and a mixture of 56% methanol and 44% water containing 1 g of heptane sulfonate and 2 mL of acetic acid.

Identification of the 9-OH-E Oxidized Product. The purified oxidized product is a deep red powder: mp 345 °C; mass spectrum, m/e 260 (M^+). The UV spectrum displays a maximal absorption at 299 nm and in the visible region at 505 nm. The NMR spectrum is in agreement with the quinone imine structure: ^1H NMR (Me_4Si in $CDCl_3$) δ 9.393 (H_1, s), 8.679 and 8.617 (H_3, d), 7.785 and 7.723 (H_4, d, J_{3-4} = 5.58 Hz), 7.508 and 7.397 (H_7, d), 6.576 and 6.465 (H_8, q), 6.807 (H_{10}, s, J_{7-8} = 9.99 Hz and J_{8-10} = 1.98 Hz).

Electron Paramagnetic Resonance Studies of Free Radicals. (a) Formation of 9-OH-E Radical in Iodine Solution. 9-OH-E solution was prepared by dissolution of the solid compound in dry Me_2SO to a concentration of 10^{-3} M. The iodine solution was prepared by dissolving solid iodine in dry Me_2SO to a concentration of 10^{-2} M. Equal volumes of 9-OH-E and iodine were mixed. The solution was flushed with argon for 15 min, frozen in liquid nitrogen, and examined in an EPR spectrometer at 110 K.

(b) Formation of Free Radicals during MPO-H_2O_2 Mediated Oxidation of Ellipticine Derivatives. Experiments were performed in 0.05 M phosphate buffer containing 10^{-4} M H_2O_2 and 25×10^{-6} M of various ellipticines. The reaction was started by the addition of 2×10^{-6} M MPO. The mixture was frozen for 10 s after the addition of the enzyme and the EPR spectrum was recorded at 110 K. In these experiments, all components of the mixtures were prepared in argon, saturated solution. All signals detected under these conditions exhibited a g factor of 2.0042 ± 0.0002, determined according the following equation: $g = h\nu/\beta Hr$, where β = Bohr magneton, Hr = applied magnetic field at resonance, ν = frequency of the radiation field oscillation, h = Planck's constant.

(c) Formation of Steady State N^2-Me-9-OH-E Radical during the Degradation of the Quinone. Experiments were performed in 0.05 M phosphate Me-buffer (pH 7.40 or 5.50) containing 10^{-4} M H_2O_2 and 2.5×10^{-6} M N^2-Me-9-OH-E. Just after the addition of 2×10^{-6} M MPO, the mixtures were transferred to the EPR cell and the spectra were recorded at room temperature. The signal observed under these conditions exhibited a g factor of 2.0046 ± 0.0002, and the extent of the signal decreased as a function of time. In order to study the kinetics of the radical decay, a series of experiments were performed in which the mixtures were frozen in liquid nitrogen for 1, 3, 5, 7, and 9 min after MPO was added to them. Spectra were recorded at 110 K, and the radical concentrations were determined by comparing them to a standard solution of spin-label material [3-(2-iodoacetamido)-2,2,5,5-tetramethyl-1-pyrolidinyloxy].

Preparation of N^2-Me-9-oxo-E Irreversibly Bound to Bovine Serum Albumin (BSA). Standard Procedure. In phosphate buffer, 0.05 M (various pH), was dissolved 10^{-4} M H_2O_2 and 10^{-4} M N^2-Me-9-OH-E containing 0.5 mCi of N^2-Me-9-OH-[10-*Me*-^{3}H]E per mmol. MPO, 10^{-7} M, was added; 30 s after the addition of MPO, 15×10^{-6} M BSA was added and the mixture was allowed to stand at 37 °C for 30 min. After the mixture cooled, the BSA was precipitated by 0.6 N perchloric acid (3:1, v/v). The tubes were centrifuged at 5000g for 20 min. The supernatant was discarded, and the precipitate was washed with 0.6 N perchloric acid. This step was repeated twice. The final precipitate was dissolved in water and dialyzed overnight against distilled water. Gel filtration on Sephadex G-75 in 0.01 M phosphate buffer (pH 7.40) showed that the radioactivity remaining after these procedures was confined on the albumin peak. Moreover, incubation of BSA containing bound N^2-Me-9-oxo-E with cold excess 9-oxo-E did not remove any radioactivity from the protein. In these experiments, radioactivity was determined by liquid scintillation counting (ABAC SL40) in a vial containing 0.5 mL of BSA and 4.5 mL of scintillation counting mixture (Packard instrument).

Cytotoxic and Antitumor Tests. Inhibition of cell growth was determined with L1210 lymphocytic leukemia cells as previously described.[19] The inhibitory efficiency against cell multiplication is expressed in terms of ID_{50}, which represents the drug concentration which reduces the rate of cell multiplication by 50% as compared to the control. The highest nonlethal dose (LD_0) was determined for each drug after a single injection into DBA/2 or Swiss mice by an intraperitoneal route. The antitumoral tests were performed on DBA/2 mice that had been inoculated with 10^5 L1210 cells and treated 24 h latter by the same route. Antitumor efficiency is expressed in termof ILS (increase in life span) over controls, $[(T - C)/C] \times 100$. The values presented in the present work have already been published.[17]

Acknowledgment. This work was supported by Research Grant ATP CNRS No. 9764. We are grateful to Dr. Gianotti (ICSN, Gif-sur-Yvette, France) for the kind guidance on EPR studies.

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In Vitro Activity of 2-Alkyl-3-hydroxy-1,4-naphthoquinones against *Theileria parva*[1]

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Received September 2, 1980

A variety of naphthoquinones have been prepared and evaluated in vitro against the causative agent of the cattle disease East Coast Fever—*Theileria parva* infection. It is concluded from structure–activity studies that a 2-hydroxyl moiety is essential for high activity. The most active compounds tested were 2-hydroxy-3-alkyl-1,4-naphthoquinones in which the alkyl moiety was cyclohexyl, cyclohexylcyclohexyl, tridecyl, or tetradecyl.

The cattle disease East Coast Fever (ECF) is endemic in Eastern and Central Africa and causes serious losses.[2] Approximately half a million cattle each year die from the disease in East Africa alone.[3] Until recently no treatment existed for ECF, which is caused by the tick-borne protozoan *Theileria parva*. However, a major development

(1) Presented in part at a Workshop on the in vitro cultivation of the Pathogens of Tropical Diseases, Nairobi, Kenya, 1979. Minutes of this meeting are reported in "The in Vitro Cultivation of the Pathogens of Topical Diseases", Schwabe and Co., A. G. Basel, 1980, p 148.

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0022-2623/81/1824-0295$01.00/0

in the search for an effective chemotherapeutic agent occurred when menoctone [2-hydroxy-3-(8-cyclohexyloctyl)-1,4-naphthoquinone, 1; Table II], developed originally as an antimalarial agent by Sterling-Winthrop, was shown to have both in vitro and in vivo activity against *T. parva*.[4] Recently, the coccidiostat halofuginone (*dl-trans*-7-bromo-6-chloro-3-[3-hydroxy-2-piperidyl)-acetonyl]-4(3*H*)-quinazolinone) has been claimed to cure *T. parva* and *T. annulata* infected cattle.[5]

Despite the promising efficacy of menoctone against *T. parva*, the drug is tedious to synthesize[6] and would be uneconomical to market as a treatment for theileriosis. Consequently, in a search for a suitable replacement, a variety of naphthoquinones have been examined against in vitro cultures of *T. parva*. The results and conclusions of this investigation are presented.

Chemistry. Synthesis of the novel chloroquinones **2**, **3**, **5**, **7–13**, **15–19** and the acetoxyquinone **38** was achieved by reaction of the appropriate carboxylic acid with 2-chloro and 2-acetoxy-1,4-naphthoquinone, respectively, in the presence of ammonium persulfate/silver nitrate according to the procedure of Jacobsen and Torsell[7] (method A). The previously reported 2-chloro-3-(3-cyclopropyl)-1,4-naphthoquinone (**6**)[8] and 2-chloro-3-dodecyl-1,4-naphthoquinone (**14**)[22] were also prepared in this manner. The chloro compounds were then hydrolyzed (method B) to the hydroxy analogues **20–37**, some of which (**22–25** and **30–37**) have previously been reported[8,9] prepared by other routes.

The hydroxynaphthoquinones **39**, **40**, and **58** were obtained using the method employed by Huyser and Amini[10] (method C) to prepare 2-cyclohexyl-3-hydroxy-1,4-naphthoquinone (**43**)—reaction of a 2-hydroxy-1,4-naphthoquinone with a cycloalkane in the presence of *tert*-butyl peroxide. Application of this reaction to cyclohexene gave a product assumed to have structure **42**, since radical formation at the carbon adjacent to the olefinic bond would be highly favored. Attempts to apply this method to the preparation of **44** from dihydropyran failed, a complex mixture resulting. The required compound, albeit in very low yield, was obtained by reaction of tris(dihydropyranyl)borane with 2-hydroxy-1,4-naphthoquinone according to the procedure of Kabalka[11] (method D). The major product isolated from this reaction was tentatively identified as 4-(3-tetrahydropyranyl)-1,2-naphthoquinone (**45**) on the basis of its ^{1}H NMR spectrum. This showed aromatic proton signals at δ 8.2 (1 H) and 7.8 (3 H) and an olefinic proton signal at δ 6.4 (1 H). The chemical shift of the latter correlates well with that reported for the 3-H in 4-methyl-1,2-naphthoquinone.[12] Method D was also employed to prepare **41** and **46**.

Synthesis of the cyclohexylthioquinone **47** was achieved via reaction of 2-chloro-3-methoxy-1,4-naphthoquinone[13] with cyclohexanethiol, with cleavage of the methyl ether to the desired hydroxy analogue occurring in situ. **48** was prepared by reaction of 2-hydroxy-1,4-naphthoquinone with formaldehyde and cyclohexanethiol according to the procedure of Moser and Paulshock.[14] The aminoquinone **50** was obtained by reduction of the amide **49**, which was prepared from 2-hydroxy-3-amino-1,4-naphthoquinone[15] according to the procedure of Podrebarac and Cheng.[16] 2-Amino-3-(cyclohexylmethyl)-1,4-naphthoquinone (**53**) was obtained from 2-chloro-3-(cyclohexylmethyl)-1,4-naphthoquinone (**5**) via the azide **52**. 2-Cycloheptyl-3-hydroxy-5,6,7,8-tetrahydro-1,4-naphthoquinone (**59**) was synthesized from the corresponding naphthoquinone **39** by application of the method of Cunningham et al.[17]

The melting points and yields of novel compounds prepared by the above routes are shown in Table I. Representative examples of these routes and preparative details of compounds **47**, **52**, **53**, and **59** are given under Experimental Section.

Biological Results and Discussion

The in vitro *T. parva* test system was employed as described previously.[24] Fourfold dilutions of compounds were assayed to determine ED_{50} values, at least two tests being conducted on each compound. The ED_{50} is the concentration of drug required (mg/L) to reduce the proportion of schizont-infected cells in the culture to 50% of that of untreated controls in the 48-h incubation period.

Initially, the effect on antitheilerial activity of replacing the hydroxyl moiety was examined. The results in Table II clearly show the hydroxyl group to be essential for high activity, quinones **5** and **53** being devoid of activity. This result parallels those obtained by Fieser[18] in his study of the antimalarial activity of 1,4-naphthoquinones. The activity of **38** is most likely due to in situ generation of small quantities of **23** in the test system.

The effect of varying the chain connecting the cycloalkyl moiety to the 2-hydroxy-1,4-naphthoquinone nucleus was studied next. The ED_{50} values of compounds containing one to eight methylene units in the connecting arm (**23–25**, **54–56** and **1**) are very similar. Introduction of a nitrogen or sulfur atom into the chain, however, results in activity being drastically reduced (see **47–50** and **57**). The most active compound in this group, 2-cyclohexyl-3-hydroxy-1,4-naphthoquinone (**43**), is approximately 10 times more active than the original compound studied, menoctone (**1**).

The activities of close analogues of **43** (**20–22**, **39–42**, **44**, **46**, and **58–61**) were examined. All of these compounds contain a cycloalkyl moiety directly attached to the quinone nucleus. The most active of these compounds remains the cyclohexylquinone **43**, although the cyclohexylcyclohexyl analogue **61** is equipotent. The cyclooctyl

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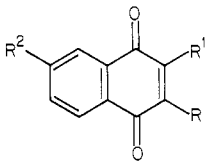
Table I. 2-Alkyl-3-substituted-1,4-naphthoquinones



no.	R	R^1	R^2	mp, °C	preparative method	% yield[a]	recrystn solvent[b]	formula
2	Cl	cyclopropyl	H	93-94	A	19	ethanol	$C_{13}H_{19}ClO_2$
3	Cl	cyclobutyl	H	70-71	A	26	ethanol	$C_{14}H_{11}ClO_2$
5	Cl	cyclohexylmethyl	H	147-149	A	56	petrol	$C_{17}H_{17}ClO_2$
7	Cl	7-cyclohexylheptyl	H	83-84	A	33	ethanol	$C_{23}H_{29}ClO_2$
8	Cl	5-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)pentyl	H	136-138	A[c]	21	toluene	$C_{25}H_{18}Cl_2O_4$
9	Cl	6-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)hexyl	H	221-222	A[c]	40	chloroform	$C_{26}H_{20}Cl_2O_4$
10	Cl	8-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)octyl	H	194-195	A[c]	29	toluene	$C_{28}H_{24}Cl_2O_4$
11	Cl	10-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)decyl	H	170-172	A[c]	41	toluene	$C_{30}H_{28}Cl_2O_4$
12	Cl	n-C_5H_{11}	H	66-68	A	41	ethanol	$C_{15}H_{15}ClO_2$
13	Cl	n-C_9H_{19}	H	79-80	A	47	ethanol	$C_{19}H_{23}ClO_2$
15	Cl	n-$C_{13}H_{27}$	H	87-88	A	22	*d*, then toluene/petrol, 1:1	$C_{23}H_{31}ClO_2$
16	Cl	n-$C_{14}H_{29}$	H	91-92	A	25	*d*, then ethanol	$C_{24}H_{33}ClO_2$
17	Cl	n-$C_{15}H_{31}$	H	90-91	A	22	*d*	$C_{25}H_{35}ClO_2$
18	Cl	n-$C_{16}H_{33}$	H	94-96	A	10	*d*	$C_{26}H_{37}ClO_2$
19	Cl	n-$C_{17}H_{35}$	H	80-82	A	22	*d*, then toluene	$C_{27}H_{39}ClO_2$
20	OH	cyclopropyl	H	118-119	B	79	toluene/petrol, 1:3	$C_{13}H_{10}O_3$
21	OH	cyclobutyl	H	134-136	B	62	petrol	$C_{14}H_{12}O_3$
26	OH	5-(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)pentyl	H	189-190	B	71	toluene	$C_{25}H_{20}O_6$
27	OH	6-(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)hexyl	H	215-216	B	60	toluene	$C_{26}H_{22}O_6$
28	OH	8-(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)octyl	H	149-151	B	76	ethanol/pyridine (10:1)	$C_{28}H_{26}O_6$
29	OH	10-(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)decyl	H	101-103	B	77	toluene	$C_{30}H_{30}O_6$
38	$OCOCH_3$	cyclohexylmethyl	H	95-96	A	17	petrol	$C_{19}H_{20}O_4$
39	OH	cycloheptyl	H	96-97	C	58	petrol	$C_{17}H_{18}O_3$
40	OH	cyclooctyl	H	75-76	C	14	petrol	$C_{18}H_{20}O_3$
41	OH	cyclododecyl	H	156-157	D	37	petrol	$C_{22}H_{28}O_3$
42	OH	2-cyclohexenyl	H	101-102	C	45	petrol	$C_{16}H_{14}O_3$
44	OH	3-tetrahydropyranyl	H	164-166	D	5	toluene	$C_{15}H_{14}O_4$
46	OH	2-norbornyl	H	131-132	D	22	petrol	$C_{17}H_{16}O_3$
48	OH	(cyclohexylthio)methyl	H	117-118	ref 14	55	toluene/petrol (1:1)	$C_{17}H_{18}O_3S$
49	OH	cyclohexanecarboxamido	H	154-155	ref 16	56	acetone/water (2:1)	$C_{17}H_{17}NO_4$
50	OH	(cyclohexylmethyl)amino	H	84-85	ref 16	58	ethanol	$C_{17}H_{19}NO_3$
58	OH	cycloheptyl	OMe	135-136	C	22	ethanol	$C_{18}H_{20}O_4$

[a] Yields are based upon isolated pure materials and are not optimized. [b] Petrol refers to petroleum ether of boiling point 60-80 °C. [c] Two molar equivalents of quinone reacted with 1 molar equiv of dicarboxylic acid. [d] Purified by column chromatography on silica gel and then neutral alumina.

derivative **40** is also highly active in contrast to the cyclopentyl analogue **22**. Introduction of heteroatoms (see **44**) or double bonds (**42** and **60**) into the cyclohexyl moiety leads to reduced antitheilerial activity. Similarly, substitution (see **58**) or reduction (**59**, ED_{50} = 10 mg/L) of the aromatic ring of the quinone nucleus has a detrimental effect.

Evaluation of 2-*n*-alkyl-3-hydroxy-1,4-naphthoquinones was also carried out—see compounds **30–37**. Activity peaks for the C_{13} (**33**) and C_{14} (**34**) members of the series and thereafter remains at a fairly constant level for higher homologues. A number of bis(quinonealkanes) (**26–29**) were also examined in the hope that against the parasite in vivo they would be metabolized more slowly than the alkyl and cycloalkyl analogues which in various species have been reported to be rapidly degraded.[19] The in vitro activity of these compounds is, however, disappointing and insufficient to warrant in vivo studies.

Conclusion

From the results discussed above it can be seen that the most active compounds tested against *T. parva* in vitro are the cycloalkylhydroxyquinones **43** and **61**, and the *n*-alkylhydroxyquinones **33** and **34**. The antitheilerial effects of **43** and **33** have been confirmed in vivo,[1,23] and the former compound, 2-cyclohexyl-3-hydroxy-1,4-naphthoquinone, has been chosen for further evaluation in field trials.

Experimental Section

Melting points, uncorrected, were recorded using an Electrothermal capillary tube melting point apparatus. Microanalyses were carried out on all compounds, and the results obtained were within 0.4% of the theoretical values. Column chromatography was performed on silica gel M.F.C. (Hopkins and Williams) or neutral alumina "Camag" M.F.C. (Hopkins and Williams). IR

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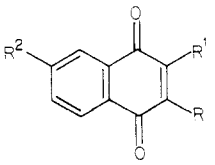
Table II. Activity of 2-Alkyl-3-substituted-1,4-naphthoquinones against *T. parva* in Vitro



no.	R	R^1	R^2	ED_{50}, mg/L
1[a]	OH	8-cyclohexyloctyl	H	0.06
5	Cl	cyclohexylmethyl	H	>10
20	OH	cyclopropyl	H	10.0
21	OH	cyclobutyl	H	10.0
22	OH	cyclopentyl	H	3.7
23	OH	cyclohexylmethyl	H	0.03
24	OH	3-cyclohexylpropyl	H	0.06
25	OH	7-cyclohexylheptyl	H	0.05
26	OH	5-(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)pentyl	H	>10
27	OH	6-(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)hexyl	H	>10
28	OH	8-(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)octyl	H	1.0
29	OH	10-(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)decyl	H	10.0
30	OH	n-C_5H_{11}	H	1.0
31	OH	n-C_9H_{19}	H	0.13
32	OH	n-$C_{12}H_{25}$	H	0.05
33	OH	n-$C_{13}H_{27}$	H	0.006
34	OH	n-$C_{14}H_{29}$	H	0.006
35	OH	n-$C_{15}H_{31}$	H	0.01
36	OH	n-$C_{16}H_{33}$	H	0.01
37	OH	n-$C_{17}H_{35}$	H	0.01
38	OCOMe	cyclohexylmethyl	H	1.0
39	OH	cycloheptyl	H	0.01
40	OH	cyclooctyl	H	0.007
41	OH	cyclododecyl	H	0.09
42	OH	2-cyclohexenyl	H	1.5
43	OH	cyclohexyl	H	0.006
44	OH	3-tetrahydropyranyl	H	6.8
46	OH	2-norbornyl	H	0.06
47	OH	cyclohexylthio	H	6.5
48	OH	(cyclohexylthio)methyl	H	>10
49	OH	cyclohexanecarboxamido	H	>10
50	OH	(cyclohexylmethyl)amino	H	>10
53	NH_2	cyclohexylmethyl	H	>10
54	OH	2-cyclohexylethyl	H	0.08
55	OH	4-cyclohexylbutyl	H	0.06
56	OH	5-cyclohexylpentyl	H	0.03
57	OH	(cyclohexylamino)methyl	H	>10
58	OH	cycloheptyl	OMe	>10
60	OH	phenyl	H	>10
61	OH	4-cyclohexylcyclohexyl (cis)	H	0.006

[a] Menoctone.

(Perkin-Elmer 157G spectrometer) and NMR (Bruker HFX-90 and Varian HA-100 and T-60 spectrometers) spectra were determined for all compounds and were consistent with the given structures.

Examples of the various routes used to prepare compounds shown in Table I are given below. Compounds 43,[10] 57[20] and 60[21] were prepared as described in the literature. 55, 56, and 61 were purchased from Aldrich Chemical Co. 1 was obtained from Sterling Drug Co.

Method A. 2-Chloro-3-cyclopropyl-1,4-naphthoquinone (2). Cyclopropanecarboxylic acid (8.6 g, 0.1 mol), 2-chloro-1,4-naphthoquinone (19.2 g, 0.1 mol), and silver nitrate (5.0 g) in acetonitrile (170 mL) were stirred vigorously and heated to reflux, and ammonium persulfate (60.0 g) in water (250 mL) was added dropwise over 1 h. After a further hour at reflux, the mixture was cooled in ice and filtered. The resulting solid was washed with chloroform (3 × 50 mL), which was combined with the original filtrate. This was washed with water (100 mL), dried ($MgSO_4$), and evaporated to an oil (16.13 g). Purification of the required product was achieved by chromatographing the oil on silica gel (200 g), eluting with toluene. This gave the product as a oil (5.44 g), which was crystallized from ethanol (35 mL) to yield 4.32 g of yellow solid (18.6% yield), melting at 93–94 °C.

Method B. 2-Cyclopropyl-3-hydroxy-1,4-naphthoquinone (20). 2-Chloro-3-cyclopropyl-1,4-naphthoquinone (2; 4.0 g, 0.017 mol) was dissolved in boiling methanol (100 mL) and a solution of potassium hydroxide (4.0 g, 0.07 mol) in water (40 mL) was added dropwise over 15 min. The solution was then cooled in ice and acidified with concentrated hydrochloric acid. The precipitated light brown solid was filtered, dried (3.15 g), and crystallized from toluene (50 mL)/petroleum ether (140 mL) to give 2-cyclopropyl-3-hydroxy-1,4-naphthoquinone as pale brown crystals, 2.90 g (79% yield), melting at 117–118 °C.

Method C. 2-Cycloheptyl-3-hydroxy-1,4-naphthoquinone (39). 2-Hydroxy-1,4-naphthoquinone (26 g, 0.15 mol), *tert*-butyl peroxide (70.0 g, 0.48 mol), and cycloheptane (580 mL) were heated at reflux for 7 h. Removal of cycloheptane (and other volatiles) by distillation at 145 °C afforded a residue which was extracted with boiling petroleum ether (3 × 200 mL). Evaporation of the combined petroleum extracts yielded crude product as a slightly oily solid (36.5 g), which crystallized from petroleum ether (130 mL) as a yellow solid, 26.17 g, melting at 83–85 °C. Further crystallization of a sample from petroleum ether resulted in the melting point rising to 96–97 °C: yield 58%.

Method D. 2-Hydroxy-3-(3-tetrahydropyranyl)-1,4-naphthoquinone (44). Dihydropyran (19.34 g, 0.23 mol) was added to a solution of borane dimethyl sulfide (5.76 g, 0.076 mol) in dry tetrahydrofuran (80 mL) under nitrogen at such a rate as to maintain gentle reflux. After a further 3 h at reflux, the reaction mixture was evaporated in vacuo, the residual solid was dissolved in tetrahydrofuran (100 mL), and water (1.8 g, 0.1 mol) was added. Air was slowly blown over the surface of the resulting solution and 2-hydroxy-1,4-naphthoquinone (10.44 g, 0.06 mol) in tetrahydrofuran (200 mL) was added over 10 min. After a further 20 min, the tetrahydrofuran was removed in vacuo from the dark red reaction mixture, which was then steam distilled. The residue was extracted with ethyl acetate (300 mL), which after washing with sodium bicarbonate solution (0.5 M, 4 × 200 mL) and water (50 mL), drying ($MgSO_4$), and evaporating yielded an oil, 12.57 g. This was chromatographed on silica gel (300 g) eluting with toluene/10% ethyl acetate. Initially, the required product, 2-hydroxy-3-(3-tetrahydropyranyl)-1,4-naphthoquinone, was obtained from the column as a yellow solid, 720 mg (4.7% yield), melting at 164–166 °C after crystallization from toluene.

Further development of the column with toluene/10% ethyl acetate gave 4-(3-tetrahydropyranyl)-1,2-naphthoquinone (45) as an orange solid, 4.08 g (28% yield), which after triturating with toluene melted at 167–169 °C. Crystallization from ethanol raised the melting point to 172–173 °C. Anal. ($C_{15}H_{14}O_3$) C, H.

2-(Cyclohexylthio)-3-hydroxy-1,4-naphthoquinone (47). 2-Chloro-3-methoxy-1,4-naphthoquinone[13] (1.85 g, 0.008 mol) was added to cyclohexanethiol (0.95 g, 0.008 mol) in dimethylformamide (8 mL), and the resulting green solution was heated at 100 °C for 6 h. After standing overnight at room tempeature, the red solution was added dropwise to water (50 mL), and the precipitated solid was filtered off and dried, yield 2.0 g. Crystallization from cyclohexane (40 mL) yielded the product as an orange solid, 1.02 g, melting at 140–143 °C. Further purification was effected by column chromatography on silica gel (30 g), eluting pure 47: 0.93 g (40% yield); mp 149–150 °C, with chloroform. Anal. ($C_{16}H_{16}O_3S$) C, H.

2-Azido-3-(cyclohexylmethyl)-1,4-naphthoquinone (52). 2-Chloro-3-(cyclohexylmethyl)-1,4-naphthoquinone (5; 1.44 g, 0.005

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mol) and sodium azide (0.65 g, 0.01 mol) were heated in ethanol (50 mL)/water (20 mL) at reflux for 1 h. The reaction mixture was cooled and the precipitated solid was filtered off, washed with water, dried, and crystallized from 2-propanol (17 mL) to give the required azide, 1.05 g (71% yield), decomposing at 123–124 °C. Anal. ($C_{17}H_{17}N_3O_2$) C, H, N.

2-Amino-3-(cyclohexylmethyl)-1,4-naphthoquinone (53). 2-Azido-3-(cyclohexylmethyl)-1,4-naphthoquinone (4.26 g, 0.014 mol) in ethanol (150 mL) was hydrogenated at ambient temperature and pressure in the presence of 10% Pd/C catalyst (500 mg) for 2.5 h. The catalyst was filtered off and the filtrate was evaporated in a current of air to yield an orange solid (3.61 g), mp 100–105 °C. This was chromatographed on silica gel (50 g), eluting the desired product as a solid (2.43 g), which was further purified by crystallization from petroleum ether (150 mL) to give amine, 1.70 g (44% yield), as orange crystals, melting at 121–122 °C. Anal. ($C_{17}H_{19}NO_2$) C, H, N.

2-Cycloheptyl-3-hydroxy-5,6,7,8-tetrahydro-1,4-naphthoquinone (59). 2-Cycloheptyl-3-hydroxy-1,4-naphthoquinone (**39**; 4.0 g, 0.015 mol) in ethanol (90 mL) was hydrogenated at 70 °C (100 atm) in the presence of Raney nickel W-6[25] (6 g) for 5 h. The catalyst was filtered off and the filtrate was evaporated in a current of air to yield a brown oil (4.75 g). This was triturated with toluene (10 mL) and an insoluble purple solid (0.46 g) was filtered off. The filtrate was chromatographed on silica gel (100 g), eluting the required product with toluene as a slightly oily solid (2.6 g). Crystallization from petroleum ether afforded pure material, 1.78 g (44% yield), as orange crystals melting at 124–125 °C. Anal. ($C_{17}H_{22}O_3$) C, H.

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Studies on Chiral Interactions of 1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane and the Corresponding *N*-Hydroxy Metabolites with Cytochrome P-450

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Received April 3, 1980

The stereoselective pharmacological behavior and metabolism of the potent psychotomimetic amine 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane have led to an investigation of the interactions of the racemic amine, its enantiomers, and the corresponding *N*-hydroxy metabolites with rabbit liver microsomal cytochrome P-450. An examination of the formation of cytochrome P-450 metabolic intermediate complexes with these species suggests that N-oxidation of the pharmacologically active (*R*)-amine is inhibited by the *S* enantiomer. Additionally, metabolic intermediate complex formation [favored by the (*R*)-amine] appears to be associated with loss of microsomal mixed function *N*-oxidase activity. The results have led to the prediction that N-hydroxylation of pure (*R*)-amine may be a qualitatively more important pathway than that observed with racemic amine even though this biotransformation may be suicidal.

The psychotomimetic properties of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (1) are reported to reside in the *R* enantiomer (**1a**).[1] Drug-induced alterations of behavior in rats[2] and hyperthermia in rabbits[3] also demonstrate a chiral dependency favoring the *R* enantiomer. Studies in our laboratory have focused on the metabolic fate of amine 1 and the characterization of metabolic pathways which may be of pharmacological importance.[4-7] We have established that racemic 1 is stereoselectively metabolized by rabbits both in vivo[4] and in vitro,[6] with the *S* enantiomer being preferentially consumed. Similar results have been reported recently for rats.[8] Analysis of the enantiomeric compositions of all but one of the metabolites derived from racemic 1 showed, as expected, an *S*/*R* ratio >1.[6] N-Oxidation of racemic 1 to the hydroxylamine **2** by 100000*g* rabbit liver microsomal preparations, however, favors the *R* enantiomer.[5] This preferential N-hydroxylation of the pharmacologically active *R* enantiomer of racemic 1 prompted us to examine the interactions of racemic 1, racemic **2**, and their individual enantiomers with rabbit liver microsomal cytochrome P-450. Binding spectra of these molecules with the oxidized form of cytochrome P-450 have been obtained by split-beam difference spectroscopy.[9] Additionally, with the aid of both difference and dual-wavelength spectroscopy[10] we have studied the possibility that these molecules, as well as other metabolites derived from 1, may undergo oxidation to species which may bind to cytochrome P-450. These so-called metabolic intermediate complexes[11] absorb maximally near 455 nm and have been observed for a number of molecules structurally similar to 1 and **2**.[11-13] Since this complexation of cytochrome P-450 is reported to result in a reduction of mixed function oxidase activity,[11] we were particularly interested in the possible stereoselective formation of this complex and the influence of complex formation on the stereoselectivity associated with the metabolism of 1.

Chemistry. The synthesis, resolution, and absolute configuration assignment of the parent amine 1 have been reported previously.[4] The preparations of the various

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