the view of differing uptake modes for 4-phenylpiperidine analgetics and fused-ring derivatives containing the same structural feature, already advanced on the basis of comparative structure–activity relationships.[13]

Experimental Section

Where analyses are indicated only by the symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Melting points, determined with a Fisher-Johns apparatus, are uncorrected. Ord curves were recorded with a Cary Model 60 photoelectric spectropolarimeter using 0.1–0.2% solutions in EtOH or H_2O (we thank Mr. K. O. Oikawa, University of Alberta, for this data). Supply of α-(−)- and β-(+)-**1e** by Dr. N. F. Albertson of the Sterling Winthrop Research Institute, Rensselaer, is gratefully acknowledged.

Isomeric 1,5,9-Trimethylbenzomorphans (1a) and Derivatives.—A mixture of α-(−)-1e (0.7 g), 40% CH_2O solution (0.45 ml), 5% Pd–C (0.16 g), and EtOH (25 ml) was shaken with H_2 (atmospheric pressure, room temperature) until gas absorption ceased. The product was filtered and the filtrate was concentrated to give α-(−)-**1a**, mp 184–186° (lit.[14] 183–184.5°). It gave a **hydrobromide,** mp 248–250° (lit.[14] 238–241°). Reductive methylation of β-(+)-1e gave β-(+)-1a, mp 181–183° (lit.[14] 183–184.5°), hydrobromide mp 274–276° dec (lit.[14] 238–242°). *Anal.* ($C_{13}H_{22}BrNO$) C, H. A mixture of α-(−)-**1a** (0.2 g), MeI (1 ml), and $CHCl_3$ (300 ml) was stirred at 38° under reflux for 5 days, concentrated to 25 ml, and diluted with Et_2O. The solid which separated was recrystallized from EtOH–Et_2O to give α-(−)-1a **methiodide,** mp 240–245°. *Anal.* ($C_{16}H_{24}INO$) C, H, N. β-(+)-1a **methiodide** was prepared similarly, mp 312–316°. *Anal.* C, H.

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A Racemic Form of 5-Ethyl-5-(3-hydroxy-1-methylbutyl)barbituric Acid as a Metabolite of Pentobarbital[1]

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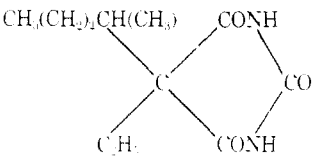
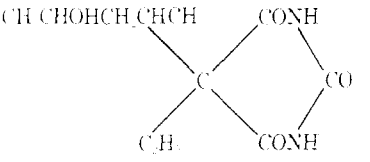
The availability of the racemic form of 5-ethyl-5-(3-hydroxy-1-methylbutyl)barbituric acid (II), recently synthesized by Dickert, Shea, and McCarty,[2] prompts us to report the isolation of this compound from the urine of dogs given anesthetic doses of pentobarbital (I). Following the removal of the (+) and (−) diastereoisomers in the reported manner,[3] the new metabolite separated from the filtrate as long, blunt needles. Elementary analysis, the uv spectrum, and a positive iodoform test suggested its probable structure. The melting points of the pure compound and its acetate derivative differed from those of the optically active isomers. However, the chromatographic behavior and ir spectrum of the racemic product were identical with those of the (+) enantiomorph. The new metabolite did not depress the melting point of the synthetic compound.



I



II

The discovery of the racemic alcohol in urine increases the probability that all four enantiomorphs of 5-ethyl-5-(3-hydroxy-1-methylbutyl)barbituric acid are involved in the metabolism of pentobarbital. Isotope dilution experiments with the optically active diastereoisomers[3,4] have undoubtedly given an erroneously low estimate of the quantitative importance of the urinary excretory products derived from penultimate oxidation of the drug. In dogs the two unconjugated alcohols accounted for about 50% of the excreted isotope.[3] Inasmuch as the usual chromatographic methods do not distinguish between the corresponding optically active and racemic forms, the estimates of Titus and Weiss[5] probably include the unknown enantiomorphs. Their data indicated that the unconjugated penultimate alcohols accounted for 62% of the recovered isotope.

Inasmuch as the sum of the conjugated and unconjugated optically active forms of 5-ethyl-5-(3-hydroxy-1-methylbutyl)barbituric acid exceeds 50% of the dose of pentobarbital,[5,6] these metabolites must be formed from different optical isomers of the drug. The strongly dextrorotatory ($[\alpha]^{28}D$ +26.6°) alcohol may be presumed to be derived from the *d* enantiomorph of pentobarbital by the addition of another *d* center. The weakly levorotatory ($[\alpha]^{28}D$ −5.6°) metabolite would then involve the *l* form of the drug and the same *d* center introduced by hydroxylation. On this basis the new metabolite may be designated as *dd* + *ll*. Since this substance depressed the melting point of the *dd* form, it is probably a racemic compound rather than a racemic mixture. An attempt to racemize the *dd* alcohol did not succeed.

Experimental Section[7]

Optically Active Isomers of 5-Ethyl-5-(3-hydroxy-1-methylbutyl)barbituric Acid (II).—The 24-hr urine from 15 dogs given 12.5 g (50 mg/kg) of sodium pentobarbital by mouth was brought to pH 6.5 and extracted continuously with ether for 48 hr. The combined extracts (750 ml) were shaken ten times with 150-ml portions of H_2O. After evaporation *in vacuo* to 15 ml and brief storage at 4°, the aqueous extract deposited 612 mg of the crude dextrorotatory alcohol. Reduction of the volume of the filtrate to 3 ml yielded 1490 mg of the crude levorotatory alcohol. Both alcohols were purified as described previously.[3]

No evidence of racemization was detected when a saturated aqueous solution of the pure dextrorotatory alcohol was heated in a sealed tube at 100° for 24 hr.

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(7) Melting points were determined on a Fisher-Johns block and not further corrected. Where analyses are indicated only by symbols of the elements, results obtained were within ±0.4% of the theoretical values.

Racemic 5-Ethyl-5-(3-hydroxy-1-methylbutyl)barbituric Acid (II).—Upon standing for a few days in a refrigerator the filtrate from the above procedure deposited 250 mg of light brown long blunt needles, mp 170–181°. After decolorization with charcoal in EtOH, repeated precipitation by heptane from Me_2CO, and recrystallization (H_2O), the product melted at 188–189°, $[\alpha]^{28}D$ 0 ± 0.1° (1.5, AcOH, $\lambda_{max}^{0.5NNaOH}$ 255 mμ (ε 6600). *Anal.* C, H, N.

The compound displayed a positive iodoform test.[8] Its ir spectrum was identical with that of the *d* alcohol[3] but different from that of the *l* alcohol.[3] It depressed the melting points of both optically active alcohols but not that of synthetic 5-ethyl-5-(3-hydroxy-1-methylbutyl)barbituric acid.[2]

Chromatography.—The ω-1 metabolites of pentobarbital were examined in four systems (Table I). The dried paper chromatograms were sprayed with 0.5 *N* NaOH and inspected in uv light. The dried thin layer plates were sprayed with a saturated solution of $HgNO_3$ to reveal the barbiturates as shiny, grayish white spots.[9] In all systems mixtures of the *d* and racemic alcohols yielded only one spot, whereas mixtures involving the *l* alcohol yielded two spots.

TABLE I
R_f VALUES OF
5-ETHYL-5-(3-HYDROXY-1-METHYLBUTYL)BARBITURIC ACID

Metabolite		System[a]			
Mp, °C	$[\alpha]^{28}D$, deg	A	B	C	D
209–210	+26.6	0.63	0.58	0.63	0.44
152–153	−5.6	0.58	0.54	0.58	0.41
188–189	0	0.63	0.58	0.63	0.44

[a] A, *i*-PrOH–28% aqueous NH_3 (4:1), Whatman No. 1; B, *n*-BuOH saturated with 0.5% aqueous NH_3, Whatman No. 1; C, *i*-PrOH–28% aqueous NH_3 (4:1), thin layer silica gel G; D, $CHCl_3$–Me_2CO (1:1), thin layer silica gel G.

Racemic 5-Ethyl-5-(3-acetoxy-1-methylbutyl)barbituric acid was prepared and purified in the usual manner[3] and melted at 136–137°. *Anal.* C, H, N.

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Potential Antimalarial Agents. Derivatives of 2-Chloro-1,4-naphthoquinone

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The chemotherapy of malaria has been stimulated by the use of several classes of effective compounds such as quinolines, naphthoquinones, and sulfones, but it has been limited appreciably by the toxicity of the various chemicals. Boldt and Goodwine[1] reported extensive studies with chloroquine as an antimalarial agent. Ter Horst and Felix[2] demonstrated the high fungistatic activity of 2,3-dichloro-1,4-naphthoquinone; Fosdick, *et al.*,[3] found that 1,4-naphthoquinones were useful inhibitors of acid formation by oral bacteria, and Fieser[4] reported naphthoquinones as potential antimalarials. DeGowin, *et al.*,[5] then showed that 4,4′-diaminodiphenyl sulfone possessed high antimalarial activity. For a number of years, we have also synthesized and studied the effects of a number of naphthoquinone derivatives as potential chemotherapeutic agents. The results have indicated that certain amine derivatives of 2-chloro-1,4-naphthoquinone may possess significant antibacterial activity *in vitro*. This study stimulated our interest in the possibility of these amine derivatives as potential antimalarial agents. The chemical structures of the amine derivatives are shown in Figure 1. The present report includes the synthesis and evaluation of 64 compounds, with analyses and tests for acute toxicity in mice for *in vivo* antimalarial activity against *Plasmodium berghei* infection in mice.

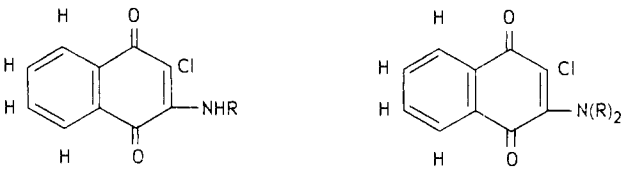

Figure 1.—Amine derivatives of 2-chloro-1,4-naphthoquinone: I, primary amine-substituted derivative, RNH = component added; II, secondary amine-substituted derivative, $(R)_2N$ = component added.

Experimental Section

General Procedure.—All the amine compounds employed were commercial preparations. The new amine derivatives were prepared by condensation of 2,3-dichloro-1,4-naphthoquinone with various primary and secondary amine compounds as follows. A 0.1-mole amount of 2,3-dichloro-1,4-naphthoquinone suspended in warm 95% EtOH (200 ml) was mixed with excess amine (0.2 mole) in EtOH (50 ml) and refluxed gently. Excess amine was employed in the system to neutralize the liberated HCl. The mixtures turned red. The basic aliphatic amines condensed readily on refluxing for 30 min. In most instances, the red crystalline product precipitated from the warm reaction mixture. Equimolecular quantities of the sulfones, amino acids, and pyridine compounds with the 2,3-dichloro-1,4-naphthoquinone had to be refluxed 15–18 hr for condensation to take place. After cooling, the insoluble condensation products were filtered and crystallized (70% EtOH). The products were fine shiny crystalline compounds obtained in yields from 70–95%. See Table I.

Acute Toxicity.—Toxicity studies on the compounds were performed in the DBA strain of mice, as maintained at the National Institutes of Health, Bethesda, Md. The chemicals were suspended in 0.25% Methocel (Methylcellulose, Dow Chemical Co., Midland, Mich.) so that the dose per 20-g mouse was contained in 0.25 ml for subcutaneous injection and the results were judged by 72-hr survival. The tolerated dose of the various preparations ranged from 500 to 2000 mg/kg. Most of the compounds were of relatively low toxicity as compared with 2,3-dichloro-1,4-naphthoquinone. The highest dose of the 2,3-dichloro-1,4-naphthoquinone tolerated by DBA mice was 250 mg/kg.

Antimalarial Activity.—All of the derivatives of 2-chloro-1,4-naphthoquinone as well as the positive control chloroquine diphosphate were evaluated subcutaneously for antimalarial activity in *Plasmodium berghei* infected mice by Dr. Leo Rane of the University of Miami. The testing procedure employed has been described previously.[6] Among the 64 compounds, bis[2-chloro-1,4-naphthoquinone-3,3′-sulfonylbis(*p*-phenylenimine)] (I) and N^4-(2-chloro-1,4-dihydro-1,4-dioxo-2-naphthyl)sulfanilamide (II) were found to possess high antimalarial activity against this parasite, as evidenced by the curative effect of I with survival of one, three, and two of five infected mice for 60 days in the mice that received doses of 40, 80, and 160 mg/kg, respectively. Higher doses of 320 and 640 mg/kg were toxic. Compound II was curative at 320, 640, and 1280 mg/kg with four, five, and

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