Notes

Potential Naphthoquinone Antimalarials. 2-Acylhydrazino-1,4-naphthoquinones[1]

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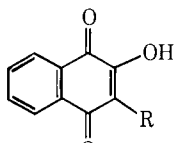
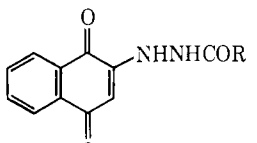
The 2-hydroxy-3-alkyl-1,4-naphthoquinones [I, R = alkyl or $(CH_2)_n$-cycloalkyl] have received considerable attention during the malaria chemotherapy programs since 1940, a remarkable feat being the synthesis and successful clinical trial of lapinone [I, R = $(CH_2)_8C(OH)(n\text{-}C_5H_9)_2$].[3] Success of lapinone stemmed largely from knowledge of metabolic disposition (fate of the side chains) and of the correlation of *in vitro* antirespiratory activity with the *critical extraction value*, pE,[4] which reflects the drug's "hydrophilic–lipophilic balance." The most active member in any active series of I generally possessed a pE value near or within the range 10–12.[3,4]



I



II

The 2-acylhydrazino-1,4-naphthoquinones [II, R = alkyl or $(CH_2)_n$cycloalkyl] are a class of compounds marked by an acidity constant (pK_a') of about 8.0–8.5.[5] It was of interest to probe whether some degree of antiplasmodial activity would be obtained from analogs of II, particularly in instances where the proper "hydrophilic–lipophilic balance," as defined by a pE value of 10–12, was incorporated into II. We report here the pE data and some biological data of two series of 2-acylhydrazino-1,4-naphthoquinones (II). Related data for certain analogs of I are included for comparison.[6]

Methods and Results

Except for IIg and IIh, the two series of 2-acylhydrazino-1,4-naphthoquinones (II) reported in Table I were synthesized by a general reaction involving condensation of a carboxylic acid hydrazide with 2-hydroxy-1,4-naphthoquinone in 80% AcOH.[5] Compounds IIg and IIh were obtained in adequate yield and purity when 80% trifluoroacetic acid was employed as solvent.

Adaptation of 2-*n*-hexanoylhydrazino-1,4-naphthoquinone (IId) to pE theory[4] was checked by calculation of pE from determinations conducted at several H^+ and quinone concentrations. The data here (Table II) supported the theory[4] with an accuracy of ± 0.05 pE unit.

Critical extraction values, pE, for each series of 2-acylhydrazino-1,4-naphthoquinones (II) increased linearly with molecular weight. Interestingly, a close similarity in pE values was observed for specific analogs of related series of I[4] and II; for example, IIm had pE 8.53 and the corresponding analog of I (R = CH_2-cyclohexyl)[4] had pE 8.43.

A sharp distinction in the physicochemical properties of specific analogs of I and II was apparent through comparison of the log K term in eq 2. The constant, K, describes the ratio of concentrations of *un-ionized* quinone distributed between the ether and aqueous phases.

The critical extraction value, pE, is defined as

$$pE = pH - 2 + \log \{[H^+](K/K_a)\} \quad (1)$$

where K connotes $[HA]^{ether}/[HA]^{water}$. It follows that,

$$(pE + 2) - pK_a = \log K \quad (2)$$

A rough value of log K can be estimated for each analog of series of I and II by assuming $pK_a^{H_2O}$ of 5.5 for I[7] and $pK_a^{H_2O}$ of 8.5 for II.[5] Comparison of calculated log K values for specific analogs of related series of I[4] and II indicated that, for comparable analogs, a distribution ratio of un-ionized I (*i.e.*, log K) exceeded that for a corresponding analog of II by an exponential factor of roughly 2.82.

Biological Data.—The 2-acylhydrazino-1,4-naphthoquinones, IIa–IIn, were evaluated as antimalarial agents against lethal *Plasmodium berghei* infections in mice [Walter Reed Army Institute of Research (WRAIR) screening program].[8] Compounds were administered subcutaneously as oil suspensions to lots of 5 mice at dosages of 40, 160, and 640 mg/kg. All analogs of II in Table I were nontoxic[9] and inactive. The mean survival time (ΔMST) of treated over control animals ranged from 0.1 to 1.3 days, but ΔMST of less than 2 days was considered insignificant.

Berberian and Slighter[10] have recently reevaluated the efficacy of series of hydroxynaphthoquinones (I) as antimalarial agents against blood-induced *P. berghei* infections in mice; the screening procedure differed, however, from the standard WRAIR assay. Regarding

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(3) (a) L. F. Fieser, J. P. Schirmer, S. Archer, R. R. Lorenz, and P. I. Pfaffenbach, *J. Med. Chem.*, **10**, 513 (1967) and references therein; (b) L. F. Fieser, "The Scientific Method," Reinhold Publishing Corp., New York, N. Y., 1964, pp 163–191.

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(6) We thank Professor Fieser for use of these data. The data were kindly searched and tabulated by Dr. R. E. Strube of WRAIR.

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TABLE I

2-ACYLHYDRAZINO-1,4-NAPHTHOQUINONES (II, R = *n*-ALKYL OR BRANCHED ALKYL SIDE CHAINS OR TERMINAL ALICYCLIC SIDE CHAINS)

Compd[a]	Side chain, R	Mp, °C[b]	Solvent for recryst[c]	pE	log K
Series 1					
IIa	$CH(CH_3)_2$	199–205	A	6.89	0.39
IIb	$(CH_2)_3CH_3$	168–171	A	7.45	0.95
IIc	$CH_2CH(CH_3)_2$	176–179	A	7.37	0.87
IId[d]	$(CH_2)_4CH_3$			7.94	1.44
IIe	$(CH_2)_6CH_3$	156–158	A	9.18	2.86
IIf	$(CH_2)_8CH_3$	140–143	A,B	10.43	3.93
IIg	$(CH_2)_{10}CH_3$	131–134	A		
IIh	$(CH_2)_{16}CH_3$	131.5–134.5	A		
Series 2					
IIi	Cyclopentyl	204–208	C	7.69	1.19
IIj[d]	CH_2-cyclopentyl			8.14	1.64
IIk[d]	Cyclohexyl			8.15	1.65
III	$(CH_2)_2$-cyclopentyl	174–179	A	8.63	2.13
IIm	CH_2-cyclohexyl	190–192	C	8.53	2.03
IIn	$(CH_2)_3$-cyclohexyl	160–164	C	9.58	3.08

[a] C, H, and N analyses agreed within 0.3% of theoretical values; except for C and N of IIh, and H and N of IIm, which were within 0.35–0.38% of theoretical values. [b] Melting points were determined on a Kofler hot stage microscope and are uncorrected. [c] A: Me_2CO; B: MeOH; C: EtOAc.

TABLE II

DETERMINATION OF pE FOR 2-HEXANOYLHYDRAZINO-1,4-NAPHTHOQUINONE (IId)

	pE found at an initial concentration in ether of (mg/250 ml)		
Buffer pH	10	30	50
8.93			7.99
9.55	7.89	7.91	7.95
10.42		7.98	
		pE_{av} = 7.94	

the screening of I in the standard WRAIR test system, Dr. R. E. Strube[6] has informed us that in a series of I [where R = $(CH_2)_nCH(CH_3)_2$, and n = 0, 3, 4, and 6] related to series 1 of II a definite activity was observed (dosage, 160–640 mg/kg; ΔMST, 4–9 days) for homolog I, n = 6 [pE = 10.20 (estimated[4]); log K = 6.70]; and, in a series of I [where R = $(CH_2)_n$-cyclohexyl, and n = 0 or 3] related to series 2 of II a definite activity (dosage, 160–640 mg/kg; ΔMST 6 days-cure) was noted for homolog I, n = 3 (pE = 9.43; log K = 5.93).

Although some of the analogs of II (series 1 and 2) exhibited a pE value near or within the range 10–12, it is noteworthy that no analog of II contained a thermodynamic condition K comparable to that found for an active, related member of I. The largest log K values measured of II in series 1 and 2 were 3.93 and 3.08 for IIf and IIn, respectively; each of these values indicated a distribution ratio amounting to roughly 0.001–0.002 of that determined for the related member of I showing definite activity in the WRAIR test procedure [*cf.* log K 6.70 and 5.93 for $(CH_2)_6CH(CH_3)_2$ and $(CH_2)_3$-cyclohexyl of I, respectively]. Higher homologs of II, *e.g.*, IIg and IIh, were prepared with the intention of incorporating a more comparable log K term, but no pE data were obtainable owing to the extreme insolubilities (ether or aq phase) of their sodium salts. In view of this limiting factor in series 1 of II, no attempt was made to prepare a homolog in series 2 of II which might afford a log K term more comparable to the $(CH_2)_3$-cyclohexyl analog of I.

Experimental Section

Carboxhydrazides.—New hydrazides are reported in Table III. Requisite carboxylic acids or esters were obtained from commercial sources.[11] Carboxylic acids were converted into Me esters by treatment with CH_2N_2,[12] the crude ester was treated with hydrazine in the usual manner,[13] and the hydrazide was purified by the means described in Table III.

TABLE III

CARBOXHYDRAZIDES

Compound[a]	Mp, °C[b]	Solvent for recrystn[c]	Analytical data[d]
$C_5H_9CONHNH_2$	118–121	A	N
$C_5H_9(CH_2)_2CONHNH_2 \cdot HCl$	96–99	B	N, Cl
$C_6H_{11}CH_2CONHNH_2$	121–122.5	A	C, H, N
$C_6H_{11}(CH_2)_3CONHNH_2$	89–90.5	A	

[a] C_5H_9 = cyclopentyl; C_6H_{11} = cyclohexyl. [b] Kofler hot stage, uncorrected. [c] A: sublimation; B: *i*-PrOH–Et_2O. [d] Unsatisfactory analyses (C, H, N) were obtained for the last compound. The hydrazide was used successfully for preparation of IIn, for which satisfactory analytical data were obtained.

Cyclopentylpropionic acid hydrazide was isolated as its HCl salt. The crude hydrazide (prepared from crude Me ester which was obtained by methylation of 25.0 g of cyclopentylpropionic acid) was dissolved in *i*-PrOH (100 ml) and dry HCl was bubbled through the solution for 15–20 min. After cooling to 25°, the mixture was diluted with Et_2O (50 ml), and the cake of cyclopentylpropionic acid hydrazide·HCl (20.0 g), mp 96–99°, was collected and washed thoroughly (Et_2O).

2-Acylhydrazino-1,4-naphthoquinones (II).—Except for IIg, IIh, and III, those analogs of II reported in Table I were prepared as previously described (procedure B).[5]

Owing to the limited solubility of $C_{11}H_{23}CONHNH_2$ and $C_{17}H_{35}CONHNH_2$ in 80% HOAc, compounds IIg and IIh were prepared by allowing 0.7 mmol of hydrazide and 0.7 mmol of 2-hydroxy-1,4-naphthoquinone per 5 ml of 80% CF_3COOH to react at 25°.

(11) Cyclopentylpropionic acid was obtained as a gift from Arapahoe Chemicals Co., Boulder, Colo. Cyclohexanebutyric acid was supplied as a gift from Columbia Organic Chemical Co., Columbia, S. C.

(12) F. Arndt, "Organic Syntheses," Collected Vol. 2, John Wiley & Sons, New York, N. Y., 1943, p 165.

(13) W. J. Hickenbottom, "Reactions of Organic Compounds," Longmans, Green, and Co., Inc., New York, N. Y., 1957, p 300.

In the case where a hydrazide·HCl was employed (*i.e.*, III), an equiv quantity of Et_3N was added to the condensing medium. Thus, a suspension of cyclopentylpropionic acid hydrazide·HCl (6.3 g, 33 mmol) and 2-hydroxy-1,4-napthoquinone (5.0 g, 29 mmol) in 80% HOAc (200 ml) containing Et_3N (3.3 g, 33 mmol) was stirred at 25° for 25 hr. The solvent was stripped *in vacuo* and the bright orange residue was freed from excess hydrazide and Et_3N·HCl using a wash solution of EtOH–H_2O–concd HCl (10:10:1). Additional successive washings with EtOH–H_2O (1:1), Me_2CO, and Et_2O gave a crude product (4.6 g) which afforded after two recrystallizations 2.73 g of III, mp 174–179°.

Critical Extraction Values (p*E*).—Extraction constants, p*E*, were determined using the standard procedures described as A and B by Fieser, *et al.*[4] Procedure B[4] was required only for IIf. Initial concentrations of naphthoquinone in Et_2O were in the order of 30–50 mg ± 0.01 mg/250 ml; which is approximately 0.4 that generally employed for the 2-hydroxy-3-alkyl-1,4-naphthoquinones.[4] This deviation from the standard procedure was necessary because of the lesser solubility of the lower molecular weight analogs of II. The concentration of naphthoquinone in the alkaline buffer was determined by measuring the absorbance at 535 mμ with a Model 2400 Beckman DU Spectrophotometer.

Antimalarials. II. Quinolinemethanols with Decreased Phototoxicity[1]

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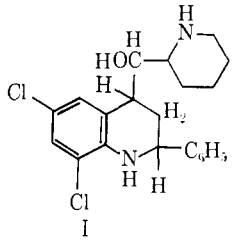
We have reported[2] that no significant decrease in phototoxicity was observed when the structures of certain 2-phenyl-4-quinolinemethanol antimalarials were modified by replacing chlorine by fluorine, or by changing the nature of the N-containing side chain.

We now report the results of a study of four additional structural modifications, three of which might be expected, on theoretical grounds, to cause a significant decrease in phototoxicity. The fourth modification involved the synthesis of a 2-phenyl-5-quinolinemethanol drug, a structure not reported previously.

Chemistry.—The synthesis route to compounds containing iodine or free phenolic groups (Table I) was the same as that used previously.[2] Six novel oxides were prepared from the appropriate cinchoninic acids and the compounds in Table I were prepared by the reaction of these oxides with the appropriate amine. During the preparation of oxides having free phenolic groups it was necessary to protect the latter by acetylation at the cinchoninic acid stage; the protecting group was removed during the reduction of the bromomethyl ketone intermediate by $NaBH_4$.

6,8-Dichloro-2-phenyl-α-(2-piperidyl)-1,2,3,4-tetrahydro-4-quinolinemethanol (I) was prepared by catalytic reduction of the well-known antimalarial 6,8-dichloro-2-phenyl-α-(2-piperidyl)-4-quinolinemethanol.[3]

The 5-quinolinemethanol III was prepared by way of II so that the antimalarial activity and phototoxicity of II could be measured also. II was prepared by our standard route from 6,8-dichloroquinoline-5-carboxylic acid, which in turn was prepared from 5-amino-2,4-dichlorobenzoic acid by a Skraup reaction.



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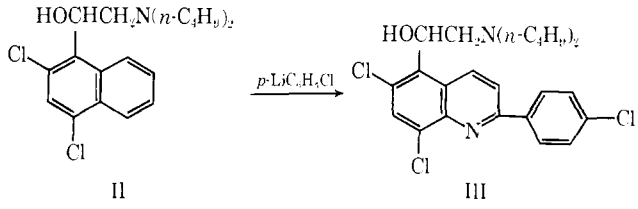

II III

Pharmacology.—Several major reviews of drug-induced phototoxicity[4–6] are available. It is apparent that phototoxic compounds fall into two classes, depending on whether the changes they evoke in tissues are oxygen-dependent or oxygen-independent. The best known oxygen-independent drugs are the furocoumarins (psoralens).[7] Recent work[8] shows that the phototoxic quinolinemethanol drugs are definitely oxygen-dependent. The photosensitizing furocoumarins are known to react with DNA in a photochemical reaction, which has been studied in detail;[9] no mechanism has yet been proposed for the photosensitizing action of the quinolinemethanols.

The possible role of long-lived triplet states in the phototoxic action of drugs[4] was the basis of our study of the iodine compounds **1–6**. It is known that molecules that contain atoms of large atomic number have a significantly decreased triplet state lifetime.[10] Examples of the effect of iodine as a "heavy atom" are available in which iodine functions in an intramolecular[11] and intermolecular[12] way.

The antimalarial activity of the iodine compounds (Table II) was equal or superior to that of analogous Cl and F compounds studied earlier. In the standard test of phototoxicity, in which the drug was administered ip, there was a significant decrease in phototoxicity (Table III) when iodine was at the 3′ or 4′ positions, but little or no decrease when iodine was at the 7 position. However, when any of these compounds was administered orally, the minimum effective phototoxic dose was 50 mg/kg or less. The high phototoxicity of the 4′,6-diiodo compound **6** seems anomalous.

The phenolic compounds **7–9** were prepared at a time when the differences in mechanism of phototoxic

(1) This work was performed under Contract DA-49-193-MD-2901 with the U.S. Army Medical Research and Development Command, Office of the Surgeon General. Contribution No. 735 of the Army Research Program on Malaria.

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