139–141 °C from dichloromethane/acetone; ^{1}H NMR δ 1.18 (3 H, s, C-13 CH_3), 1.26 (3 H, s, C-13 CH_3), 2.16 (3 H, s, C-20 CH_3), 3.25 (1 H, t, J = 9.3 Hz, 17β-H), 5.30 (1 H, d, J = 0.7 Hz, C-4 H). Anal. ($C_{21}H_{30}O_3$) C, H.

[^{3}H]Ouabain Radioligand Binding Assay. Hearts from pentobarbital-anesthetized mongrel dogs were removed and immediately immersed in Krebs–Henseleit buffer (mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.4, $NaHCO_3$ 26, glucose 11) equilibrated with 95% O_2–5% CO_2 at room temperature. Within 60 min the ventricles were chopped with scissors and disintegrated by Polytron treatment (Brinkman Instruments Inc.) in ice-cold 50 mM Tris buffer, pH 7.4. The suspension was passed through a coarse screen to remove the bulk of connective tissue and the filtrate was centrifuged at 34000g for 45 min at 4 °C. The supernatant was discarded and the pellet resuspended in the buffer for radioligand binding assay (see below) and stored at −20 °C. Once thawed, the tissue suspension was used immediately or discarded. The buffer was 45 mM Tris, 5 mM Tris phosphate, 3 mM $MgCl_2$, pH 7.4. Duplicate assay tubes contained 2.0 nM [^{3}H]ouabain in 0.1 mL of buffer, approximately 10 mg of tissue (wet weight) in 0.1 mL of buffer, and steroids (see below). Nonspecific binding (about 10% of total binding) was determined in the presence of 0.1 mM ouabain. Following incubation at 0 °C for 90 min, assay tubes were centrifuged at 2700g for 25 min at 4 °C and the supernatants aspirated off. Pellets were dissolved in 0.3 mL of 2 N KOH and 0.2-mL aliquots dispensed into vials for liquid scintillation counting. Data were expressed as percent of specifically bound [^{3}H]ouabain (i.e., difference between total binding and nonspecific binding). The steroids dissolved in a few microliters of 95% ethanol were added to reaction tubes; this volume of ethanol alone had no effect on specific binding of [^{3}H]ouabain. Similar potencies were obtained for the steroids in the radioligand binding assay when the ethanolic solutions were evaporated to dryness in the reaction tube, tissue suspension was added, and the tube was vortexed for 1 min.

Measurement of Contractility of Isolated Cardiac Tissue. Dogs of either sex, weighing between 5 and 12 kg were anesthetized with sodium pentobarbital (35 mg/kg ic), and the heart was removed. Thin (<1-mm diameter), free-running trabecula were tied to the base of a perspex holder which had embedded electrodes for punctate stimulation. The opposite end of the tissue was connected to a Grass FT-03C isometric force transducer and a Grass Polygraph. A Pulsar 6i stimulator (F Haer Co.) connected to a custom-built computer-controlled programmable pulse generator[15] provided square wave stimuli of 3-ms duration to the trabecula. Stimulus amplitude was adjusted to about 10–20% above threshold. The resulting tension was increased until the maximum active tension in response to electrical stimulation was evoked. The basic stimulus interval was 2000 ms. Tissues were placed in a 10-mL vertical tissue bath containing Krebs–Henseleit solution bubbled with 95% O_2 and 5% CO_2, and the bath temperature was maintained at 37.0 ± 0.2 °C. The muscle was equilibrated for 1 h prior to the experiment.

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Endoperoxides as Potential Antimalarial Agents

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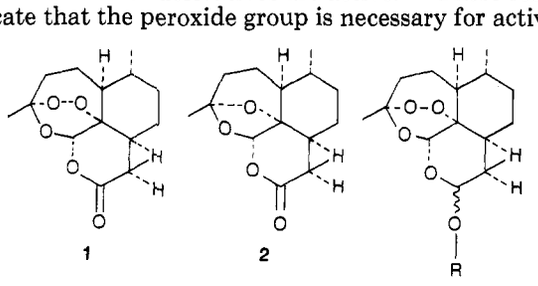
A number of mono- and bicyclic endoperoxides were prepared and tested for antimalarial activity in search of a simplified analogue of the 5-oxygen-substituted 1,2,4-trioxane ring structure of the naturally occurring antimalarial qinghaosu. The compounds were assayed in an in vitro system for antimalarial activity against chloroquine-susceptible and chloroquine-resistant strains of *P. falciparum*. The most active compound in this assay was 2-[((butyloxy)carbonyl)oxy]-1,1,10-trimethyl-6,9-epidioxy-Δ^7-octalin (**17a**), which showed an IC_{50} of 100 and 57 ng/mL, respectively. For comparison, qinghaosu exhibits a mean IC_{50} < 3.4 ng/mL.

Artemisinin (qinghaosu, **1**), a novel sesquiterpene lactone antimalarial drug, shows antimalarial activity in both animals and humans against chloroquine-sensitive and -resistant strains of *Plasmodium falciparum*.[1-3] Structure–activity relationship studies on **1** show that desoxyqinghaosu (**2**) is inactive whereas dihydroqinghaosu (**3**) and its ether and ester derivatives are active.[1,3] These results indicate that the peroxide group is necessary for activity.



1 **2** **3**

3a, R = H

Since no information about the necessity of the remainder of the qinghaosu molecule is available, we have undertaken an investigation to prepare three types of model compounds for antimalarial evaluation: (1) cyclic peroxides, (2) 1,2,4-trioxanes, and (3) 1,2,4-trioxanes containing a 5-oxygen substituent. These three types of compounds separate the 5-oxa-1,2,4-trioxane ring of **1** into its component parts while maintaining the peroxide function. In this paper we present the syntheses and antimalarial evaluation of members of the first set of compounds, cyclic peroxides.

Chemistry

1,5,5-Trimethyl-6-(3-acetoxybutyl)-3,6-epidioxycyclohexene (**7**) was prepared in order to explore the efficacy of a peroxide group in a simple monocyclic structure for

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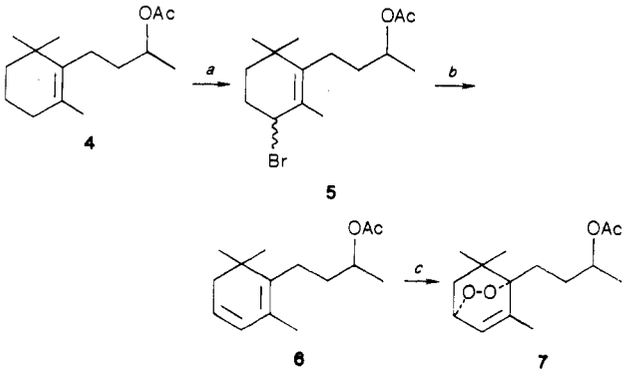
Chart I



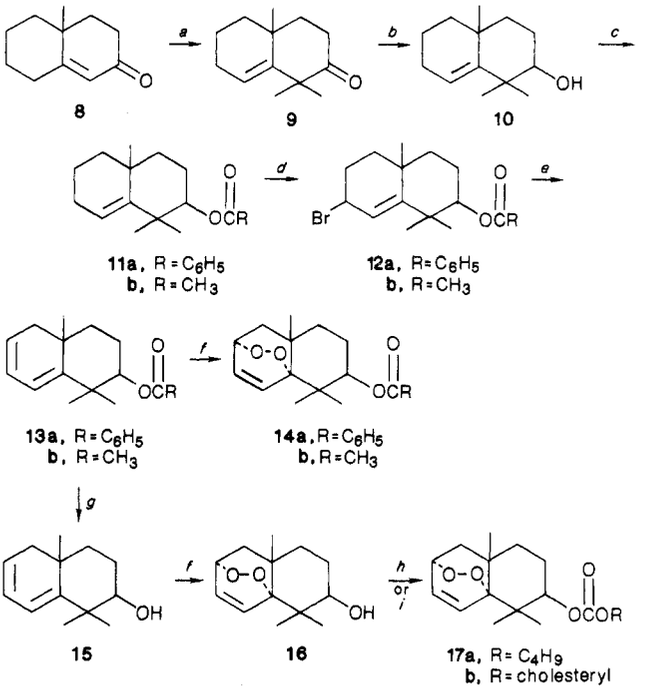
[a] NBS, CCl_4. [b] *N,N*-Dimethylaniline. [c] O_2, rose bengal.

antimalarial activity. The preparation of this compound (see Chart I) has been briefly described in the literature,[4] but without experimental details or spectral data. The light-catalyzed reaction of dihydro-β-ionol acetate (**4**) with *N*-bromosuccinimide afforded allylic bromide **5**, which was not isolated but treated with *N,N*-dimethylaniline (DMA) to afford the diene acetate **6**.[5] The rose bengal sensitized photooxygenation of **6** afforded the target compound **7**. The photooxygenation reaction gave a cleaner, more easily purified product when polymer-bound rose bengal rather than rose bengal itself was used as sensitizer. ^1H NMR analysis of **7** suggests that it is a 2:1 mixture of diastereomers. The allylic methyl group appears as two doublets (J = 1.8 Hz) at 1.95 and 1.98 ppm in a 2:1 ratio, respectively, and the C-3′ hydrogen appears as a doubled multiplet at 4.8 and 4.96 ppm in a 1:2 ratio, respectively.

The epidioxy-Δ^7-octalins (**14a,b**, **16**, and **17a,b**) were prepared in order to study the effect of the peroxide group in bicyclic ring systems. Even though compound **14a** as well as the intermediates for its preparation was reported in the literature,[6,7] neither experimental details nor physical characteristics were given. Thus, it was necessary to develop synthetic methods for the preparations of **14a** as well as **14b**, **16**, and **17**. The general scheme is shown in Chart II. 2-Keto-10-methyl-$\Delta^{1,9}$-octalin (**8**)[8,9] was treated with an excess of potassium *tert*-butoxide and iodomethane to yield the corresponding 1,1-dimethyl derivative **9**.[10] The ketone **9** was reduced with sodium borohydride to alcohol **10**. The benzoate **11a** was prepared by esterification of **10** with benzoyl chloride in the presence of pyridine.

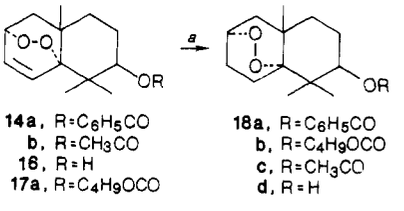
The ester **11a** was converted to the allylic bromo derivative **12a** with *N*-bromosuccinimide using benzoyl peroxide catalyst. The crude **12a** was heated with DMA to give the diene benzoate **13a**. When **13a** was subjected to photooxygenation using eosin as sensitizer in absolute alcohol according to the literature procedure,[7] 7% of **14a** was obtained along with a complex mixture of decomposition products. If the photooxygenation was carried out with polymer-bound rose bengal as sensitizer, however, compound **14a** was obtained in 63% yield. The ^1H NMR

Chart II



[a] *t*-BuOK, *t*-BuOH, MeI. [b] $NaBH_4$. [c] C_6H_5COCl, Pyr, Et_2O or $(CH_3CO)_2O$, Pyr. [d] NBS, CCl_4, $h\nu$. [e] DMA, heat. [f] $^1O_2$. [g] $LiAlH_4$. [h] Pyr, $ClCO_2C_4H_9$ [i] Pyr, cholesteryl chloroformate.

Chart III



[a] PADA, CH_3OH–AcOH.

spectrum of **14a** showed a resonance at 4.56 ppm for the proton attached to carbon bearing the epidioxide.

The acetate **11b** was prepared by esterification of **10** with acetic anhydride in pyridine. This ester was converted to the corresponding allylic bromo analogue **12b** with *N*-bromosuccinimide using benzoyl peroxide catalyst. The diene acetate was prepared from the crude bromo derivative **12b** by heating with DMA. Rose bengal sensitized photooxygenation of **13b** gave the target compound **14b**. Compound **14b** was characterized by its ^1H NMR spectrum, which showed resonances at 6.61 ppm for the olefinic proton and 4.56 ppm for the proton attached to the epidioxide carbon.

Preparation of diene **15** by the bromination–dehydrobromination of **10** was not successful. Consequently, the acetate **13b** was used as the starting compound for the synthesis of compounds **16** and **17**. Thus, **13b** was converted to the alcohol **15** by LAH reduction. The alcohol **15** was oxygenated in the presence of polymer-bound rose bengal to give the target compound **16**. Since the product tends to decompose under the experimental conditions, the reaction was stopped when approximately 50–60% of the starting material had been consumed. The product **16** was characterized by its ^1H NMR spectrum, which showed resonances at 6.56–6.68 ppm for the olefinic protons and at 4.51 ppm for the proton attached to the epidioxide carbon. Compounds **17a** and **17b** were obtained by treating **16** with butyl chloroformate or cholesteryl

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chloroformate in the presence of pyridine.

Since all the epidioxy-Δ^7-octalins were unstable when left at ambient temperatures for several days, these compounds were converted to their corresponding 2-substituted 1,1,10-trimethyl-6,9-epidioxydecalins (**18a–d**) with the hope of improving their chemical stability. Since the reduction of double bonds in the presence of the endoperoxy function using diimide had been reported,[11] diimide reduction of **14a** was investigated (see Chart III).

With diimide prepared from potassium azodicarboxylate (PADA) or azodicarboxamide,[12-14] we initially failed to achieve complete reduction of **14a**. This was a serious problem because of the difficulty encountered in separating **14a** from **18a**. We found that complete reduction could be achieved if PADA was prepared according to Berson et al.,[15] a 20-fold excess of PADA was used, and the reaction time was extended to 18 h. Using these conditions we were able to convert all the epidioxy-Δ^7-octalins to their corresponding saturated compounds **18a–d** in 74–87% yields. Compounds **18a–d** were more stable than their dehydro derivatives; however, storage at ambient temperature resulted in decomposition.

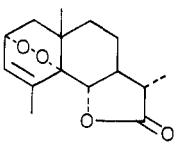
Biological Testing

Compounds **14a,b**, **16**, **17a,b**, and **18c,d** were assayed in an in vitro system for antimalarial activity against chloroquine-susceptible and chloroquine-resistant strains of *P. falciparum* by the method reported by Desjardins et al.[16] The activity in this test is expressed as concentration (ng/mL) causing 50% inhibition (IC_{50}) of the uptake of [^{3}H]hypoxanthine by *P. falciparum*. Compound **17a**, which showed an IC_{50} of 100 and 57 ng/mL, respectively, for the susceptible and resistant strains, was the most active of the compounds assayed. For comparison, qinghaosu exhibits a mean IC_{50} < 3.4 ng/mL against both strains.[17]

Compounds **7**, **14a,b**, **16**, **17a,b**, and **18a–d** were tested for blood schizonticidal activity against *P. berghei* in mice.[18] Testing was carried out at the Rane Laboratory, University of Miami, Miami, FL. The compounds were all inactive in the standard *P. berghei* screen at doses of 40, 160, and 640 mg/kg.

In summary, some simple cyclic peroxides were prepared and evaluated for antimalarial activity in order to gain information concerning what portions of the qinghaosu ring are necessary for activity. The absence of significant activity indicates that the peroxide linkage alone is insufficient for activity. However, we would like to point out that all of the epidioxides reported in this study were relatively unstable at ambient temperature. In contrast, qinghaosu is stable even when heated to 150 °C in neutral solvents.[19] Other investigators have also found qinghaosu to be surprisingly stable.[20,21]

The recent report[22] that 2,5α-epidioxy-6,11βH-eudesm-3-en-6,13-olide (**19**) was inactive when assayed against *P. Berghei* in mice and *P. falciparum* in vitro also indicates that the cyclic peroxide alone is insufficient for antimalarial activity.



19

Experimental Section

Melting points were determined on a Koffler hot stage. Infrared (IR) spectra were recorded on a Perkin-Elmer 457 spectrophotometer. Ultraviolet spectra were run on a Varian Model 2290 spectrophotometer. Proton magnetic resonance (^{1}H NMR) spectra were obtained on a Bruker 250 spectrometer. Chemical shifts were reported in δ values relative to tetramethylsilane (Me_4Si). Carbon magnetic resonance (^{13}C NMR) spectra were determined at 22.4 MHz on a JEOL FX-90Q spectrometer. Chemical shifts are reported in parts per million (ppm), and the δ scale is referenced to the Me_2SO-d_6 solvent, using 39.5 ppm relative to Me_4Si.

2,6,6-Trimethyl-1-(3-acetoxybutyl)cyclohexa-1,3-diene (6).[4] To a refluxing solution of carbon tetrachloride (400 mL) was added dihydro-β-ionol acetate (**4**)[5] (17.0 g, 0.66 mol) and *N*-bromosuccinimide (14.0 g, 0.77 mol). The mixture was irradiated with a 150-W light for 15 min. GLC analysis (3% SE-30 column 6 ft × 25 mm, at 140 °C) indicated that the reaction had gone to completion. The solid suspension was filtered, and the filtrate and washings were combined and treated directly with *N,N*-dimethylaniline (75 mL) and heated to reflux for 1 h. The product was taken up in ether, and the ethereal phase was washed with 10% (v/v) hydrochloric acid solution followed by dilute $NaHCO_3$ solution and water. The organic phase was dried (Na_2SO_4), filtered, and concentrated to give 23 g of crude reaction product, which was purified by silica gel column chromatography to provide 10 g of the diene acetate **6**: ^{1}H NMR ($CDCl_3$) δ 0.95 (s, 6, geminal CH_3), 1.2 (d, 3, J = 6 Hz, terminal CH_3), 2.0 (br s, 6, olefinic CH_3 overlapped with the acetate), 4.86 (m, 1, CHOAc), and 5.6 (br s, olefinic H); UV (MeOH) λ_{max} 263 nm.

GLC analysis showed that the diene acetate **6** was about 90% pure.

1,5,5-Trimethyl-6-(3-acetoxybutyl)-3,6-epidioxycyclohexene (7).[4] The diene acetate **6** (3.0 g, 0.012 mol) was dissolved in 80 mL of CH_2Cl_2 containing 1.0 g of polymer-bound rose bengal sensitizer. The solution was irradiated with a GE quartzline BWY-650 lamp for 3.5 h. The rose bengal dye was removed by filtration and washed with CH_2Cl_2. The concentrated filtrate was purified by silica gel column chromatography (5% EtOAc in CH_2Cl_2) to provide 1.8 g of pink oil. Repeated silica gel column chromatography afforded 1.2 g of **7** as a colorless oil. Along with the desired product **7**, some unreacted diene and possibly a secondary photooxygenation product were also isolated: ^{1}H NMR ($CDCl_3$) δ 0.83 (s, 3, geminal CH_3), 1.24 (s, 3, geminal CH_3), 1.23 (d, 3, J = 6 Hz, terminal CH_3), 1.95 (d, 2, J = 1.8 Hz, olefinic CH_3), 1.98 (d, 1, J = 1.8 Hz, olefinic CH_3, second isomer), 2.0 (s, 3, acetate CH_3) 4.45 (m, 1, CHOO), 4.8–4.96 (m, 1, CHOAc), and 6.24 (br d, 1, J = 5 Hz, olefinic H); GC-CI mass spectrum, m/z 269 (M + 1), 209 [M + 1 − 60 (acetic acid)]; EI mass spectrum, m/z 268 (M^+), 236 ($M^+ - O_2$). Anal. ($C_{15}H_{24}O_4$) C, H.

2-Keto-1,1,10-trimethyl-Δ^8-octalin (9).[10] To a cooled (10–15 °C) solution of **8**[7,9] (10.25 g, 0.0625 mol) and potassium *tert*-butoxide (21 g, 0.186 mol) in *t*-BuOH (60 mL) was added a solution of iodomethane (56 g, 0.4 mol) in *t*-BuOH (60 mL). After the addition, the reaction mixture was refluxed for 1 h. Most of the solvent was removed under reduced pressure, and the residue was dissolved in ethyl ether. The ether solution was washed with

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saturated NaCl solution and dried ($MgSO_4$). The oily substance obtained after removal of the solvent was chromatographed on silica gel (600 g). Elution with 10% ether–hexane gave 10 g of pure **9**. GLC (SE-30 6-ft column at 180 °C) showed a single peak: ^1H NMR ($CDCl_3$) δ 1.23 (s, 3, C_{10}-CH_3), 1.30 (s, 6, C_1-CH_3), 2.57 (t, 2, CH_2CO), and 5.57 (t, 1, CH=C).

2-(Benzoyloxy)-1,1,10-trimethyl-Δ^8-octalin (11a).[6] To a stirred solution of **9** (10 g, 0.052 mol) in 100 mL of MeOH at 0 °C was added $NaBH_4$ (5.5 g) portionwise. Stirring was continued for 40 min. Most of the solvent was removed under reduced pressure, and excess $NaBH_4$ was decomposed with dilute hydrochloric acid. The mixture was extracted with ether, and the extracts were washed with NaCl solution and dried (Na_2SO_4). Removal of the solvent gave the alcohol **10** (9.8 g), which was used directly for the preparation of the benzoate.

A solution of benzoyl chloride (16.5 g, 0.0117 mol) in 50 mL of $CHCl_3$ was added dropwise to a solution of the alcohol **10** in 150 mL of $CHCl_3$ and 25 mL of pyridine, and the reaction mixture was heated to reflux for 3 h. More benzoyl chloride (14 g, 0.1 mol) was added during this period. The mixture was cooled and washed successively with water, dilute hydrochloric acid, saturated Na_2CO_3 solution, and NaCl solution. The dried ($MgSO_4$) $CHCl_3$ solution was evaporated to a waxy solid. The mixture containing the desired benzoate and benzoic anhydride was dissolved in hexane and stirred for 3 h while dry NH_3 was passed into the solution. The precipitate was removed by filtration, and the filtrate was evaporated to dryness. The residue was crystallized from MeOH to give 10.76 g (69%) of **11a**: mp 74–76 °C; ^1H NMR ($CDCl_3$) δ 1.13 (s, 3, C_{10}-CH_3), 1.23 (s, 3, C_1-CH_3), 1.27 (s, 3, C_1-CH_3), 4.8 (m, 1, C_2-H), 5.53 (t, 1, C_8-H), and 7.05–8.1 (m, 5, aromatics).

2-(Benzoyloxy)-1,1,10-trimethyl-6,8-hexalin (13a).[6] A solution of **11a** (540 mg, 2 mmol) and *N*-bromosuccinimide (390 mg, 2.2 mmol) along with a catalytic amount of benzoyl peroxide in CCl_4 (10 mL) was heated to reflux for 15 min. The cooled solution was filtered, and the filtrate was evaporated to dryness. The crude bromo analogue (**12a**) was dissolved in *N,N*-dimethylaniline (2 mL) and was heated on a steam bath for 30 min. The solvent and *N,N*-dimethylaniline were removed under reduced pressure, and the residue was dissolved in ether. The ether solution was washed with dilute hydrochloric acid and saturated NaCl solution. The crude product obtained on evaporation was chromatographed on silica gel (30 g). Elution with $CHCl_3$ gave 0.27 g (51%) of **13a** as a waxy solid: ^1H NMR ($CDCl_3$) δ 1.16 (s, 3, C_{10}-CH_3), 1.23 (s, 3, C_1-CH_3), 1.36 (s, 3, C_1-CH_3), 4.93 (t, 1, CHO), 5.95 (m, 3, olefinic), and 7.46–8.2 (m, 5, aromatics).

This reaction was repeated on larger scales to give 50–54% yields.

2-(Benzoyloxy)-1,1,10-trimethyl-6,9-epidioxy-Δ^7-octalin (14a). Polymer-bound rose bengal (100 mg) was suspended in a solution of diene benzoate **13a** (500 mg, 1.63 mmol) in 70 mL of methylene chloride and irradiated in the presence of oxygen at room temperature by using a GE quartzline BWY-650 lamp for 2.5 h. The polymer-bound dye was separated by filtration and the filtrate evaporated to dryness. The residue was dissolved in 5% EtOAc–CH_2Cl_2, applied to a silica gel column, and eluted with the same solvent system. The pure fractions were pooled and evaporated to give 0.32 g (63%) of pure **14a**. The product was recrystallized from methylene chloride–pentane: mp 156–158 °C (lit.[7] mp 168 °C); ^1H NMR ($CDCl_3$) δ 1.08 (s, 3, C_1-CH_3), 1.09 (s, 3, C_1-CH_3), 1.35 (s, 3, C_{10}-CH_3), 4.56 (m, 1, HCOO), 5.29 (m, 1, CHO), 6.59–6.71 (m, 2, olefinic), and 7.41–8.05 (m, 5, aromatics). Anal. ($C_{20}H_{24}O_4$) C, H.

The experiment was repeated with 8.4 g of diene benzoate **13a** to give 5.05 g (55%) of the target compound. When eosin was used as sensitizer according to the reported procedure,[7] a lower yield was obtained.

2-Acetoxy-1,1,10-trimethyl-Δ^8-octalin (11b). The alcohol **10** prepared from 24.55 g (0.13 mol) of **9** was dissolved in 40 mL of pyridine, cooled in an ice bath, and treated with 40 mL of acetic anhydride. The mixture was stirred at room temperature overnight. The excess anhydride was decomposed by careful addition of water, and the resulting mixture was extracted with ether. The ether extracts were washed successively with dilute hydrochloric acid, 5% $NaHCO_3$ solution, and saturated NaCl solution. The dried ($MgSO_4$) ether extracts were concentrated under reduced pressure, and the residue was chromatographed on silica gel using 10% EtOAc in petroleum ether as eluant to give 17.95 g (49%) of pure acetate (**11b**) as a clear thick liquid: ^1H NMR ($CDCl_3$) δ 0.96 (s, 3, C_{10}-CH_3), 1.02 (s, 3, C_1-CH_3), 1.09 (s, 3, C_1-CH_3), 1.96 (s, 3, CH_3CO), 4.41 (m, 1, CHO), and 5.47 (t, 1, olefinic); IR 1734 (C=O) cm^{-1}.

2-Acetoxy-1,1,10-trimethyl-6,8-hexalin (13b). A solution of **11b** (17.5 g, 0.074 mol), *N*-bromosuccinimide (13.17 g, 0.074 mol), and 200 mg of benzoyl peroxide in 200 mL of carbon tetrachloride was heated to reflux for 15 min. The cooled mixture was filtered, and the residue was washed with a minimum amount of carbon tetrachloride. The filtrate and washings were evaporated under reduced pressure. The crude bromo analogue **12b** was dissolved in 30 mL of *N,N*-dimethylaniline and was heated on a steam bath for 40 min. The excess *N,N*-dimethylaniline was removed under reduced pressure. The resulting residue was dissolved in ether and washed with dilute hydrochloric acid followed by saturated NaCl solution. The crude product obtained on evaporation was chromatographed on silica gel (300 g) and eluted with 10% ether in hexane to give 10.95 g (63%) of **13b** as a viscous liquid: IR ($CHCl_3$) 1735 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.05 (s, 3, C_{10}-CH_3), 1.18 (s, 3, C_1-CH_3), 1.31 (s, 3, C_1-CH_3), 2.01 (s, 3, CH_3CO), 4.43 (m, 1, CHO), and 5.78 (m, 3, olefinic).

This product was used in the next step without further purification.

2-Acetoxy-1,1,10-trimethyl-6,9-epidioxy-Δ^7-octalin (14b). Polymer-bound rose bengal (1.0 g) was suspended in a solution of diene acetate **13b** (8.5 g, 0.036 mol) in 70 mL of CH_2Cl_2 and irradiated at room temperature by using a GE quartzline BW-Y-650 lamp while oxygen was passed through the solution for 3 h. The dye was separated by filtration, and the filtrate was evaporated to dryness. Recrystallization of the residue from ether–hexane gave 4.1 g of **14b**: mp 122–124 °C. An additional 1.8 g of **14b** was obtained by column chromatography of the mother liquor on silica gel (200 g, using 10% ether in hexane as eluant) to give a total yield of 5.9 g (59%): ^1H NMR ($CDCl_3$) δ 1.01 (s, 3, C_{10}-CH_3), 1.03 (s, 3, C_1-CH_3), 1.18 (s, 3, C_1-CH_3), 2.04 (s, 3, CH_3CO), 4.56 (m, 1, HCOO), 5.05 (m, 1, CHO), and 6.57–6.65 (m, 2, olefinic). Anal. ($C_{15}H_{22}O_4$) C, H.

2-Hydroxy-1,1,10-trimethyl-6,8-hexalin (15). To a stirred suspension of $LiAlH_4$ (4.0 g, 0.105 mol) in 50 mL of ether was added dropwise a solution of **13b** (6.53 g, 0.028 mol) in 50 mL of ether. The mixture was heated to reflux for 2.5 h. The excess $LiAlH_4$ was destroyed by successive addition of 4 mL of H_2O, 4 mL of 15% NaOH, and 12 mL of H_2O. The resulting solid material was removed by filtration and washed with ether. The combined filtrate and washings were dried over Na_2SO_4 and evaporated to dryness under reduced pressure to give 4.27 g (81%) of **15**. The material proved to be pure enough to be used in the next step.

2-Hydroxy-1,1,10-trimethyl-6,9-epidioxy-Δ^7-octalin (16). A solution of **15** (3.45 g, 0.018 mol) in 50 mL of CH_2Cl_2 was irradiated in the presence of polymer-bound rose bengal while O_2 was passed through the solution for 2.5 h. At this point approximately 50–60% of the starting material had reacted. The dye was separated by filtration, and the filtrate was evaporated under reduced pressure. The crude residue obtained was chromatographed on silica gel (200 g) using 20% EtOAc–CH_2Cl_2 as eluant. The first compound eluted from the column was unreacted starting material (1.54 g, 45%). Evaporation of the solvent from the fraction containing the second component gave 0.915 g (41%) of **16**. Recrystallization from CH_2Cl_2–hexane gave 0.76 g (34%) of pure **16**: mp 127–129 °C; ^1H NMR ($CDCl_3$) δ 1.01 (s, 3, C_{10}-CH_3), 1.11 (s, 6, C_1-CH_3), 3.81–3.87 (m, 1, C*H*OH), 4.50–4.55 (m, 1, 6, C*H*OO), and 6.56–6.68 (m, 2, olefinic). Anal. ($C_{13}H_{20}O_3$) C, H.

This reaction was repeated on a 0.072-mole scale and gave 38% yield.

2-[((Butyloxy)carbonyl)oxy]-1,1,10-trimethyl-6,9-epidioxy-Δ^7-octalin (17a). To a stirred solution of **16** (2.3 g, 0.0103 mol) and pyridine (1.00 g, 0.013 mol) in 25 mL of CH_2Cl_2 was added dropwise a solution of butyl chloroformate (2.7 g, 0.020 mol). After 3 h the mixture was washed with saturated NaCl solution and dried over Na_2SO_4. The drying agent was removed by filtration, and the filtrate was evaporated under reduced pressure. The resulting residue was purified by silica gel chromatography using first CH_2Cl_2 and then EtOAc–CH_2Cl_2 as eluant. Compound **17a** (2.85 g, 86%) was obtained as a viscous syrup:

IR ($CHCl_3$) 1740 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.94 (t, 3, CH_2CH_3), 1.02 (s, 3, C_1-CH_3), 1.07 (s, 3, C_1-CH_3), 1.20 (s, 3, C_{10}-CH_3), 4.13 (t, 2, OCH_2), 4.54 (m, 1, C_6-H), 4.75 (m, 1, C_2-H), and 6.58 (m, 2, olefinic). Anal. ($C_{18}H_{28}O_5$) C, H.

2-[((Cholesteryloxy)carbonyl)oxy]-1,1,10-trimethyl-6,9-epidioxy-Δ^7-octalin (17b). The sample of **17b** was prepared in a manner analogous to that described above for **17a**. Thus, 3.36 g (0.015 mol) of **16** in 5 mL of pyridine was treated with cholesteryl chloroformate (9.0 g, 30 mmol) to give, after purificiation by column chromatography and crystallization from CH_2Cl_2–hexane, 3.08 g of **17b**: mp 175–176 °C; IR ($CHCl_3$) 1740 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.67 (s, 3, $C_{18'}$-CH_3), 0.86 (d, 6, $C_{26'}$-CH_3 and $C_{27'}$-CH_3), 0.91 (d, 3, $C_{20'}$-CH_3), 1.00 (s, 3, C_1-CH_3), 1.01 (s, 3, C_1-CH_3), 1.07 (s, C_{10}-CH_3), 1.19 (s, 3, $C_{19'}$-CH_3), 4.53 (m, 1, C_3-H), 4.90 (dd, 1, C_2-H), 5.38 (m, 1, C_6-H), and 6.55–6.69 (m, 3, olefinic). Anal. ($C_{41}H_{64}O_5$) C, H.

2-(Benzoyloxy)-1,1,10-trimethyl-6,9-epidioxydecalin (18a). To a flask containing a stirred mixture of 50 mL of CH_2Cl_2 and 50 mL of MeOH cooled in an ice–NaCl bath (~−10 °C) were added **14a** (2.3 g, 0.007 mol) and potassium azodicarboxylate (27.3 g, 0.14 mol). Acetic acid (8.4 g) in 50 mL of CH_2Cl_2 was added dropwise over a period of 45 min and stirred overnight while the temperature was allowed to rise to 23–25 °C. The solid residue was removed and washed with CH_2Cl_2. The filtrate and washings were combined and evaporated to dryness to give 2.31 g of product. Recrystallization from CH_2Cl_2–pentane gave 1.94 g (84%) of **18a**: mp 146–148 °C; ^1H NMR ($CDCl_3$) δ 1.05 (s, 3, C_1-CH_3), 1.18 (s, 3, C_1-CH_3), 1.29 (s, 3, C_{10}-CH_3), 4.12 (m, 1, HCOO), 5.14 (m, 1, HCO), and 7.39–8.03 (m, 5, aromatics). Anal. ($C_{20}H_{26}O_4$) C, H.

2-[((Butyloxy)carbonyl)oxy]-1,1,10-trimethyl-6,9-epidioxydecalin (18b). The reduction of the olefinic analogue **17a** was carried out as described for the benzoyloxy analogue **14a**. Thus, to a stirred suspension of **17a** (3.24 g, 0.010 mol) and potassium azodicarboxylate (39.1 g, 0.2 mol) in 120 mL of a mixture of 50% CH_2Cl_2 and MeOH at 5 to 0 °C was added acetic acid (12.0 g, 0.2 mol) in 50 mL of CH_2Cl_2 over a period of 3.5 h. The stirred reaction mixture was allowed to warm to 23–25 °C and remained in that temperature range overnight. Enough water was added to the mixture to dissolve all of the solids. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (50 mL). The combined extracts were washed with NaCl solution and dried ($MgSO_4$). Evaporation of the solvent gave 3.16 g (97%) of a waxy substance, which solidified under vacuum. Recrystallization from hexanes gave 2.60 g (80%) of the target compound **18b**: mp 46–48 °C; ^1H NMR ($CDCl_3$) δ 0.93 (t, 3, CH_2CH_3), 1.03 (s, 6, C_1-CH_3), 1.23 (s, 3, C_{10}-CH_3), 4.11 (m, 3, OCH_2 and C_6-H), and 4.74 (dd, 1, C_2-H). Anal. ($C_{18}H_{30}O_5$) C, H.

2-Acetoxy-1,1,10-trimethyl-6,9-epidioxydecalin (18c). The reaction was carried out as described for **14a**. Thus, 2.95 g (0.011 mol) of **14b** was reduced with 42.0 g (0.22 mol) of potassium azodicarboxylate and 13.2 g (0.22 mol) of acetic acid. After workup, the residue was recrystallized from CH_2Cl_2–hexanes to give 2.21 g (74%) of **18c**: mp 131–133 °C; ^1H NMR ($CDCl_3$) δ 0.97 (s, 3, C_1-CH_3), 1.02 (s, 3, C_1-CH_3), 1.24 (s, 3, C_{10}-CH_3), 2.02 (s, 3, $COCH_3$), 4.1 (m, 1, HCOO), and 4.89 (dd, 1, C_2-H). Anal. ($C_{15}H_{24}O_4$) C, H.

2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin (18d). Compound **18d** was prepared as described for **18a–c**. Thus, **16** (2.8 g, 0.0125 mol) with potassium azodicarboxylate (46.8 g, 0.24 mol) and acetic acid (14.4 g, 0.24 mol) gave, after recrystallization from CH_2Cl_2–pentane, 2.51 g (87%) of **18d**: mp 148–149 °C; ^1H NMR ($CDCl_3$) δ 0.95 (s, 3, C_1-CH_3), 1.08 (s, 3, C_1-CH_3), 1.22 (s, 3, C_{10}-CH_3), 3.68 (dd, 1, CHOH), and 4.08–4.18 (m, 1, HCOO). Anal. ($C_{13}H_{22}O_3$) C, H.

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Registry No. **4**, 108511-73-5; **6**, 57069-88-2; **7** (isomer 1), 108511-74-6; **7** (isomer 2), 108590-48-3; **8**, 826-56-2; **9**, 4668-61-5; **10**, 91975-35-8; **11a**, 102454-53-5; **11b**, 108511-75-7; **12a**, 108511-75-7; **12a**, 108511-76-8; **12b**, 108511-77-9; **13a**, 94759-83-8; **13b**, 101100-51-0; **14a**, 108511-78-0; **14b**, 108511-79-1; **15**, 100532-28-3; **16**, 108511-80-4; **17a**, 108511-81-5; **17b**, 108511-82-6; **18a**, 108511-83-7; **18b**, 108533-24-0; **18c**, 108511-84-8; **18d**, 108511-85-9; MeI, 74-88-4; C_6H_5COCl, 98-88-4; $ClCO_2C_4H_9$, 592-34-7; cholesteryl chloroformate, 7144-08-3.

Synthesis and Biological Activities of Arginine-vasopressin Analogues with 4-Hydroxyproline in Position 7[†,‡]

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Three arginine-vasopressin (AVP) analogues in which the proline residue in position 7 was substituted with 4-hydroxyproline were synthesized by solid-phase techniques, and their biological activities were evaluated by antidiuretic, pressor, and uterotonic bioassays. The [7-*trans*-4-hydroxy-L-proline]AVP, the 1-desamino[7-*trans*-4-hydroxy-L-proline]AVP, and the 1-desamino[7-*cis*-4-hydroxy-L-proline]AVP analogues showed a high antidiuretic and strikingly high uterine activity, a sharp decrease in pressor activity, and a better antidiuretic and uterine to pressor selectivity than the parent compound, arginine-vasopressin. The uterine activities are the highest so far assayed in AVP analogues with replacements in position 7.

Analogues of the neurophypophyseal hormone arginine-vasopressin that have substitutions for proline in position 7 (the first residue in the tripeptide -Pro-Arg-GlyNH_2 "tail" portion of this molecule) are of considerable interest for structure–activity studies. The proline residue is important in positioning the "tail" portion of vasopressin correctly in relation to the "ring" portion of this molecule and thus is very important for biological activity. All replacements of the naturally occurring proline that have been made to date drastically alter the activity and/or specificity of vasopressin. Some 7-substituted analogues, such as [7-glycine]AVP and [7-leucine]AVP[1,2] have de-

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† Abbreviations follow the recommendations of IUPAC–IUB Commission on Biochemical Nomenclature (*Int. J. Pept. Protein Res.* **1984**, *24*, 9). Additional abbreviations are as follows: MeBzl, methylbenzyl; *N*-MeAla, *N*-methylalanine; Hyp, 4-hydroxyproline; AcOH, acetic acid; 1-BuOH, 1-butanol; *i*-PrOH, 2-propanol; DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide; HOBt, 1-hydroxybenzotriazole; DIPEA, diisopropylethylamine; AVP, arginine–vasopressin; d, 1-desamino; TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography.

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