Occurrence and Pharmacological Significance of Metabolic Ortho-Hydroxylation of 5- and 8-Hydroxy-2-(di-*n*-propylamino)tetralin

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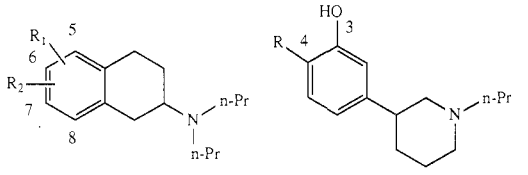
Aromatic ortho-hydroxylation in the liver might be one of several possible reasons for the low bioavailabilities of the potent, centrally acting dopaminergic and serotoninergic agonists 5- and 8-hydroxy-2-(di-*n*-propylamino)tetralin, respectively. In vitro and in vivo experiments showed that such an oxidative metabolism did indeed take place. However, the amount of hydroxylated metabolites found in the brain was estimated to represent only 0.3% of the total amount of drug administered. The O-methylation rates of these catechols were also measured in vitro and showed that 5,6-dihydroxy-2-(di-*n*-propylamino)tetralin is a poor substrate for catechol *O*-methyltransferase (COMT) and that its 7,8-dihydroxy isomer is virtually devoid of substrate activity. No O-methylated metabolites were detected in the in vivo samples analyzed. A new synthetic strategy was applied to achieve the isomeric catechols studied. 5-Methoxy- or 8-methoxy-2-(di-*n*-propylamino)tetralin was lithiated in the ortho position and the metalated species was subsequently quenched in nitrobenzene, yielding the methoxy hydroxy isomers, which were heated in 48% aqueous HBr to achieve the corresponding catechols.

5-Hydroxy- and 8-hydroxy-2-(di-*n*-propylamino)tetralin (5- and 8-OH-DPAT; **1** and **2**, respectively) are potent, centrally acting dopamine (DA) and serotonin (5-HT) receptor agonists, respectively.[1-3] Both **1** and **2** have poor bioavailability when administered orally.[4,5] One of several possible reasons for this might be high first pass elimination via aromatic hydroxylation to the catechol analogues 5,6- and 7,8-di-OH-DPAT (**3** and **4**, respectively) and the subsequent O-methylation to their possible catechol *O*-methyltransferase (COMT) products **5**, **6** and **7**, **8**, respectively.



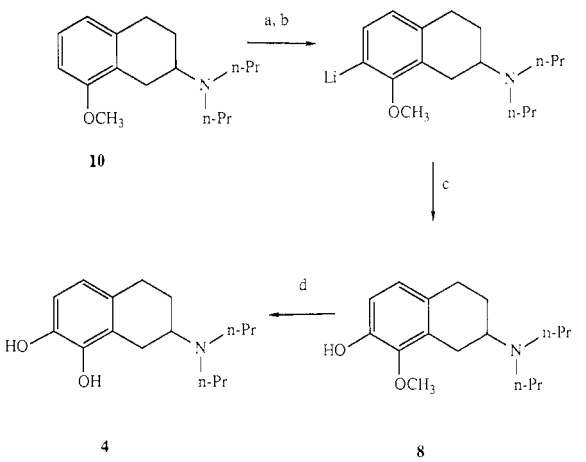
compd	R_1	R_2
1	5-OH	H
2	8-OH	H
3	5-OH	6-OH
4	7-OH	8-OH
5	5-OMe	6-OH
6	5-OH	6-OMe
7	7-OMe	8-OH
8	7-OH	8-OMe
9	5-OMe	H
10	8-OMe	H
11	6-OH	H
12	7-OH	H
13	6-OH	7-OMe

compd	R
14	H
15	OH

Previous studies of the pharmacological relevance of catechol formation from the two enantiomers of 3-(3-hydroxyphenyl)-*N*-*n*-propylpiperidine (3-PPP; **14**) indicated a 1–5% conversion of the phenol to the catechol.[6,7] The catechols thus formed were immediately O-methylated by COMT, leaving essentially no catechol in the brain regions studied.[6,7]

In order to study the importance of these potential metabolic events for compounds **1** and **2**, we prepared the catechols **3** and **4** via the methoxy hydroxy isomers **5** and **8**. Compounds **3** and **4** have previously been reported to be dopaminergic agonists.[1,2,8] Due to its obvious structural similarity to 8-OH-DPAT (**2**), we decided to investigate whether compound **4** might also have 5-HT receptor agonist properties. In addition, the potential central effects of the methoxy hydroxy analogues **5** and **8** were studied. The isomeric methoxy hydroxy analogues **6** and **7** were not prepared and are just mentioned in relation to **5** and **8** in this study.

Scheme I. Synthetic Pathway to Compounds 4 and 8[a]



[a] (a) *n*-BuLi, (b) TMEDA, (c) nitrobenzene, (d) 48% HBr.

Compound **3** is presently used as a nonradioactive ligand in our laboratories to label postsynaptic D2 receptors in vivo.[9] This constituted a second impetus for us to find

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Table I. Physical Data

compd	yield,[a] %	mp,[b] °C	formula
3	87	228–230[c]	$C_{16}H_{25}NO_2$·HBr
5	26	170–190 dec	$C_{17}H_{27}NO_2$·HCl
4	76	216–218[d]	$C_{16}H_{25}NO_2$·HBr
8	20	220–230 dec	$C_{17}H_{27}NO_2$·HCl

[a] The yield notation comes from the last step. [b] Recrystallization solvents were EtOH/ether for compounds 3, 5, and 8 and MeOH/ether for compound 4. [c] Literature[2] 220–223 °C. [d] McDermed et al.[8] reported the melting point for 4·HCl to be 160–163 °C. We basified 4·HBr with 10% Na_2CO_3 and extracted the base to CH_2Cl_2. Usual workup yielded an oil, which was converted to its hydrochloride with HCl-saturated EtOH. The solvents were removed at reduced pressure, and recrystallization (EtOH/ether) gave crystals melting at 240–243 °C. To understand this discrepancy, we have reanalyzed, by GC and GC/MS, a sample of authentic material (pyridine Ac_2O derivatization), kindly supplied by Dr. McDermed, and have concluded that it contains a large amount of the potent DA receptor agonist 7-OH-DPAT. This contaminant would almost certainly have been reflected in the pharmacological results they reported for compound 4.[8]

a more convenient synthesis of this valuable pharmacological tool than that previously reported.[8]

Chemistry

The syntheses of the target catechols 3 and 4 are outlined in Scheme I. Either 5- or 8-OMe-DPAT (9 or 10, respectively) was treated with *n*-BuLi in anhydrous ether, with *N,N,N',N'*-tetramethylethylenediamine (TMEDA) as a cosolvent. The presence of TMEDA was crucial for the success of the metalation reaction. The lithiation is known to take place in a position ortho to the methoxy group, either due to the stabilizing factor of the complexation of lithium by this group or to the acidity of the ortho-hydrogen atom.[10] Several possibilities for exchanging lithium for oxygen exist and, in our hands, the procedure described by Buck et al.[11] using nitrobenzene gave the best yields. Ether cleavage of the methoxy hydroxy isomers 5 and 8 gave the catechols 3 and 4, respectively (Table I).

Pharmacology

Biochemistry. The in vivo biochemical test utilizes the well-established phenomenon of receptor-mediated feedback inhibition of the presynaptic neuron.[12] The synthesis rate of the catecholamines DA and norepinephrine (NE) is inhibited by agonists (and activated by antagonists) at dopaminergic and α-adrenergic receptors, respectively. Similarly, the synthesis rate of 5-HT is inhibited by 5-HT receptor agonists.[13,14] The Dopa accumulation, following decarboxylase inhibition by means of (3-hydroxybenzyl)-hydrazine (NSD 1015), was used as an indicator of the DA synthesis rate in the DA rich areas (i.e. limbic system and corpus striatum) and the NE synthesis rate in the NE-rich hemispheres (mainly cortex). The 5-HTP accumulation was taken as an indicator of the 5-HT synthesis rate in the three brain areas (Table II).

Locomotor Activity. Postsynaptic effects of the test compounds were assessed by the increase in locomotor activity (reserpine pretreatment). Motor activity recordings were carried out as previously described with the use of motility meters[2] (Table II).

Brain Concentrations and in Vitro O-Methylation. Concentrations of the catechol isomers 3 and 4 were measured by HPLC with electrochemical detection in three brain regions after the administration of the test compounds (1 and 2) with or without pretreatment with the COMT inhibitor tropolone. The rate of O-methylation was measured in vitro by incubation of 3 and 4 with COMT and the methyl donor *S*-adenosyl-L-methionine and estimation of the *O*-methyl derivatives formed (5 and 6 from 3 and 7 from 4; see footnotes *a* and *b* of Table IV) was carried out by means of HPLC and electrochemical detection, as previously described[6,7] (Tables III and IV).

Results and Discussion

Compound 4 has previously been described to be a centrally acting DA receptor agonist.[1] As seen from Table II, compound 4 has both dopaminergic and serotoninergic properties, of much less potency, however, than compounds 1 and 2, respectively. Gross behavior observations of the animals revealed mixed DA and 5-HT agonist syndromes. In addition, the effects on Dopa accumulation measured in the hemispheres indicate α-adrenergic properties of this compound. Compound 4 is a racemic mixture, and when the potential pharmacological effects of the *R*- and *S*-7-OH-DPAT (*R*- and *S*-12) and *R*- and *S*-8-OH-DPAT (*R*- and *S*-2) moieties inherent in racemic 4 are considered, it might be speculated that there would be a possibility that *S*-4 might be a selective 5-HT receptor agonist, since it contains the 5-HT receptor agonist *S*-8-OH-DPAT and the nondopaminergic *S*-7-OH-DPAT. *R*-4 should have effects on both receptor types, since it contains the 5-HT receptor agonist *R*-8-OH-DPAT and the dopaminergic *R*-7-OH-DPAT.[3,15]

As seen from Table II, compound 5 is a potent and selective DA receptor agonist with an ED_{50} value that is similar to that of 6-OH-DPAT (11). Its 7-OH-8-OMe isomer (8) also retains potent dopaminergic properties but is less active as a central 5-HT receptor agonist than 8-OMe-DPAT (10).

The concentrations of compounds 1 and 3, and 2 and 4 after sc administration to rats of compounds 1 and 2, respectively, were measured in three brain regions 15, 45, and 90 min after administration of the test compounds. Table III shows the concentrations 45 min after injection. The highest levels of compound 2 were found 15 min after injection (about 16 nmol/g after 15 μmol/kg), which decreased to about 10 nmol/g 45 min after injection. It seems as if compound 2 is relatively slowly eliminated, since 90 min after the injection with 15 μmol/kg, levels of 2 are still about 3–5 nmol/g. There was no regional distribution of compounds 1 and 2, since their striatal concentrations did not differ significantly from the concentrations in cortex and cerebellum. Brain concentrations of compounds 1 and 2, reached after 45 min, represent about 0.3–0.5% of the dose administered,[16] which is in the same range as that

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(16) These values were calculated from Table III according to the following. Compound 1: assuming the rat brain weighs 1.5 g and the concentration measured is 10 nmol/g, this gives 15 nmol/brain. Since the dose is 25 μmol/kg, this gives 5000 nmol/rat (rat weight 200 g). Hence, the amount of 1 in the brain represents 15/5000 × 100% = 0.3% of the total amount of 1 administered. Compound 2: at 15 μmol/kg (3000 nmol/rat) the brain concentration of 2 is about 10 nmol/g. Hence, the amount of 2 in the brain represents 15/3000 × 100% = 0.5% of the total amount of 2 administered.

Table II. Biochemical and Motor Activity Effects in Reserpinized Rats

	Dopa accumulation: ED_{50}, nmol/kg			5-HTP accumulation: ED_{50}, nmol/kg			motor activity	
compd	limbic system	striatum	hemispheres	limbic system	striatum	hemispheres	dose, μmol/kg	acc[a]
1[b]	11	9	I[c]	I	I	I		
9[b]	13	18	I	I	I	I		
3[b]	7	7	I	I	I	I	0.25	529 ± 10*[d]
5	160–432[e]	125–578	I	I	I	I	12.5	204 ± 24*[f]
11[g]	180 ± 14	170 ± 20	I	I	I	I		
2[g]	I	I	I	48 ± 9	45 ± 5	68 ± 3		
10[h]	I	I		340 ± 190	290 ± 125	230 ± 60		
4[i]	986–1276	977–1230	1750–3141	1535–7834	1393–4966	1052–13122	3.15	31 ± 18*
8	314–607	350–520	I	1493–6109	2056–11143	1618–6471	12.5	149 ± 31*

[a] Accumulated counts 0–30 min after drug treatment. [b] Data taken from ref 2. [c] I means inactive. No effects were seen at the highest dose tested (50 μmol/kg). [d] Statistics according to the Student's *t* test. (*) $p \leq 0.05$. [e] The intervals given were recalculated from pED_{50} ± SEM values achieved by the statistics program package RS-1. [f] Accumulated counts 65–95 min after drug administration. [g] Data taken from ref 3. [h] Data taken from ref 19. [i] The effect seen in the hemispheres indicates α-adrenergic properties of this compound.

Table III. Brain Concentrations (nmol/g) of Compounds 1 and 3, and 2 and 4 45 min after Sc Administration of Compounds 1 and 2

			brain concentration, nmol/g ± SEM, $n = 4$[a]					
			no tropolone			tropolone		
compd administered	dose, μmol/kg	compd monitored	striatum	cerebellum	cortex	striatum	cerebellum	cortex
1	25 (sc)	1	10.8 ± 0.7	8.5 ± 0.2	12.1 ± 1.1	11.9 ± 0.9	9.4 ± 0.4	12.7 ± 0.8
1	25 (sc)	3	0.2 ± 0.05	0.08 ± 0.02	0.08 ± 0.01	0.43 ± 0.05	0.30 ± 0.01	0.37 ± 0.02
2	15 (sc)	2	10.3 ± 1.1	8.1 ± 1.0	10.7 ± 1.0	12.3 ± 1.3	9.0 ± 0.9	13.2 ± 1.4
2	15 (sc)	4	0.65 ± 0.02	0.50 ± 0.05	0.64 ± 0.03	0.58 ± 0.06	0.54 ± 0.04	0.70 ± 0.05

[a] No hydroxy methoxy metabolites were detected in these in vivo samples.

Table IV. Apparent Kinetic Constants for the Aromatic Hydroxylation of Compounds 1 and 2 and for the O-Methylation of Compounds 3 and 4

substrate	enzymatic reaction	product	K_m, mM	V_{max}, pmol min^{-1} (mg of protein)$^{-1}$	V_{max}/K_m
1	hydroxylation	3	0.06	1175	19583
2	hydroxylation	4	0.08	750	9375
3	O-methylation	5 + 6[a]	0.08	400	5000
4	O-methylation	7[b]	2.4	280	117
S-15[c]	O-methylation		0.077	37000	485000

[a] The second component monitored is anticipated to be the 5-OH-6-OMe isomer, compound 6. [b] The product monitored in this case is anticipated to be the 8-OH-7-OMe isomer, compound 7, since its isomer 8 was not detected in these assays. [c] Data taken from ref 7.

found for other monohydroxylated 2-aminotetralins[17] and for 3-PPP (14).[6,7]

One of several possible metabolic routes of phenols beside conjugation is aromatic hydroxylation. It has been shown for 3-PPP (14) that catechol formation, although being a minor metabolic pathway, can take place in vivo.[6,7] Measurement of the in vitro hydroxylation rates showed that compounds 1 and 2 are both hydroxylated, virtually to the same extent (Table IV). These in vitro findings were confirmed by the in vivo experiments, which demonstrated the presence of the catechols 3 and 4 after the administration of 1 and 2, respectively (Table III). The amounts of 3 and 4 (after tropolone pretreatment) formed in rat brain after subcutaneous administration of 1 and 2, respectively, represent about 6% of the brain levels of the parent compounds, which is 1 order or magnitude higher than the levels found for the catechol derivative of 3-PPP (15) after the subcutaneous administration of 3-PPP (14) itself.[6,7]

It might be expected that the catechols 3 and 4 formed are further metabolized by O-methylation with COMT, and consequently we investigated this possibility. Tropolone pretreatment, which blocks the COMT activity, did not have any significant effect upon the levels of 4 after the administration of 2 as compared to no pretreatment (Table III). This indicates that 4 is an extremely poor substrate for COMT and does not, or only very slowly, undergo O-methylation in vivo. Catechol 3, on the other hand, is O-methylated by COMT, showing about 5 times higher values after tropolone pretreatment, although it is a poor substrate, as compared to other catecholamines (e.g. compound 15, which is included as a reference compound in Table IV),[7] the levels of which can be increased more than 1000-fold when COMT is inhibited. In vitro data on the rates of O-methylation were in excellent agreement with these in vivo results. Comparison of the kinetic constants of COMT for compounds 3 and 4 (Table IV) and for other catecholamines[18] shows that 3 is a poor substrate and 4 is hardly a substrate at all, as indicated by its extremely high K_m value.

Since the isomeric methoxy hydroxy derivatives 6 and 7 were not available, all the O-methylated metabolities could not be identified with certainty. However, a comparison of chromatographic and electrochemical characteristics of the one *O*-methyl derivative detected in the in vitro incubation samples of compound 4 with those of the synthesized compound 8, one of the two possible methoxy hydroxy isomers, showed that these were not identical. In other words, it is likely, but not yet proved, that compound 4 is O-methylated in vitro exclusively to the other isomer, the 7-O-methylated compound 7. Compound 3 is methylated to two O-methylated derivatives, one being identical with authentic 5, the second one in all likelihood being the isomer 6. As expected from the very low O-methylation rates of 3 and 4, we were not able to detect any methoxy hydroxy metabolites in the tissue samples of rats injected with compounds 1 and 2, respectively.

In conclusion, the low bioavailabilities of the monophenolic 2-aminotetralins 1 and 2 are probably not due to ortho-hydroxylation of these compounds to the catechol

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derivatives **3** and **4**, respectively, and their subsequent O-methylation, since the amount of hydroxylated metabolities found in rat brain was estimated to be only 0.3% of the total amount of drug administered. Consequently, other metabolic pathways like sulfatation, glucuronidation, and N-dealkylation should be considered when attempting to explain the low bioavailabilities of the pharmacologically important compounds **1** and **2**. In addition, the pharmacological effects of compounds **1** and **2** are likely to be caused by these compounds per se and not be any potent metabolities.

Experimental Section

Chemistry. Melting points (uncorrected) were determined with a melting point microscope (Reichert Thermovar). ^{1}H NMR spectra were recorded with a Bruker 270 MHz and a Varian EM-360 MHz instrument (Me_4Si). GC was performed with a HP 5830A instrument with a flame-ionization detector. A fused silica column (11 m, 0.22 mm i.d.) coated with cross-linked SE-54 (film thickness 0.3 μm, gas H_2, gas velocity 40 cm/s) was used throughout.

GC/MS spectra were recorded on a HP5970A Mass Selective Detector working at 70 eV and interfaced with a HP 5700A gas chromatograph. High-resolution MS spectra were recorded on a ZAB-HF mass spectrometer (VG-Analytical) with direct inlet, working in the EI mode (60 eV). All spectra were in accordance with the assigned structures.

For purity tests, TLC was performed on fluorescent silica gel plates developed in at least two different systems. For all the compounds, only one spot (visualized by UV light and I_2 vapor) was obtained.

6-Hydroxy-5-methoxy-2-(di-*n*-propylamino)tetralin (5). 5-Methoxy-2-(di-*n*-propylamino)tetralin hydrochloride (**9**·HCl)[2] (500 mg, 1.68 mmol) was suspended in anhydrous ether (10 mL) under Ar(g). The mixture was chilled with ice, and *n*-BuLi in *n*-hexane (1.6 M, 10 mL, 16 mmol) and anhydrous TMEDA (1 mL, 6.7 mmol) were added from a syringe. The reaction mixture was stirred at 0 °C for 10 min, the ice bath was removed, and the mixture was stirred at room temperature overnight. The reaction mixture was then chilled to −80 °C (CO_2/ether) and added with a syringe to a solution of anhydrous nitrobenzene (1 mL, 9.8 mmol) in anhydrous ether (10 mL), still at −80 °C. The reaction mixture was stirred at this temperature for 4 h before the CO_2/ether bath was removed and the reaction quenched with 10% H_2SO_4 (25 mL). The phases were separated, and the organic layer was reextracted with another portion (5 mL) of the acid. The combined acidic water phase was extracted four times with ether and was then basified with a concentrated NH_4OH solution. The water phase was extracted four times with CH_2Cl_2, the organic layer was dried (Na_2SO_4) and filtered, and the solvent was removed by evaporation. The crude product was chromatographed (SiO_2) with EtOAc with 1% triethylamine as eluant. The fractions containing pure **5** were pooled, and the solvent was removed by evaporation. The residue was converted to its hydrochloride salt with HCl-saturated EtOH and treated with charcoal. The solution was filtered and the solvent was evaporated. Recrystallization (EtOH/ether) gave crystals (137 mg, 26%) of the desired product **5**·HCl. High-resolution MS was performed and gave $C_{17}H_{27}NO_2$: calcd 277.2043 and found 277.204 ± 10. No impurities were detected by GC or TLC.

7-Hydroxy-8-methoxy-2-(di-*n*-propylamino)tetralin (8). The free base of 8-methoxy-2-(di-*n*-propylamino)tetralin (**10**)[3] (600 mg, 2.3 mmol) was treated with *n*-BuLi in *n*-hexane (1.6 M, 10 mL, 16 mmol) and TMEDA (1 mL, 6.7 mmol) and subsequently reacted with nitrobenzene (1 mL, 9.8 mmol) as described for the preparation of compound **5** above. Chromatographic purification (SiO_2) of the crude product was accomplished with petroleum ether/EtOAc (9:1) as eluant. The pure product base was converted to its hydrochloride salt and recrystallized (EtOH/ether), as described for the preparation of **5**·HCl above, yielding crystals (145 mg, 20%) of the desired product **8**·HCl. High-resolution MS was performed and gave $C_{17}H_{27}NO_2$: calcd 277.2043 and found 277.204 ± 10. No impurities were detected by GC or TLC.

Demethylation of Methoxy Compounds. The catechols were obtained by heating the appropriate methoxy compounds in 48% aqueous HBr for 2 h at 125 °C under nitrogen. The hydrobromic acid was evaporated and the residue was crystallized from MeOH/ether.

Assignment of Catechol Position in Compounds 3 and 4. The specificity of the ortho lithiation[10] was determined by NMR studies (Bruker 270 MHz) of the target compounds **3** and **4**. In both compounds the two aromatic protons couple in a typical AB spectrum (J = 8 Hz), indicating either ortho or para substitution. If para substitution would prevail in the lithiation reaction, both final products would be identical, i.e. 5,8-dihydroxy-2-(di-*n*-propylamino)tetralin, which was not the case (differences in melting points and NMR spectra: the HBr salts of compounds **3** and **4** in CD_3OD show shift differences for the two ortho protons of 36 and 44 Hz, respectively). 5,8-Dihydroxy-2-(di-*n*-propylamino)tetralin has been reported to have the melting point 233–235 °C (HBr salt),[19] which is not very different from the melting point of compound **3**·HBr (Table I). Identification with the literature melting point was possible for compound **3** (HBr salt) but not for compound **4** (HCl salt) (see footnote *d* of Table I).

Pharmacology. Animals used in the biochemical and motor activity experiments were male rats of a Sprague-Dawley strain (ALAB, Sollentuna, Sweden), weighing 200–300 g. All substances to be tested were dissolved in saline immediately before use, occasionally with the addition of a few drops of glacial acetic acid and/or moderate heating in order to obtain complete dissolution. Reserpine was dissolved in a few drops of glacial acetic acid and made up of volume with 5.5% glucose. Injection volumes were 5 or 10 mL/kg, and all solutions had neutral pH values (except for the solutions of reserpine).

Biochemistry. The biochemical experiments and the determinations of Dopa and 5-HTP by means of HPLC with electrochemical detection were performed as previously described.[20] Separate dose–response curves based on four to six dose levels for each substance (sc administration) and each brain area were constructed. From these curves, the dose of the drug yielding a half-maximal decrease (ED_{50} value) of the Dopa and the 5-HTP levels were estimated separately (Table II).

Motor Activity. The motor activity was measured by means of photocell recordings (M/P 40 Fc Electronic Motility Meter, Motron Products, Stockholm) as previously described.[2] Eighteen hours prior to the motility testing (carried out between 9 a.m. and 1 p.m.), the rats were subcutaneously injected in the neck region with reserpine (5 mg/kg). The different test compounds were also administered subcutaneously in the neck region (n = 4). Immediately after drug administration, the rats were placed in the test cages (one rat/cage) and put into the motility meters. Motor activity was then followed and recorded for the subsequent 30 min (Table III).

Brain Concentrations. Male Wistar rats (CDL, Groningen, The Netherlands) were injected sc in the neck region in the volume 1 mL/kg with various doses of the test compounds **1** and **2**, which had been dissolved in saline. Control animals received a sc saline injection. Rats were killed by decapitation after 15, 45, or 90 min and the brains were taken out. Striatal, cerebellar, and cortical tissue was rapidly dissected and immediately frozen on dry ice and kept at −80 °C until assayed.

Perchloric acid extracts of the brain tissue samples were prepared and purified over Sephadex G10, as reported earlier.[6] The formic acid eluate, containing the amines, was injected with a Rheodyne injection valve with a 20-μL loop onto a Nucleosil 5 C18 column (150 × 4.6 mm). The mobile phase was a phosphate/citrate buffer, pH 4, either with 10–12.5% 2-propanol (for the determination of compounds **1** and **2**) or with 22% MeOH and 0.05% heptanesulfonic acid (for the determination of compounds **3** and **4**) and was delivered with a flow rate of 1 mL/min with a Waters 6000A pump. The detection of the test compounds was made with a rotating carbon paste electrode set at 900 mV

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for monitoring compounds 1 and 2 and set at 500 mV for monitoring compounds 3 and 4. The peak heights gave the tissue concentrations (nmol/g of tissue) from calibration curves. These were prepared from standard solutions (authentic samples dissolved in 0.02 M formic acid). Corrections were made for recoveries (for compound 1, 79.6%, SEM = 2.3%, n = 10; for compound 2, 84.3%, SEM = 1.7%, n = 12; for compound 3, 89.7%, SEM = 1.5%, n = 14; for compound 4, 86.8%, SEM = 1.2%, n = 21).

In Vitro Aromatic Hydroxylation. Compounds 1 and 2 were incubated with crude rat liver microsomes in 10-mL glass tubes at 37 °C as previously described.[6] Incubation constituents were NADPH (0.1 mM), $MgCl_2$ (2.5 nM), Tris buffer, pH 7.4 (0.1 M), microsomes (0.5 mg of protein/mL), and H_2O to a final volume of 1.0 mL. After incubation for 3 min, the reaction was stopped by adding 50 μL of perchloric acid (60%). Supernatants were assayed for compounds 3 and 4 (as described above) without further purification. In control incubation samples (one without microsomes and one without test compound) compounds 3 and 4 were not detectable. Apparent kinetic constants (K_m and V_{max}) were calculated from Lineweaver–Burk plots with three to four concentrations, each measured in triplicate.

In Vitro O-Methylation. Incubations of compounds 3 (0.025–0.5 mM) and 4 (0.05–1 mM) with rat liver COMT were performed essentially as described earlier.[6] After 15 min, 50 μL of perchloric acid (60%) was added to the incubation mixture, and the O-methylated products were measured in the supernatant by the same method as described above for the determination of compounds 1 and 2, except for the electrode potential, which now was set to 750 mV. Compounds 5 and 8 had retention times of (10% *i*-PrOH) 13 and 7 min, respectively. The only methoxy hydroxy compound detected in the incubation samples of compound 4 had a retention time of 12 min. This suggests that compound 4 is in vivo methylated to compound 7 instead of 8. In incubation samples of compound 3, two methoxy hydroxy compounds were detected, with retention times of 13 min (5) and 9 min (probably 6). Concentrations of compounds 6 and 7 were calculated from the calibration curves of their isomers 5 and 8, respectively, assuming an identical electrochemical sensitivity for both isomers in each case. Apparent kinetic constants were calculated from Lineweaver–Burk plots with three to four concentrations, each measured in triplicate.

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Protoberberine Alkaloids as Antimalarials

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The protoberberine alkaloids berberine (1), palmatine (2), jatrorrhizine (3), and several berberine derivatives (4–10) were tested for antimalarial activity in vitro against *Plasmodium falciparum* and in vivo against *Plasmodium berghei*. The berberine derivatives 4–10 were designed and synthesized to maximize structural diversity within a modest set of compounds. Palmatine (2) and jatrorrhizine (3) were isolated as their chlorides from *Enantia chlorantha*. None of the protoberberine alkaloids was active in vivo, although compounds 1, 2, 3, 5, and 6 exhibited a potency comparable to that of quinine in vitro.

Berberine (1) is probably the most widely distributed of all alkaloids, having been found in plants of the nine botanical families, Berberidaceae, Papaveraceae, Runnunculaceae, Rutaceae, Menispermaceae, Rubiaceae, Rhamnaceae, Magnoliaceae, and Annonaceae. This and allied protoberberine alkaloids such as palmatine (2) have been used extensively in folk medicine.[1] At the present time, the only practical therapeutic application of berberine is in the treatment of cutaneous leishmaniasis,[2-9] although various claims have been made concerning its use in the treatment of malaria.[1,5,10-18] Whereas several investigators found berberine to be inactive in experimental and clinical malaria,[5,14] others claimed it to be valuable alone[16,17] or as an adjunct to quinine.[12,13] Berberine has also been claimed to be beneficial in the treatment of the splenomegaly of malaria.[10,11] In a recent in vitro study,[18] berberine chloride at 50 μM was found to completely block protein synthesis in *Plasmodium falciparum*. Finally, aqueous extracts from the African plant *Enantia chlorantha* Oliver, which contains the protoberberine alkaloids palmatine, columbamine, and jatrorrhizine,[19] have been claimed to be therapeutically useful in the treatment of malaria.[20]

Although antimalarial effects have been ascribed to berberine, little quantitative experimental data exist to substantiate such claims. In this paper we present data on the antimalarial properties of berberine (1) and its

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