregates formed. This growth phenomenon indicates that the gelatin matrix and its protein moiety were capable of controlling the nucleation, growth, and orientation of calcium oxalate crystals.

Like silica gel, gelatin gel provided a favorable growth supporting medium on which the slowly diffusing calcium and oxalate ions nucleated; but by virtue of the stereospecificity of its nucleating sites, calcium oxalate crystals developed in the gelatin into such a degree of organization. The obtained results add experimental evidence for the important role of protein-structured substrate in dictating growth and specific orientation in the formation of calcium oxalate concretions. They also support Gebhardt's (13) assumption that epitaxial nucleation of stone components on suitably structured substrate (*e.g.*, collagen fibers) is the primary factor in stone formation.

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Synthesis of (4-Quinolinoamino)aminoalkyltetrahydronaphthalene Derivatives for Possible Antimalarial Activity

I. NABIH [x], S. ISMAIL, and M. NASR

Abstract □ (4-Quinolinoamino)aminoalkyltetrahydronaphthalene derivatives were synthesized in an attempt to introduce new agents with antimalarial activity.

Keyphrases □ (4-Quinolinoamino)aminoalkyltetrahydronaphthalene derivatives—synthesized and screened for antimalarial activity □ 4-Aminoquinoline compounds—synthesized and screened for antimalarial activity □ Antimalarial activity—(4-quinolinoamino)aminoalkyltetrahydronaphthalene derivatives

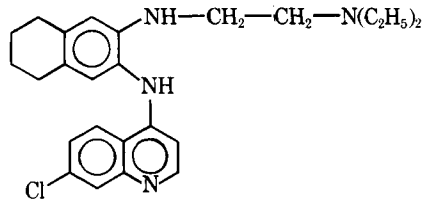
Compounds of the 4-aminoquinoline type still play a major role in the treatment of malaria. The development of resistance by some strains to most of the present antimalarials gave the initiative for several trials of new chemotherapeutic agents that may overcome this problem. In tropical countries where malaria eradication activities are still lacking, the provision of effective chemoprophylaxis and treatment represents a major problem.

DISCUSSION

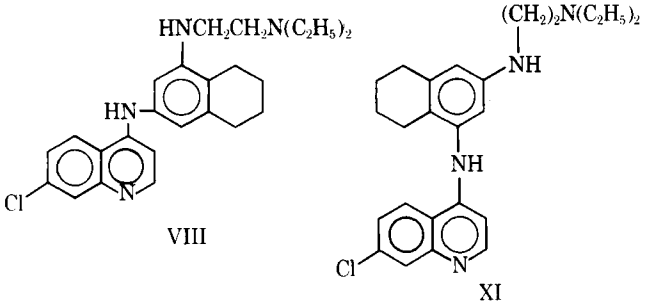
In previous articles (1, 2), the synthesis and biological activity of a new compound 4-(7-chloro-4-quinolylamino)-2-diethylaminomethyl-5,6,7,8-tetrahydro-1-naphthol (I) were described. Structurally, the compound is related to 4-aminoquinoline and bears a substituted tetrahydronaphthalene (tetralin) system as a side chain. The rationale for including the tetralin system is that it may undergo metabolic transformation to a naphthoquinone type of structure.

Derivatives of this type have shown considerable antimalarial activity. They appear to act by inhibiting the respiration of plasmodia (3). New compounds that would structurally include both the nitrogen heterocycle and a naphthoquinone structure might be useful as antimalarials, since the resistance of the parasite to one should not imply resistance to the other because both act through different mechanisms.

Biological studies based on the response to the product by *Plasmodium berghei* in mice revealed that oral doses of I at 5–100 mg/kg are curative. In addition, the agent proved to give complete protection against later exposure to massive infection by the parasite through subcutaneous application (2), thus providing effective chemoprophylaxis beside chemotherapeutic activity.



IV



VIII XI

The present work illustrates the synthesis of new compounds of the 4-aminoquinoline type, incorporating the tetrahydronaphthalene system whereby a diamino side chain is located at different positions on the aromatic part. In these structures the OH group on the aromatic part of the tetralin system was not included in order to investigate the biological significance for the presence of the phenolic OH on this part of the molecule.

Thus, it was decided to synthesize compounds structurally represented as IV, VIII, and XI. Reduction of 2-amino-3-nitro-5,6,7,8-tetrahydronaphthalene with stannous chloride and concentrated hydrochloric acid at about 150° gave 2,3-diamino-5,6,7,8-tetrahydronaphthalene (II) (4). Condensation of II with 4,7-dichloroquinoline gave 2-*N*-(7-chloro-4-quinolylamino)-3-amino-5,6,7,8-tetrahydronaphthalene (III). When III was reacted with β-diethylaminoethyl chloride, it gave 2-*N*-(7-chloro-4-quinolylamino)-3-(β-diethylaminoethylamino)-5,6,7,8-tetrahydronaphthalene (IV).

Condensation of 1-amino-3-nitro-5,6,7,8-tetrahydronaphthalene (V) with β-diethylaminoethyl chloride gave 1-(β-diethylaminoethylamino)-3-nitro-5,6,7,8-tetrahydronaphthalene (VI). Catalytic reduction of VI using 5% palladium-on-charcoal gave 1-(β-diethylaminoethylamino) - 3 - amino - 5,6,7,8-tetrahydronaphthalene (VII). On condensation with 4,7-dichloroquinoline, VII gave 1 - (β - diethylaminoethylamino) - 3-*N*-(7-chloro-4-quinolylamino)-5,6,7,8-tetrahydronaphthalene (VIII).

Condensation of V with 4,7-dichloroquinoline gave 1-*N*-(7-chloro - 4 - quinolylamino) - 3 - nitro - 5,6,7,8 - tetrahydronaphthalene (IX). Catalytic hydrogenation of IX (5% palladium-on-charcoal) gave 1-*N*-(7-chloro-4-quinolylamino)-3-amino-5,6,7,8-tetrahydronaphthalene (X). Treatment of X with diethylaminoethyl chloride yielded 1-*N*-(7-chloro-4-quinolylamino)-3-(β-diethylaminoethylamino)-5,6,7,8-tetrahydronaphthalene (XI).

EXPERIMENTAL[1]

Compound II—Compound II was prepared through reduction of 2-amino-3-nitro-5,6,7,8-tetrahydronaphthalene with stannous chloride and concentrated hydrochloric acid to give XXXVI, mp 135° [lit. (4) mp 136°].

Compound III—A mixture of 1 g (0.00617 mole) of II and 1.22 g (0.00617 mole) of 4,7-dichloroquinoline was refluxed in 10 ml of ethanol for 10 hr. Then this mixture was cooled and treated with ammonium hydroxide (25%) solution, and the precipitated product was filtered and recrystallized from ethanol to give 1.6 g (80% yield) of III, mp 300°; IR: 720 (Ar—Cl), 1590 (C=N), and 3250 (NH) cm^{-1}.

Anal.—Calc. for $C_{19}H_{17}ClN_3$: C, 70.81; H, 5.28; Cl, 10.87. Found: C, 70.53; H, 5.55; Cl, 11.01.

Compound IV—A mixture of 1 g (0.00308 mole) of III and 0.417 g (0.00308 mole) of β-diethylaminoethyl chloride was refluxed in 10 ml of ethanol for 10 hr. Then the reaction mixture was cooled and treated with 25% potassium hydroxide solution. The formed precipitate was recrystallized from ethanol to give 1.2 g of IV (91% yield), mp 180°; IR: 720 (Ar—Cl), 1590 (C=N), and 3250 (NH) cm^{-1}.

Anal.—Calc. for $C_{25}H_{31}ClN_4$: C, 71.10; H, 7.34; Cl, 8.41. Found: C, 71.03; H, 6.95; Cl, 8.81.

Compound V—Compound V was prepared by reduction of 1,3-dinitro-5,6,7,8-tetrahydronaphthalene with sodium sulfide and sodium bicarbonate, mp 77° [lit. (4) mp 78°].

Compound VI—A mixture of 1 g (0.0052 mole) of V and 0.7 g (0.0052 mole) of β-diethylaminoethyl chloride was refluxed in 10 ml of ethanol for 10 hr. The reaction was then cooled and treated with 25% ammonium hydroxide solution, the mixture was concentrated and then extracted with ether, the residual oil remaining after distillation of the ether was dissolved in absolute ethanol, and a stream of dry hydrogen chloride was allowed to pass in the solution. A hygroscopic hydrochloride was formed. The product VI was identified as the picrate, which melted after recrystallization from dilute ethanol at 128°; IR: 3340 (NH) and 3010 (cyclohexyl) cm^{-1}.

Anal.—Calc. for $C_{16}H_{25}N_3O_2 \cdot C_6H_3N_3O_7$: C, 50.58; H, 5.74; N, 16.09. Found: C, 50.75; H, 5.78; N, 16.10.

Compound VII—A mixture of 2 g (0.00686 mole) of VI (dissolved in 30 ml of absolute ethanol) and 0.3 g of 5% palladium-on-charcoal was hydrogenated. The solvent was concentrated, the charcoal was filtered, and a dry stream of hydrogen chloride was allowed to pass through the filtrate. A hygroscopic hydrochloride formed.

Product VII was identified as the picrate, which melted after recrystallization from dilute ethanol at 124–125°; IR: 3340 (NH), 3017 (cyclohexyl), and 3300 (NH_2) cm^{-1}.

Anal.—Calc. for $C_{16}H_{27}N_3 \cdot 2(C_6H_3N_3O_7)$: C, 47.63; H, 4.76; N, 17.14. Found: C, 47.31; H, 4.57; N, 17.20.

Compound VIII—A mixture of 1 g (0.00383 mole) of VII and 0.758 g (0.00383 mole) of 4,7-dichloroquinoline was refluxed in 10 ml of ethanol for 10 hr. The reaction mixture was neutralized with 25% ammonium hydroxide solution, and the formed precipitate was filtered and recrystallized from ethanol to give 1.5 g (93% yield) of VII, mp 115–117°; IR: 720 (Ar—Cl), 1590 (C=N), and 250 (NH) cm^{-1}.

Anal.—Calc. for $C_{25}H_{31}ClN_4$: C, 71.10; H, 7.34; Cl, 8.20. Found: C, 71.41; H, 7.12; Cl, 7.89.

Compound IX—A mixture of 1 g (0.00258 mole) of V and 1.02 g (0.00258 mole) of 4,7-dichloroquinoline was refluxed in 10 ml of ethanol for 10 hr. The reaction mixture was cooled and treated with 25% potassium hydroxide solution, and the formed precipitate was filtered and recrystallized from ethanol to give 1.5 g (85% yield) of IX, mp 129–130°; IR: 720 (Ar—Cl) and 250 (NH) cm^{-1}.

Anal.—Calc. for $C_{19}H_{16}ClN_3O_2$: N, 11.89; Cl, 9.91. Found: N, 11.85; Cl, 10.18.

Compound X—A mixture of 5 g (0.013 mole) of IX (dissolved in 100 ml of absolute ethanol) and 0.5 g of 5% palladium-on-charcoal was hydrogenated. The catalyst was filtered, and the solvent was concentrated. The formed precipitate was recrystallized from ethanol to give 4 g (85% yield) of X, mp 182–184°; IR: 720 (Ar—Cl) and 3250 (NH) cm^{-1}.

Anal.—Calc. for $C_{19}H_{18}ClN_3$: C, 70.58; H, 5.57; Cl, 10.83. Found: C, 70.30; H, 5.61; Cl, 10.51.

Compound XI—A mixture of 1 g (0.00308 mole) of X, 0.4 g (0.00308 mole) of XLIV, and 0.4 g (0.00308 mole) of β-diethylaminoethyl chloride was refluxed for 7 hr. The formed precipitate was isolated and recrystallized from ethanol to give 1.1 g (77% yield) of XI, mp 300° dec.; IR: 720 (Ar—Cl), 1590 (C=N), and 3250 (NH) cm^{-1}.

Anal.—Calc. for $(C_{25}H_{31}ClN_4 \cdot HCl)_2H_2O$: C, 64.34; H, 7.06; Cl, 15.20. Found: C, 64.13; H, 6.84; Cl, 14.99.

Biological Testing—Compounds IV, VII, and XI were submitted to biological testing. The test was based on the evaluation of response of *P. berghei* in mice (5). The compounds were tested by oral and subcutaneous routes, and none of the compounds showed any activity at the tolerated levels.

[1] Melting points were taken in open capillary tubes with a Gallenkamp electric melting-point apparatus and are uncorrected. The IR spectra were recorded with a Carl Zeiss Infra-cord spectrophotometer, model UR 10. Microanalyses were performed by the microanalytical laboratory, National Research Center, Cairo, U.A.R., and the Spang Microanalytical Laboratory, Ann Arbor, Mich.

These findings suggest that the presence of the naphtholic OH group, as in I, is still necessary for biological activity within this group. Apparently, due to the possibility of chelation with the cations necessary for the metabolic functions of the parasite, the oxygen of this group and the nitrogen of the neighboring side chain may contribute in the cation complex formation.

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Synthesis and Biological Properties of Alkyl Esters of Polyene Antibiotics

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Abstract □ Several new alkyl esters of polyene antibiotics were prepared by an improved general procedure, and their toxicity and microbiological activity were tested. Some of these alkyl esters were more active than the nonesterified polyenes against *Candida albicans* but were less effective than the known methyl esters. Their toxicity was much less than that of the parent compounds.

Keyphrases □ Polyene antibiotics, alkyl esters—synthesis, antifungal activity □ Antifungal agents—synthesis and testing of alkyl esters of polyene antibiotics

The first attempt to reduce the toxicity of the macrolidic polyenes consisted of reacting the amino groups to give the corresponding *N*-acyl derivatives (1, 2), but these compounds were shown to be less active than the original antibiotics so chemotherapeutic employment was out of the question.

Since most natural polyenes have amphoteric characteristics, a new approach blocking the carboxyl group was developed and the first derivative, partricin methyl ester, was more active and less toxic than the parent antibiotic (3, 4). The methyl esters of other polyenes also gave good results (5, 6).

To continue this research, some homologous alkyl esters were prepared to determine whether other factors, such as the structure of the substituent group, could influence the biological activity of these polyene derivatives. The compounds were prepared by allowing the natural polyene to react with excess diazoalkane in the presence of ammonium hydroxide or other organic bases; surprisingly, the polyenes formed fewer by-products and thus biologically more active esters when in a basic medium.

The esterification of the carboxyl was confirmed by IR and NMR spectra, while TLC (Table I) on silica gel confirmed the quality of the products.

The polyene esters are pale-yellow to dark-yellow solids, which are practically insoluble in water, alkali, and the usual organic solvents and are very soluble in dimethyl sulfoxide, 2-methoxyethanol[1], and pyridine. The UV spectra show the typical absorbance patterns of the initial heptaenes and tetraenes (7).

All compounds were tested for their antifungal activity and acute toxicity in mice (LD_{50}); the results are reported in Table I in comparison with the parent substances and the known methyl esters. Since partricin is also active against protozoa, its esters were tested for their antitrichomonal activity in a preliminary manner.

EXPERIMENTAL

General Procedure of Synthesis—Concentrated ammonium hydroxide (0.5 ml) and then a 1.5% solution of diazoalkane in ether (10 ml) were added dropwise with stirring to a solution of polyene antibiotic (1 g) in dimethyl sulfoxide (20 ml). The mixture was kept at room temperature for 3 hr, and then ether (200 ml) was added to give a pasty solid. The precipitate was treated with acetone–ether, giving a high yield of the required ester.

TLC—TLC was carried out on silica gel[2] 60 F_{254}, using butanol–ethanol–acetone–32% ammonium hydroxide (2:5:1:3) as eluent and detecting the spots by exposure to UV light.

Antifungal Activity—The compounds were tested against *Candida albicans*, strain 200[3]. The test strain was cultured in Sabouraud medium[4] for 18 hr at 36° and diluted to 10% transmittance at 580 nm. Then about 0.15 ml of the diluted test strain was inoculated in 100 ml of Sabouraud medium so that the final test tubes contained 10^6 cells.

The substances were dissolved in dimethyl sulfoxide and serially diluted with sterile distilled water; 0.5 ml of each dilution was added to 4.5 ml of the inoculated broth, and all tubes were incubated for 24 hr at 36°. The results are reported as the minimum inhibitory concentration (MIC), *i.e.*, the lowest concentration of polyene antibiotic at which no visible growth was observed.

Acute Toxicity—The approximate LD_{50} was determined in groups of five female Swiss mice, 20–24 g. The compounds were

[1] Methyl cellosolve.
[2] Merck.
[3] Società Prodotti Antibiotici collection.
[4] Difco.