1,2,4-Trioxanes as Potential Antimalarial Agents

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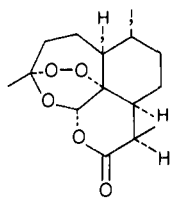
A number of 1,2,4-trioxanes were prepared and tested for antimalarial activity in search of a simplified analogue of the naturally occurring antimalarial qinghaosu. The compounds were assayed in an in vitro system for antimalarial activity against chloroquine-susceptible and chloroquine-resistant strains of *Plasmodium falciparum*. The most active compounds were methyl 2-(2,4a-epidioxy-4a,5,6,7,8,8a-hexahydro-5,5,8a-trimethyl-2*H*-1-benzopyran-2-yl)acetate (**3b**), which showed IC_{50}'s of 96 and 39 ng/mL, respectively, and 2,4a-epidioxy-3,4,4a,5,6,7,8,8a-octahydro-2-[2-(benzoyloxy)propyl]-5,5,8a-trimethyl-2*H*-1-benzopyran (**12**), which showed IC_{50}'s of 24 and 99 ng/mL, respectively. For comparison, qinghaosu exhibits an IC_{50} of 1 ng/mL for both strains.

Chinese workers have reported the isolation and characterization of a novel sesquiterpene lactone, artemisinine (qinghaosu, **1**), which is active against both chloroquine-sensitive and resistant strains of *Plasmodium falciparum*[1-3] and appears to be especially effective in treatment of malaria infections of the brain.[3] Structure–activity studies



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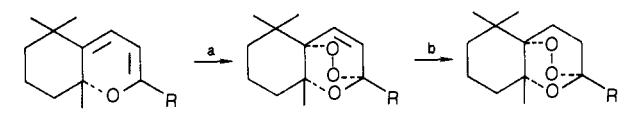
on a number of derivatives of **1** have established the necessity of the peroxide group for antimalarial activity.[1,3] No information concerning the necessity of the rest of the molecule is available.

We have undertaken an investigation to attempt to determine the minimum requirements for antimalarial activity of qinghaosu-like molecules that focuses on the unique 5-oxygen-substituted 1,2,4-trioxane ring of **1**. Specifically, the investigation of three types of compounds for antimalarial evaluation is being undertaken: (1) cyclic peroxides, (2) 1,2,4-trioxanes, and (3) 1,2,4-trioxanes containing a 5-oxygen substituent. In an earlier paper we described the synthesis and antimalarial activity of a number of simple cyclic peroxides,[4] in this paper we describe the synthesis and antimalarial activity of some compounds containing a 1,2,4-trioxane ring.

Chemistry. Compound **3a** was prepared as described in the literature.[5] Compound **3b** was prepared similarly by singlet oxygen addition to the known tetrahydrobenzopyran **2b**.[6] Diimide[7] reduction of **3a** and **3b** afforded their saturated analogues **4a**[8,9] and **4b** (Scheme I). The stereochemistry of the singlet oxygen addition is assigned

Scheme I[a]

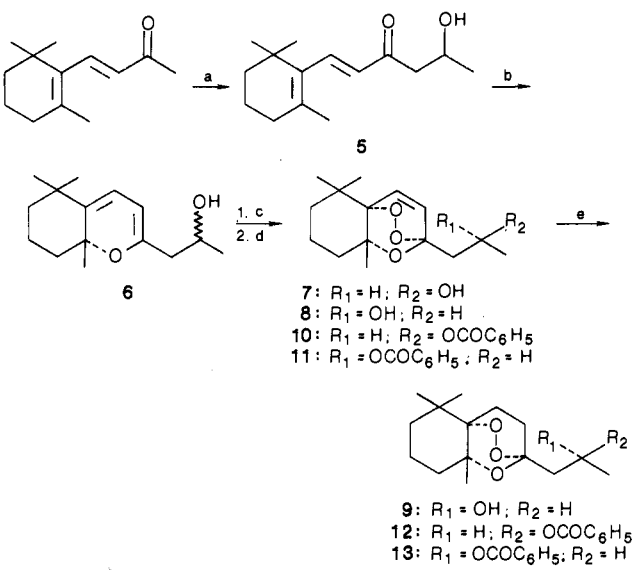


2a: R = CH_3
b: R = CH_2COOCH_3

3 **4**

[a] (a) *hν*, O_2, rose bengal; (b) potassium azodicarboxylate (PADA)/HOAc.

Scheme II[a]



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7: R_1 = H; R_2 = OH
8: R_1 = OH; R_2 = H
10: R_1 = H; R_2 = $OCOC_6H_5$
11: R_1 = $OCOC_6H_5$; R_2 = H

9: R_1 = OH; R_2 = H
12: R_1 = H; R_2 = $OCOC_6H_5$
13: R_1 = $OCOC_6H_5$; R_2 = H

[a] (a) BuLi, diisopropylamine, CH_3CHO; (b) *hν*, Et_3N; (c) *hν*, O_2, rose bengal; (d) C_6H_5COCl, Py, $CHCl_3$; (e) potassium azodicarboxylate/HOAc.

as shown on the basis of comparison of the ^1H NMR spectra of the olefins **3a** and **3b** with their corresponding saturated analogues **4a** and **4b**. The 8a-methyl resonances of **3a** and **3b** are shifted 0.14 and 0.25 ppm upfield from the corresponding methyl resonances of their saturated analogues. This upfield shift is attributed to the diamagnetic anisotropy of the 3,4-double bond of **3a** and **3b**.[10] This type of shift has been observed in the related bicyclo[2.2.2]octene system.[11] The magnitude of the shift (0.13–0.25 ppm) agrees reasonably well with the average value of 0.23 ppm calculated by the method of Yamaguchi et al.[10,11] from the energy-minimized structure of **3a** generated by using the SYBYL software of Tripos Associates. Compounds **7–13** were prepared from tetrahydrobenzopyran **6** as shown in Scheme II. Reaction of β-ionone with

(1) Klayman, D. L. *Science (Washington, D.C.)* **1985**, *228*, 1049.
(2) (a) Liu, J.-M.; Ni, M.-Y.; Fan, J.-F.; Tu, Y.-Y.; Wu, Z.-H.; We, Y.-L.; Chu, W.-S. *Huaxu Xuebao* **1979**, *37*, 129. (b) Qinghaosu Research Group *Sci. Sin., Ser. B (Engl. Ed.)* **1980**, *23*, 380.
(3) Qinghaosu Antimalaria Coordinating Research Group *Chin. Med. J. (Beijing, Engl. Ed.)* **1979**, *92*, 811.
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(8) Etoh, H.; Kazuo, I.; Iguchi, M. *Agric. Biol. Chem.* **1973**, *37*, 2241.
(9) The preparation of **4a** by the catalytic reduction of **3a** has been reported,[8] but the product was insufficiently characterized to allow a comparison with **4a** obtained from the diimide reduction of **3a**.
(10) Yamaguchi, S.; Okuda, S.; Nakagawa, N. *Chem. Pharm. Bull.* **1963**, *11*, 1465.
(11) Tori, K.; Takamo, Y.; Kitachonaki, K. *Chem. Ber.* **1964**, *97*, 2798.

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Table I. In Vitro Antimalarial Activity against *P. falciparum*[a]

no.	IC_{50}, ng/mL chloroquine susceptible	IC_{50}, ng/mL chloroquine resistant	no.	IC_{50}, ng/mL chloroquine susceptible	IC_{50}, ng/mL chloroquine resistant
1	1.13	1.00	**9**	389	416
3a	>1200	>1200	**10**	733	1250
3b	96	39	11	789	1430
4a	1350	1000	**12**	23.9	99.3
4b	83	75	**13**	1120	184
8	4930	4760			

LDA and acetaldehyde gave the condensation product **5**. Photochemical cyclization of **5** in the presence of triethylamine[6] gave tetrahydrobenzopyran **6**. Rose bengal sensitized photooxygenation of **6** gave a mixture of diastereomeric alcohols **7** and **8**. The stereochemistry of the singlet oxygen addition to **6** was assigned by comparison of the 8a-methyl resonances of the olefins **8**, **10** and **11** with their saturated analogues **9**, **12**, and **13**, respectively (vide supra). One of the alcohols, with the arbitrarily assigned stereochemistry depicted by structure **8**, was isolated by crystallization. The second alcohol, **7**, was purified through its crystalline benzoate **10**. Esterification of **8** afforded benzoate **11**. The saturated compounds **9**, **12**, and **13** were prepared by reduction of the corresponding olefin with diimide.[7]

Biological Testing. The compounds were assayed in an in vitro system for antimalarial activity against chloroquine-susceptible and chloroquine-resistant strains of *P. falciparum* by using the method reported by Desjardins et al.[12] 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*. The results are shown in the table. The most highly active compounds are **3b**, which showed an IC_{50} of 96 and 39 ng/mL, respectively, for the chloroquine-susceptible and chloroquine-resistant strains, and **12**, which showed an IC_{50} of 24 and 99 ng/mL, respectively, for the chloroquine-susceptible and -resistant strains. For comparison, qinghaosu exhibits an IC_{50} of 1 ng/mL against both strains. Although the most active compounds are 1 order of magnitude less active than the qinghaosu, some observations can be made. The compounds bearing the longer side chain, except for alcohol **8**, are more active than the methyl side chain compounds. Reduction of the double bond either increases the activity by 1 order of magnitude [compare **8** and **9**, **10** and **12**, and **11** and **13** (chloroquine-resistant strains)] or has little effect [compare **2a** and **3a**, **2b** and **3b**, and **11** and **13** (chloroquine-susceptible strain)]. The stereochemistry of the hydroxyl group had little effect on the activity of the olefins **10** and **11** but a marked effect on the dihydro derivatives **12** and **13** where **12** was 45 times more active than **13** in the chloroquine-sensitive strain but only twice as active as **13** in the chloroquine-resistant strain. Compounds **3b** and **13** show considerable more activity toward the chloroquine-resistant strain of *P. falciparum* than the chloroquine-susceptible strain. The remainder of the compounds are more like qinghaosu in that they show approximately equal activity against both strains, except for the ester **12**, which is about 4 times more active against the chloroquine-susceptible strain.

Compounds **8**, **3a**, **3b**, **4a**, and **4b** were tested for blood schizonticidal activity against *Plasmodium berghei* in mice.[13] 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. These results are not surprising since qinghaosu is not active in this screen.

In summary, some simple compounds containing a 1,2,4-trioxane ring were prepared and evaluated for antimalarial activity in order to gain information concerning what portions of the qinghaosu ring is necessary for activity. In general, the compounds prepared were more active than the simple endoperoxides studied earlier,[4] but the most active 1,2,4-trioxanes had only the same magnitude of activity as the most active endoperoxides.

The general lack of high activity indicates that the 1,2,4-trioxane ring system alone is not sufficient for antimalarial activity. These results, coupled with the lack of significant activity in the simple endoperoxides, indirectly suggest that the 5-oxygen substituent of the 1,2,4-trioxane ring system of qinghaosu is important to its antimalarial activity.

Experimental Section

Melting points were determined on a Koffler hot stage apparatus. Infrared (IR) spectra were recorded on a Perkin-Elmer 457 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).

Molecular models were constructed with a molecular modeling system, which consists of an Evans and Sutherland PS330 graphics system linked to a Digital Equipment Corp. microVAX work station. Software employed was the SYBYL program (version 3.4) from Tripos Associates, St. Louis, MO.

Energy minimization of the molecules was carried out by use of the MAXIMIN molecular mechanics methods within the SYBYL software package. MAXIMIN is a minimization method that uses the simplex algorithm but allows such options as fixed geometries in parts of the molecule. Bond length and valence angle minimization is performed simultaneously with slight variation of the torsional angles to get a minimum-energy geometry of a certain conformer.

2,4a-Epidioxy-3,4,4a,5,6,7,8,8a-octahydro-2,5,5,8a-tetramethyl-2*H*-1-benzopyran (4a). The endoperoxide **3a** (3.1 g, 0.014 mol) in 150 mL of methanol was cooled in an ice bath. Under nitrogen and with vigorous stirring, potassium azodicarboxylate (PADA)[14] (35 g, 0.17 mol) was added in one portion. To the yellow suspension was added dropwise a solution of methanol and acetic acid (30 mL, 1:1) over a period of 2 h. Vigorous gas evolution was noted. The bright yellow color of the suspension gradually faded to white. The precipitate was separated by filtration, and the filtrate and washings were combined and concentrated to give 2.6 g (83%) of yellow solid. Recrystallization from cold methanol gave 2.0 g (63%) of **4a** as a white solid: mp 55–57 °C; IR ($CHCl_3$) 3000–2800 (CH), 1385 (geminal dimethyl) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.94 (s, 3 H, C_5-CH_3), 0.96 (s, 3 H, C_5-CH_3), 1.25 (s, 3 H, C_{8a}-CH_3), and 1.38 (s, 3 H, C_2-CH_3). Anal. ($C_{13}H_{22}O_3$) C, H.

Methyl 2-(2,4a-Epidioxy-4a,5,6,7,8,8a-hexahydro-5,5,8a-trimethyl-2*H*-1-benzopyran-2-yl)acetate (3b). The pyran **2b**[6] (6.0 g, 0.024 mol) was dissolved in 100 mL of methylene chloride containing 1.0 g of polymer-bound rose bengal sensitizer. The solution was irradiated in the presence of oxygen at room temperature with a GE quartzline BWY-650 lamp for 4 h. The rose bengal dye was removed by filtration and washed with CH_2Cl_2. The filtrate was evaporated to dryness, and the residue was purified by silica gel column chromatography (CH_2Cl_2) to give 3.5 g (52%) of a colorless oil, which solidified upon cooling to −16 °C in a refrigerator. Further trituration with cold pentane afforded **3b** as a white crystalline solid: mp 54.5–56 °C; IR ($CHCl_3$) 1740

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(C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.01 (s, 3 H, CH_3), 1.12 (s, 3 H, CH_3), 1.14 (s, 3 H, CH_3), 2.79 (AB q, 2 H, J = 14.8 Hz, CH_2COOMe), 3.70 (s, 3 H, OCH_3), 6.54 (d, 1 H, J = 8.8 Hz, olefinic H), 6.78 (d, 1 H, J = 8.8 Hz, olefinic H); mass spectrum M^+ calculated for $C_{15}H_{22}O_5$ m/z 282.1467, found m/z 282.1468. Anal. ($C_{15}H_{22}O_5$) C, H.

Methyl 2-(2,4a-Epidioxy-3,4,4a,5,6,7,8,8a-octahydro-5,5,8a-trimethyl-2*H*-1-benzopyran-2-yl)acetate (4b). The peroxide **3b** (4.6 g, 0.016 mol) in 150 mL of methanol was cooled in an ice bath. Under nitrogen and with vigorous stirring, PADA (40.5 g, 0.20 mol) was added in one portion. To the yellowish suspension was added dropwise a solution of methanol and acetic acid (1:1, v/v, 30 mL) over a period of 2 h. Vigorous gas evolution was noted. The bright yellow color of the suspension gradually faded to white. The precipitate was filtered, and the filtrate and washings were combined and concentrated to give 3.5 g of pale yellow solid, which was recrystallized from cold methanol to give 2.4 g (52%) of **4b** as a white solid: mp 97–98 °C; IR ($CDCl_3$) 3000–2800 (CH), 1740 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.95 (s, 3 H, C_5-CH_3), 0.97 (s, 3 H, C_5-CH_3), 1.40 (s, 3 H, C_{8a}-CH_3), 2.55 (s, 2 H, CH_2COOMe), and 3.67 (s, 3 H, OCH_3). Anal. ($C_{15}H_{24}O_5$) C, H.

2-Hydroxy-4-oxo-6-(2,6,6-trimethyl-1-cyclohexen-1-yl)-5-hexene (5). To a solution of diisopropylamine (5.3 g, 0.05 mol) in freshly distilled THF (100 mL) at 0 °C was added BuLi (2.3 M, 23 mL). After being stirred for 30 min, the mixture was cooled to −78 °C in a dry ice–2-propanol bath, and β-ionone (9.6 g, 0.05 mol) in 50 mL of THF was added dropwise, and stirring was continued for 45 min. A solution of acetaldehyde (2.5 g, 0.055 mol) in 10 mL of THF was added dropwise to this stirred solution at −78 °C. After 1 h, the cold bath was removed, and the temperature was allowed to rise. Water was added carefully, and the mixture was extracted with ether. The organic layer was washed successively with dilute hydrochloric acid, 5% $NaHCO_3$ solution, and saturated NaCl solution. The dried (Na_2SO_4) extracts were evaporated to a syrupy liquid. The residue was chromatographed on silica gel (400 g) and eluted with 10% EtOAc–CH_2Cl_2 to give 8.75 g (74%) of **5** as a syrup: ^1H NMR ($CDCl_3$) δ 1.12 [s, 6 H, $C_6(CH_3)_2$], 1.20 (d, 3 H, $HOCHCH_3$), 4.20 (m, 1 H, *H*COH), 6.00 (d, 1 H, C*H*=CHCO), and 7.25 (=CHCO). This material was used without further characterization. When this experiment was repeated on a 0.1 mol scale, a 67% yield of **5** was obtained.

5,6,7,8-Tetrahydro-2-(2-hydroxypropyl)-5,5,8a-trimethyl-8a*H*-1-benzopyran (6). A solution of **5** (7.08 g, 0.03 mol) and triethylamine (3.03 g, 0.03 mol) in 80 mL of 50% CH_2Cl_2–pentane was irradiated with a 350-W Hg lamp for 4 h at room temperature. The mixture was evaporated and applied to a silica gel column (300 g) and eluted with 5% EtOAc–CH_2Cl_2 to give 5.02 g (71%) of **6**: ^1H NMR ($CDCl_3$) δ 1.05 (s, 3 H, CH_3), 1.10 (s, 3 H, CH_3), 1.20 (d, 3 H, $HOCHCH_3$), 1.36 (s, 3 H, C_{8a}-CH_3), 3.96 (m, 1 H, *H*COH), 4.95 (d, 1 H, C_4H), and 5.6 (d, 1 H, C_3H). This material was used without further characterization.

When this experiment was repeated on a 0.07 mol scale, a 50% yield of **6** was obtained.

2,4a-Epidioxy-4a,5,6,7,8,8a-hexahydro-2-(2-hydroxypropyl)-5,5,8a-trimethyl-2*H*-1-benzopyran (α-Isomer, 8). A solution of **6** (8.2 g, 0.035 mol) in 100 mL of CH_2Cl_2 was mixed with polymer-bound rose bengal (3.0 g) and irradiated at room temperature with a GE quartzline BWY-650 lamp for 4 h. The dye was removed by filtration, and the filtrate was evaporated to a syrup. The residue was chromatographed on silica gel (500 g) and eluted with 10% ethyl acetate–CH_2Cl_2. Evaporation of the pure fraction gave 4.30 g of a waxy substance. Crystallization from ether–petroleum ether gave 1.63 g (18%) of **8**, mp 100–102 °C. The analytical sample prepared by recrystallization from ether–petroleum ether had mp 101–102 °C: ^1H NMR ($CDCl_3$) δ 1.01 (s, 3 H, CH_3), 1.12 (s, 3 H, CH_3), 1.17 (s, 3 H, C_{8a}-CH_3), 1.22 (d, 3 H, $HCOHCH_3$), 4.22 (m, 1 H, C*H*OH), 6.50 (d, 1 H, C_4H), 6.59 (d, 1 H, C_3H). Anal. ($C_{15}H_{24}O_4$) C, H.

2,4a-Epidioxy-4a,5,6,7,8,8a-hexahydro-2-[2-(benzoyloxy)propyl]-5,5,8a-trimethyl-2*H*-1-benzopyran (β-Isomer, 10). In a repeat experiment, photooxidation of 22 g of **6** afforded 5.06 g of crude **7** which was isolated as an oil by concentration of the mother liquor from the crystallization of **8**. Then, **7** was dissolved in 50 mL of $CHCl_3$ containing 5 mL of pyridine. This solution was cooled (−10 to −15 °C), and a solution of benzoyl chloride (4.05 g, 0.029 mol) in 25 mL of $CHCl_3$ was added dropwise. After stirring overnight, the reaction mixture was diluted with 300 mL of $CHCl_3$ and washed successively with H_2O, 2% NaOH solution, 2 N HCl solution, and saturated sodium chloride solution. The chloroform solution was dried (Na_2SO_4) and concentrated to a solid under vacuum. Recrystallization from ether–petroleum ether gave 6.05 g (86%) of **10**: mp 56–58 °C; ^1H NMR ($CDCl_3$) δ 0.98 (s, 3 H, CH_3), 1.04 (s, 3 H, CH_3), 1.06 (s, 3 H, C_{8a}-CH_3), 1.43 (d, 3 H, J = 7 Hz, $OCHCH_3$), 5.53 (m, 1 H, HCO), 6.46 and 6.58 (2 d, 2 H, CH=CH), 7.2–8.05 (m, 5 H, Ar H). Anal. ($C_{22}H_{28}O_5$) C, H.

2,4a-Epidioxy-4a,5,6,7,8,8a-hexahydro-2-[2-(benzoyloxy)propyl]-5,5,8a-trimethyl-2*H*-1-benzopyran (α-Isomer, 11). Benzoylation of **8** was carried out as described for **7**. Thus, benzoylation of 2.5 g (0.009 mol) of **8** gave after purification by column chromatography on silica gel (10% EtOAc–hexanes) 2.6 g (75%) of a waxy solid, which on trituration under pentane at −78 °C gave 2.11 g (61%) of **11** as a white amorphous powder: mp 58–60 °C; ^1H NMR ($CDCl_3$) δ 0.99 (s, 3 H, CH_3), 1.04 (s, 3 H, CH_3), 1.08 (s, 3 H, CH_3), 1.41 (d, 3 H, J = 5 Hz, $OCHCH_3$), 5.42–5.57 (m, 1 H, CHO), 6.52 and 6.65 (2 d, 2 H, olefinic), and 7.21–8.06 (m, 5 H, Ar H). Anal. ($C_{22}H_{28}O_5$) C, H.

2,4a-Epidioxy-3,4,4a,5,6,7,8,8a-octahydro-2-(2-hydroxypropyl)-5,5,8a-trimethyl-2*H*-1-benzopyran (α-Isomer, 9). 2,4a-Epidioxy-4a,5,6,7,8,8a-hexahydro-2-(2-hydroxypropyl)-5,5,8a-trimethyl-2*H*-1-benzopyran (**8**) (2.21 g, 0.008 mol) was dissolved in 40 mL of CH_2Cl_2 under argon. The solution was cooled to 0 °C, and then 28.9 g (0.165 mol) of potassium azodicarboxylate was added in one portion with vigorous stirring. A solution of acetic acid (17.1 mL, 0.033 mol) was slowly added dropwise (over ca. 3 h). The bright yellow reaction mixture was allowed to warm to room temperature and stirred for 36 h. The white suspension was filtered through Celite and concentrated to a yellow oil, which crystallized slowly on standing to yield 2.21 g (99%) of analytically pure **9**: mp 50–51 °C; IR (KBr) 1130, 1290, 1380, 1465, 1760, 2955, 3350; ^1H NMR ($CDCl_3$) δ 0.97 (s, 3 H, C_5-CH_3), 0.99 (s, 3 H, C_5-CH_3), 1.15 (d, 3 H, J = 6, $CHOHCH_3$), 1.42 (s, 3 H, $OCCH_3$), 1.64 (m, 6 H), 2.19 (m, 6 H), 3.26 (s, 1 H, OH) and 4.16 (m, 1 H, C*H*OH). Anal. ($C_{15}H_{26}O_4$) C, H.

2,4a-Epidioxy-3,4,4a,5,6,7,8,8a-octahydro-2-[2-(benzoyloxy)propyl]-5,5,8a-trimethyl-2*H*-1-benzopyran (β-Isomer, 12). 2,4a-Epidioxy-4a,5,6,7,8,8a-hexahydro-2-[2-(benzoyloxy)propyl]-5,5,8a-trimethyl-2*H*-1-benzopyran (**10**) (2.50 g, 0.0067 mol) was dissolved in 40 mL of CH_2Cl_2 under argon. The solution was cooled to 0 °C, and then 26.7 g (0.134 mol) of PADA was added in one portion with vigorous stirring. A solution of acetic acid (15.3 mL, 0.269 mol) was slowly added dropwise (over ca. 3 h). The bright yellow reaction mixture was allowed to warm to room temperature and stirred for 36 h. The white suspension was filtered through Celite and concentrated to a yellow oil, which crystallized slowly on standing to yield 2.38 g (95%) of **12**: mp 94–96 °C; IR (KBr) 720, 970, 1030, 1075, 1100, 1120, 1195, 1270, 1285, 1320, 1360, 1385, 1395, 1450, 1725, 2940, 3000; NMR ($CDCl_3$) δ 0.94 (s, 6 H, $(CH_3)_2$), 1.34 (s, 3 H, $OCCH_3$), 1.38 (d, 3 H, J = 6 Hz, $CH(OR)CH_3$), 1.54 (m, 2 H), 1.71 (m, 4 H), 2.07 (m, 6 H), 5.44 (t of q, 1 H, J = 1.6 Hz, $CH(OR)CH_3$), 7.43 (m, 2 H, Ar), 7.55 (m, 1 H, Ar), 8.02 (m, 2 H, Ar). Anal. ($C_{22}H_{30}O_5$) C, H.

2,4a-Epidioxy-3,4,4,5,6,7,8,8a-octahydro-2-[2-(benzoyloxy)propyl]-5,5,8a-trimethyl-2*H*-1-benzopyran (α-Isomer, 13). The α-benzoate **11** (1.91 g, 5.13 mmol) was dissolved in 35 mL of methylene chloride in a three-neck flask equipped with a mechanical stirrer and addition funnel. PADA (28.0 g, 0.14 mmol) was added in one portion, followed by dropwise addition of a solution of acetic acid (17.0 mL, 295 mmol) in 40 mL of CH_2Cl_2 over a period of 3 h. This suspension was maintained at ambient temperature for 26 h, when the white precipitate was removed by filtration through Celite. The filtrate was concentrated to a light cream solid, yielding 1.71 g (89%) of **13** as white crystals: mp 70–73 °C; IR (cm^{-1}) 720, 970, 1120, 1280, 1380, 1450, 1710, 1180, 1950; ^1H NMR ($CDCl_3$) δ 0.94 (s, 3 H, CH_3), 0.95 (s, 3 H, CH_3), 1.28 (d, 3 H, J = 1 Hz, C_{8a}-CH_3),[15] 1.45–1.67 (br m, 4 H),

(15) For long-range coupling of tertiary methyl groups, see Jackman, L. M.; Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, 2nd ed.; Pergamon: New York, 1969; Chapter 3-4, p 337.

1.79 (dd, 2 H, J = 4, 15 Hz), 2.02–2.21 (br m, 6 H), 5.41 (m, 1 H, $CHCO_2C_6H_5$), 7.45 (m, 3 H, Ar), 8.05 (dd, 2 H, J = 1.5, 8 Hz, Ar). Anal. ($C_{22}H_{30}O_5$) C, H.

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Synthesis and Copper-Dependent Antimycoplasmal Activity of 1-Amino-3-(2-pyridyl)isoquinoline Derivatives. 1. Amides

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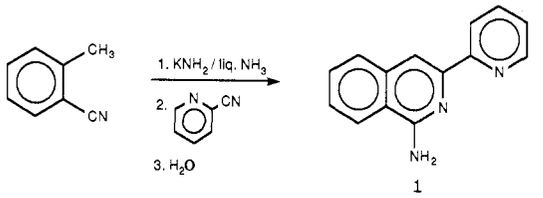
In order to investigate the antimycoplasmal activity of compounds structurally related to 2,2′-bipyridyl, a series of both aliphatic and aromatic amides derived from 1-amino-3-(2-pyridyl)isoquinoline were synthesized. The most active compounds appeared to be as active as Tylosin, an antimycoplasmal therapeutic that is used in veterinary practice, in the presence of a small nontoxic amount of copper. Furthermore, it was found that antimycoplasmal activity depends on the hydrophobic fragmental value of the amide residue. A quantitative structure–activity relationship established the optimal hydrophobic fragmental value of the amide residue to be 0.30.

Mycoplasmas are known to be causative agents of many infectious diseases not only in plants and animals but in humans as well.[1-3] Broad-spectrum antibiotics from the small polyene type (34–37 carbon atoms) and the tetracycline type are inhibitory to mycoplasmas in vitro as well as in vivo. Unfortunately, these broad-spectrum antibiotics induce resistance rapidly.[1] Tylosin, a macrolide antibiotic is often used in therapy of mycoplasmal infections in poultry.[1] Furthermore, Pijper et al.[4] have shown that in the presence of a small and nontoxic amount of copper certain 2,2′-bipyridyl derivatives are highly active against mycoplasmas. Due to the low activity of these compounds in the absence of copper, it was concluded that growth inhibition is caused by their copper complexes rather than by these compounds themselves. In a study on the mechanism of action of these copper complexes, Smit et al.[5] and Gaisser et al.[6,7] discovered that copper itself is the ultimate toxic agent, whereas ligands facilitate copper transport across the membrane through the formation of lipophilic complexes. The toxicity of copper is most probably based on the inhibition of enzymes involved in the energy providing metabolism like NADH-oxidase and lactate dehydrogenase.[7]

In a recent study from our laboratory, Linschoten et al.[8] reported on the antimycoplasmal activity of a series of amides and amidines derived from 4-amino-2-(2-pyridyl)quinazoline. It was found that the most active compound, *N*-[2-(2-pyridyl)quinazolin-4-yl]-2-pyridinecarboxamidine, was on a molar basis 40 times as active as Tylosin, which was used as a reference compound. On the basis of these results, we decided to continue our search for new antimycoplasmal therapeutics with the synthesis of amides and amidines derived from 1-amino-3-(2-pyridyl)isoquinoline (**1**), which has certain advantages over the structurally related 4-amino-2-(2-pyridyl)quinazoline from a synthetic point of view.

Scheme I



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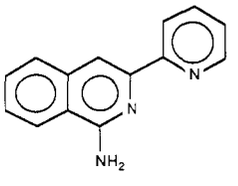

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In the present paper, we report on the synthesis and antimycoplasmal activity of both aliphatic and aromatic amides derived from 1-amino-3-(2-pyridyl)isoquinoline (**1**). In order to establish the structure with optimal activity, we used the efficient method proposed by Topliss.[9] This method is an application of the Hansch approach and is based on a proper selection of an initial small group of compounds. Analysis of the potency order provides a rational basis for the selection of more potent analogues.

Chemistry. A general method for the synthesis of amides consists of the acylation of amines by agents like acyl chlorides.[10] While these acyl chlorides can be obtained from the corresponding acids very easily,[11,12] our major

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