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Synthesis of New 2-Alkylamino-1,4-naphthoquinones as Inhibitors of Coenzyme Q and as Antimalarials†

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As based on the essentiality of coenzyme Q_8 in the metabolism of *Plasmodium*, new lipoidal 1,4-naphthoquinones have been synthesized as potential inhibitors of the biosynthesis and/or function of coenzyme Q_8 in the metabolism of *Plasmodium* and as potential antimalarials. Eight new 2-alkylamino-1,4-naphthoquinones have been synthesized and tested for antimalarial activity against *Plasmodium berghei* in the mouse. Three compounds (one new, and two previously prepared derivatives) showed evidence of activity by $T - C$ values ranging from 1.3 to 3.7 at 640 mg/kg dose level without toxicity by antimalarial assay against *P. berghei* in the mouse. The 2-alkylamino-1,4-naphthoquinones, represented in the assays by 7 of the 8 new compounds and 3 previously prepared derivatives, showed no significant inhibition of either the NADH- or succinoxidase enzyme systems. In contrast, our recently prepared 7-alkyl-6-hydroxy-5,8-quinolinequinones and the isomeric 6-alkyl-7-hydroxy-5,8-quinolinequinones were highly potent inhibitors.[1,2] The former derivatives were found to be powerful inhibitors of both *in vitro* mitochondrial enzyme systems, whereas the latter compounds were inhibitory only in succinoxidase.[1] Similarly a number of 6-alkylamino-5,8-quinolinequinones were inhibitory to both the NADH-oxidase and the succinoxidase systems, and inhibition of the NADH-oxidase system could be completely reversed by CoQ_{10}.[3]

The previous research which constitutes the background for this work has been described.[1-3] Recently, a series of new 7-alkyl-6-hydroxy-5,8-quinolinequinones and 6-alkyl-7-hydroxy-5,8-quinolinequinones have been synthesized and tested for antimalarial activity against *Plasmodium berghei* in the mouse.[2] Antimalarial activity accompanied by no observable toxicity at the highest levels tested was demonstrated for a number of these lipoidal 5,8-quinolinequinones. Many of these compounds were also evaluated in mitochondrial NADH- and succinoxidase systems for inhibition of coenzyme Q, and these *in vitro* data were compared with the data obtained from testing, *in vivo*, these compounds against *P. berghei* in the mouse.[1,2]

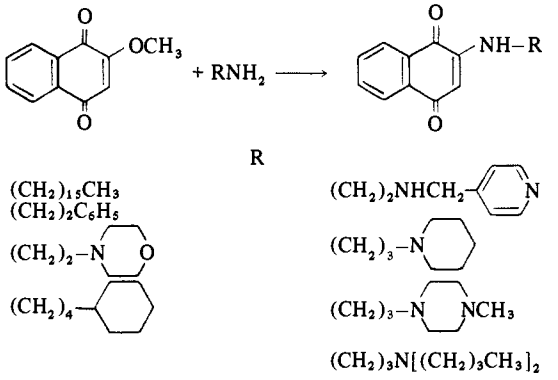
A series of 15 6-alkylamino-5,8-quinolinequinones (and one 7-alkylaminomethyl-6-hydroxy-5,8-quinolinequinone) have also been synthesized and tested for antimalarial activity against *P. berghei* in the mouse.[3] Ten of the total of 16 compounds showed definite antimalarial activity against *P. berghei* in the mouse. Three of the compounds met the arbitrary criterion of effectiveness to be declared "active," and none of these 3 showed toxicity at the highest level tested (640 mg/kg). Representative compounds were evaluated in mitochondrial NADH- and succinoxidase systems for inhibition of coenzyme Q. Seven 6-alkylamino-5,8-quinolinequinones were highly inhibitory to both NADH- and succinoxidase systems.

The syntheses and biological activities of 8 new 2-alkylamino-1,4-naphthoquinones are described herein. In view of the promising antimalarial activity of our newly synthesized 5,8-quinolinequinones, it was of current interest to prepare analogous naphthoquinone derivatives, *i.e.*, 2-alkylamino-1,4-naphthoquinones with alkyl side chains designed to increase the lipoidal character of the molecule and with alkyl side chains containing varying numbers of heteroatoms in an attempt to design molecules which could function as antimetabolites of the highly lipoidal coenzyme Q_8 of *Plasmodium* species.

†Coenzyme Q. 147.

Organic Syntheses. The synthesis of the 8 new 2-alkylamino-1,4-naphthoquinones was accomplished by treating 2-methoxy-1,4-naphthoquinone[4] in EtOH with the appropriate alkylamine as indicated in Scheme I. The MeO group

Scheme I

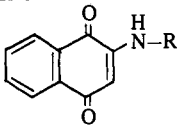


of 2-methoxy-1,4-naphthoquinone could be replaced by an amino group upon direct interaction with the appropriate alkylamine.[4,5] Generally, the amines reacted very readily with 2-methoxy-1,4-naphthoquinone, particularly the short chain aliphatic primary amines. Each of the 2-alkylamino-1,4-naphthoquinones was a red or orange crystalline substance with a sharp melting point (Table I).

Results of Antimalarial Assays. These compounds were tested for antimalarial activity against *P. berghei* in mice.[7] A single dose at the desired level was given sc 72 hr after the mice were infected with *P. berghei*. A minimum mean survival time of 13.0 days was required for the compound to be declared "active;" control mice exhibited a mean survival time of 6.2 days. Mice living 60 days or more after treatment were considered as cured.

One of the 8 new 1,4-naphthoquinones (**9**) showed definite activity ($T - C = 3.1$ at 640 mg/kg) in the *in vivo* antimalarial test against *P. berghei* in the mouse by procedures devised by Rane.[7] However, this compound was not declared

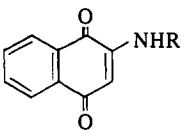
Table I. Chemical Data on Certain 2-Alkylamino-1,4-naphthoquinones and Results of Assays for Antimalarial Activity

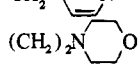
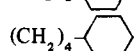
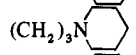
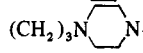


No.	R	Mp, °C	% yield[c]	*In vivo* antimalarial activity in mouse test (*P. berghei*)[a] $T - C$,[b] mg/kg	Toxicity, mg/kg
1[f]	$(CH_2)_{11}CH_3$	94-95	47[e]	3.7 at 640	
2[f]	$(CH_2)_{13}CH_3$	95-96	44[e]	0.5 at 640	
3	$(CH_2)_{15}CH_3$	94-96	38[e]	0.9 at 640	
4[f, j]	$(CH_2)_{17}CH_3$	96-98	38[e]	0.3 at 640	
5[i]	$(CH_2)_2C_6H_5$	139-140	68[d]	0.2 at 640	
6[g]	$CH_2C_5H_4N$	168-170	32[d]	1.3 at 640	
7[h]	$(CH_2)_2N$	145-146	69[d]	0.7 at 640	
8	$(CH_2)_4$	141-142	67[e]	0.1 at 640	
9	$(CH_2)_2NHCH_2C_5H_4N$	146-147	61[e]	3.1 at 640	
10	$(CH_2)_3N$	102-104	47[e]	0.7 at 160	5/5 deaths at 320
11[k]	$(CH_2)_3N$  $N-CH_3$	130-131	42[e]	0.5 at 160	5/5 deaths at 320
12[k]	$(CH_2)_3N[(CH_2)_3CH_3]_2$	76-77	47[e]	0.1 at 640	

[a] All compds were administered sc, in graded doses, to groups of 5 mice. [b] $T - C$ = change in survival time, in days, of treated and nontreated (control) mice. [c] Yields were based on starting quinone. [d] In this reaction, there was a molar excess of starting amine. [e] In this reaction, there was a molar excess of starting quinone. [f] Previously prepd.[6] [g] Previously prepd.[8] [h] Recrystd from EtOH-EtOAc-$CHCl_3$. [i] Recrystd from EtOH-$CHCl_3$. [j] Recrystd from Et_2O-EtOH-$CHCl_3$ and Et_2O-EtOH-hexane. [k] Recrystd from Et_2O.

Table II. *In Vitro* Assay of Certain 2-Alkylamino-1,4-naphthoquinones for Inhibition in the CoQ_{10}-Enzyme Systems DPNH-oxidase and Succinoxidase



R	DPNH-oxidase[c] Specific activity[a]	DPNH-oxidase[c] Inhibitor concentration[b]	Succinoxidase[c] Specific activity[a]	Succinoxidase[c] Inhibitor concentration[b]
$(CH_2)_{11}CH_3$	0.36		0.34	
	0.30	170	0.31	170
$(CH_2)_{15}CH_3$	0.37		0.37	
	0.22	190	0.34	190
$(CH_2)_{17}CH_3$	0.37		0.37	
	0.35	190	0.35	190
$(CH_2)_2C_6H_5$	0.32		0.34	
	0.40	170	0.30	170
	0.36		0.37	
	0.28	170	0.30	170
	0.29		0.34	
	0.37	170	0.31	170
	0.36		0.37	
	0.39	170	0.29	170
	0.23		0.20	
	0.24	150	0.20	190
	0.23		0.19	
	0.25	150	0.18	150
$(CH_2)_3N[(CH_2)_3CH_3]_2$	0.37		0.35	
	0.41	190	0.31	190

[a] Microatoms of O/min per mg of protein. [b] Nanamoles of inhibitor/mg of protein. [c] Reaction mixt contd no coenzyme Q.

"active" by the arbitrary criterion of 100% increase or greater in the survival time for antimalarial activity against *P. berghei*; no toxicity was evident at the highest dose level tested (640 mg/kg). Two derivatives, **10** and **11**, showed rather high toxicity at the 320 mg/kg dose level. Apparently, the length of the alkyl side chain and/or the placement of additional heteroatoms in the side chain contribute to the toxicity of these latter 2 compounds.

2-*n*-Dodecylamino-1,4-naphthoquinone[6] (**1**), and 2-(4-picolylamino)-1,4-naphthoquinone[8] (**6**), both previously prepared (see footnotes *f* and *g* in Table I), also showed marginal antimalarial activity against *P. berghei* in mice with $T - C$ = 3.7 at 640 mg/kg and $T - C$ = 1.3 at 640 mg/kg, respectively.

Inhibition of CoQ_{10}-Enzyme Systems. All of the 2-alkylamino-1,4-naphthoquinones in Table I, except **2** and **9**, were tested in these assays and showed no significant inhibition of either the NADH- or succinoxidase systems at levels tested (Table II). In contrast, certain 7-alkyl-6-hydroxy-5,8-quinolinequinones and the isomeric 6-alkyl-7-hydroxy-5,8-quinolinequinones were highly potent inhibitors. The former derivatives were found to be powerful inhibitors of both *in vitro* mitochondrial enzyme systems, whereas the latter compounds were inhibitory only in succinoxidase.[1] Similarly a number of 6-alkylamino-5,8-quinolinequinones were inhibitory to both the NADH-oxidase and the succinoxidase enzyme systems, and inhibition of the NADH-oxidase system could be completely reversed by CoQ_{10}.[3]

Experimental Section

General Procedures. All melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

Background for the methodology for the testing of these 2-alkylamino-1,4-naphthoquinones has been described.[9] The assays were conducted in the Warburg respirometer using beef heart mitochondria. Each flask contd 0.2 ml of KOH (20%) in the center well and 0.2 ml of enzyme in the side arm. The order of addn of reagents and quantities used were as follows: Tris-chloride (0.1 *M*; pH 7.5), 1 ml; sucrose (1 *M*), 0.5 ml; mitochondrial phospholipids (12.8 mg/ml), 0.05 ml; inhibitor, 0.05 ml (in abs EtOH); EDTA (0.8 μM/ml), 0.1 ml; cytochrome *c* (3 μg/ml H_2O), 0.05 ml; abs EtOH (total vol not to exceed 0.1 ml).

2-Alkylamino-1,4-naphthoquinones. Eight new 2-alkylamino-

1,4-naphthoquinones were prepd by treating 2-methoxy-1,4-naphthoquinone in EtOH with the appropriate alkylamine at room temp in a manner similar to that previously described[5] (Table I). After stirring at room temp, the reaction mixt was dild with Et_2O or hexane or both and placed in the freezer. Generally, a dark red or orange ppt was collected and recrystd repeatedly from a suitable solvent(s), usually EtOH (charcoal) or EtOH-Et_2O (charcoal).

Acknowledgments. We wish to thank Mrs. Alice Ma for excellent technical assistance with the *in vitro* studies. This work was supported by U. S. Army Medical Research and Development Command Contract No. DADA 17-69-C-9067. This is Contribution No. 992 from the Army Research Program on Malaria.

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Stereochemical Studies of Antimuscarinic Agents. Diastereoisomeric Esters of 3-Tropanol, 1,3-Dimethyl-4-piperidinol, and Related Compounds

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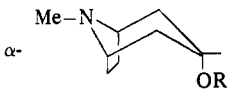
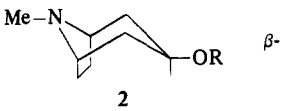
The anticholinergic properties of a series of isomeric 3-tropanol and 1,3-dimethyl-4-piperidinol esters (diphenylacetates and/or benzilates) and related compounds have been determined by measuring their ability to antagonize ACh-induced contractions of guinea pig ileum (pA_2 values) and cause mydriasis in mice eyes. In the pA_2 experiments, isomers with the smaller $^+N\cdots OCOR$ separation (acyloxy function axial in preferred piperidine chair conformation) were the more potent by factors of 2 to 3 in most cases, while differences in the mydriatic ED_{50} values of isomeric pairs were insignificant. A Me substituent α to the acyloxy group substantially reduced the cholinolytic potencies of 4-piperidyl esters but addition of a β group was advantageous in this respect. Potency differences between hydrohalide–methiodide pairs were small except in the case of 1-methyl-3-piperidyl benzilate where N-methylation reduced potency by over a half in the ileum test. The relevance of the structure–activity variations observed to the characteristics of receptors for cholinergic agonists and antagonists in the guinea pig ileum and circular muscles of the mouse eye are discussed.

This paper is concerned with the possible relationship between preferred geometry and antimuscarinic activity in atropine and related basic esters, attention being focussed upon the conformation of the amino alcohol moiety of cholinolytic esters. In atropine and other solanaceous alkaloids, the acyloxy function at C-3 is cis to the 2,6-bimethylene bridge of the tropane skeleton (**1b**). There have been

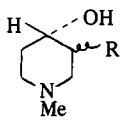




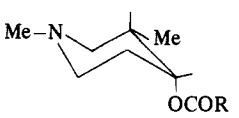
1 2

a, R = H
b, R = COR′
c, R = C(OH)Ph_2

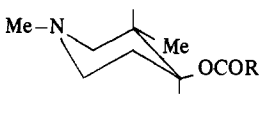
only a few studies of the pharmacology of analogs in which the acyloxy group is trans in the above sense, *e.g.*, reports on benzoate and tropate esters of pseudotropine **2a**,[1,2] and the chief object of the present work was to compare the anticholinergic activities of pairs of isomers which differed in the orientation of the OCOR function. One tropine–pseudotropine pair of esters has been examined but most esters tested are derivatives of 1-methyl-4-piperidinol. The piperidinol **3a** is a simplified version of the 3-tropanols in that it lacks the 2,6-bimethylene bridge, and it forms esters with diphenylacetic and benzilic acids which have high antimuscarinic activities: corresponding esters of 1-methyl-3-piperidinol **6** have similar pharmacological properties.[3-5]



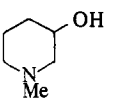
3a, R = H, b, R = Me



cis
4



trans
5



6

Introduction of a 3-Me substituent into **3** leads to cis and trans isomers which have the preferred conformation **4** and **5**, respectively.[6] The esters **4** are therefore steric analogs of tropyl esters (axial OCOR function) while those of **5** are related to pseudotropyl derivatives (equatorial OCOR).

Experimental Section

When analyses are indicated, analytical results obtd for those elements were within ±0.4% of the theoretical values. All benzilates were prepd by ester exchange between basic alcohols and methyl benzilate according to Cannon's procedure.[7] Salts were crystd from EtOH-Et_2O unless otherwise stated. Pmr spectra were recorded on a Varian A-60 spectrometer with $CDCl_3$ or DMSO-d_6 as solvents and TMS as standard.

Tropane Derivatives. Benzilate esters of tropine and pseudotropine·HCl were obtd by a reported method;[6] the former base gave a **methiodide**, mp 278° [*Anal.* ($C_{23}H_{28}INO_3$) C, H], and the latter a **methiodide**, mp 246–247° from acetone (*Anal.* C, H). Ester