Antimalarial Agents. 1. α-Santonin-Derived Cyclic Peroxide as Potential Antimalarial Agent

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An α-santonin-derived cyclic peroxide (7) related to qinghaosu (1) has been synthesized and tested for antimalarial activity in vitro against the chloroquine-resistant (Smith) isolates of *Plasmodium falciparum* as well as in vivo against *Plasmodium berghei* in mice and was found to be devoid of activity.

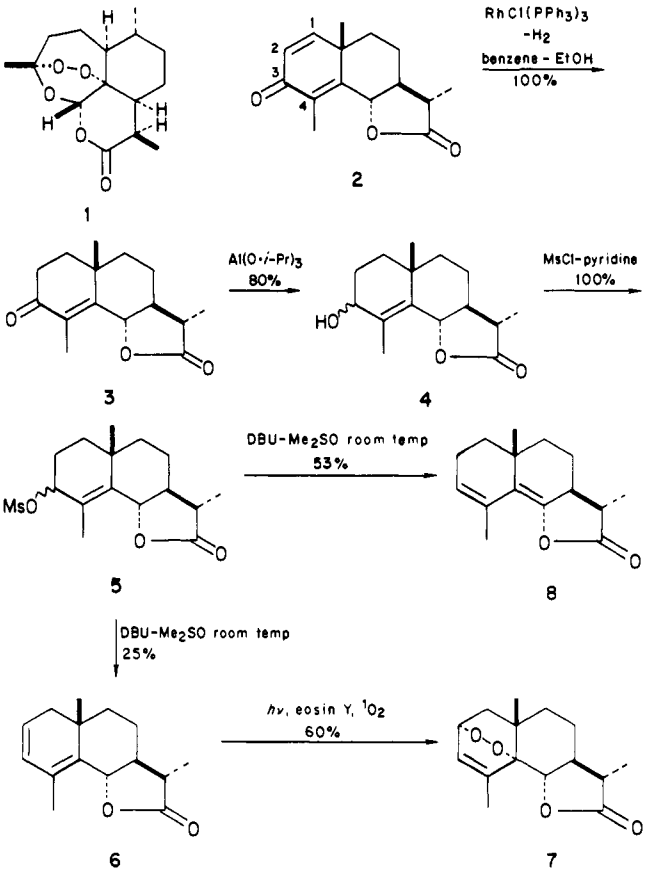
Qinghaosu (artemisinin) (1) has recently been used as a new type of antimalarial drug with rapid action and low toxicity against chloroquine-resistant *Plasmodium falciparum* in mainland China.[1] Clinical studies of different qinghaosu preparations were carried out involving over 2000 cases of malaria infected with *Plasmodium vivax* and *P. falciparum*. All patients were clinically cured.[2-4] Qinghaosu, isolated from *Artemisia annua*,[1,5] is a novel sesquiterpene lactone bearing an unusual cyclic peroxide function, destruction of which eliminates the antimalarial properties of the compound.[6] Our previous screening of cyclic peroxy ring lacking sesquiterpene lactones and related derivatives including helenalin,[7] acetyl helenalin,[8] 2,3-dihydrohelenalin,[7] 2,3-epoxyhelenalin,[9] plenolin,[7] bis(helenalinyl) malonate,[10] bis(tetrahydrohelenalinyl) malonate,[10] tenulin,[11] and eupatolide[12] have demonstrated to be devoid of antimalarial activity.[19] Thus it appeared to be of interest to examine further sesquiterpene lactones with the introduction of a cyclic peroxide function as potential antimalarial agents. This paper describes the synthesis and the antimalarial activity of a peroxide (7) derived from α-santonin (2).

Chemistry. The synthetic route leading to the target compound 7 is illustrated in Scheme I. α-Santonin (2), which is commercially available, is selected as a model compound. 1,2-Dihydro-α-santonin (3) was prepared by selective catalytic hydrogenation of 2 with tris(triphenylphosphine)rhodium chloride according to a literature method[13] in quantitative yield. Reduction of 3 with aluminum isopropoxide in 2-propanol, based upon Corey and Hortmann's procedure[14] in their synthesis of an analogous compound, furnished the allylic alcohol (4) as a mixture of two epimers in 80% yield. Without further separation, the mixture of compounds of 4 was transformed to a homoannular diene (6) by an initial quantitative conversion of 4 into a mesylate (5) with mesyl chloride in triethylamine, followed by treatment of 5 with 1,8-diazabicyclo[5.4.0]undec-7-ene in dimethyl sulfoxide at room temperature in 25% yield. Attempted improvement of the yield from 5 to 6 under various reaction conditions failed, as this reaction also gave rise to a heteroannular diene (8). Photooxygenation involving the irradiation of a solution of 6 and eosine Y in benzene with a 200-W photoflood lamp while a finely dispersed stream of purified oxygen was bubbled through the reaction mixture according to Barrett and Büchi[15] afforded the peroxide 7 in 60% yield.

Biological Results and Discussion

Compound 7 was evaluated for antimalarial activity[20] *in vitro* against the chloroquine-resistant (Smith) isolates of *Plasmodium falciparum*, using the semiautomated microdilution technique according to Desjardins et al.,[16]

Scheme I



as well as in vivo against *Plasmodium berghei* in mice, using the method of Osdene et al.,[17] and was inactive in

(1) Qinghaosu Antimalaria Coordinating Research Group, *Chin. Med. J. (Beijing, Engl. Ed.)* **1979**, *92*, 811.
(2) Wan, Y. D.; Zhang, Q. Z. *Yaoxue Tongbao* **1981**, *16*, 9 and literatures cited therein.
(3) China Cooperative Research Group on Qinghaosu and Its Derivatives as Antimalarials, *J. Trad. Chin. Med.* **1982**, *2*, 45.
(4) Xiao, P. In "Natural Products as Medicinal Agents"; Beal, J. L., Reinhard E., Eds.; Hippokrates Verlag Stuttgart Press: Stuttgart, 1981; p 351.
(5) Klayman, D. L.; Lin, A. J.; Acton, N.; Scovill, J. P.; Hoch, J. M.; Milhous, W. K.; Theoharides, A. M.; Dobek, A. S. *J. Nat. Prod.* **1984**, *47*, 715.
(6) Zhu, D. Y. *Zhongguo Yaoli Xuebao* **1983**, *4*, 194.
(7) Lee, K. H.; Furukawa, H.; Huang, E. S. *J. Med. Chem.* **1972**, *15*, 609.
(8) Lee, K. H.; Meck, R.; Piantadosi, C.; Huang, E. S. *J. Med. Chem.* **1973**, *16*, 299.
(9) Lee, K. H.; Kim, S. H.; Furukawa, H.; Piantadosi, C.; Huang, E. S. *J. Med. Chem.* **1975**, *18*, 59.
(10) Lee, K. H.; Ibuka, T.; Sims, D.; Muraoka, O.; Kiyohawa, H.; Hall, I. H.; Kim, H. L. *J. Med. Chem.* **1981**, *24*, 924.
(11) Waddell, T. G.; Austin, A. M.; Cochran, J. W.; Gerhart, K. G.; Hall, I. H.; Lee, K. H. *J. Pharm. Sci.* **1979**, *68*, 715.

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0022-2623/85/1828-1743$01.50/0

both assays. Compound 7 showed an ED_{50} value of 728.59 ng/mL and toxicity at the dosage levels tested (160 and 640 mg/kg) in the in vitro and the in vivo assays, respectively, while 1 demonstrated an ED_{50} value of <3.4 ng/mL in the same isolates.[5] These results would seem to indicate that the requirement of the cyclic peroxide function for potent antimalarial activity among the sesquiterpene lactones might be quite specific and could involve a unique C–O–O–C–O–C–O–C–O linkage as found in 1. Further investigation along this line is currently in progress.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Perkin-Elmer 257 grating spectrophotometer. ^1H nuclear magnetic resonance (^1H NMR) spectra were recorded on a Bruker 250 MHz spectrometer and are given in parts per million (δ) downfield from an internal tetramethylsilane (Me_4Si) standard. The abbreviations s, d, t, q, and m refer to singlet, doublet, triplet, quartet, and multiplet, respectively. Mass spectra were determined on a V. G. Micromass 70-70 instrument at 70 eV using a direct inlet system. Silica gel for column chromatography refers to Merck silica gel 60 (70–230 mesh). Silica gel for preparative thin-layer chromatography (PTLC) refers to Analtech silica gel G (1000 μm). Compounds were visualized by ultraviolet light or spraying with 1% $Ce(SO_4)_2$–10% H_2SO_4 solution followed by heating. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

3-Hydroxy-6,11βH-eudesm-4-en-6,13-olide (4). This compound was prepared on the basis of Corey's procedure[14] in his synthesis of an analogous compound. A solution of 11.7 g (0.0471 mol) of 1,2-dihydro-α-santonin (3)[13] and aluminum isopropoxide (26 g) in dry 2-propanol (150 mL) was allowed to boil gently in a 250-mL flask fitted with a Vigreux column. The acetone vapor formed was allowed to escape from the reaction mixture. After 6 h, the 2-propanol was distilled and the reaction mixture was reduced to ca. a 50-mL volume. Cold 1 N hydrochloric acid was added, and the resulting acidic solution was extracted with methylene chloride. The extracts were washed with saturated sodium bicarbonate and saturated sodium chloride solutions and dried over sodium sulfate to afford an oily mixture (4) in ca. 80% yield. Compound 4: IR ($CHCl_3$) 3620 (OH), 3030 (C=C), 1775, 1765 cm^{-1} (mixture lactone C=O) cm^{-1}; NMR ($CDCl_3$) δ 1.18 and 1.11 [3 H (3:1), s each, Me-10], 1.21 (3 H, J = 7.5 Hz, Me-11), 1.93 and 1.98 [3 H (3:1), s each, Me-4], 2.85 (br, s, OH) (disappeared upon addition of D_2O), 3.94 (1 H, t, J = 7.5 Hz, H-3), 4.62 (1 H, d, J = 9 Hz, H-6), 2.32 (1 H, m, H-11).

6,11βH-Eudesm-2,4-dien-6,13-olide (6). This compound was synthesized from 4 via 5. To a solution of 4 (3 g) in anhydrous tetrahydrofuran (50 mL) was added 4 mL of triethylamine. The mixture was cooled to <5 °C and treated with mesyl chloride (2 g). Stirring was continued for 2 h at <5 °C. The resulting triethylamine hydrogen chloride salt was filtered from the solution and the filtrate was evaporated in vacuo. The residue was added to water and extracted with methylene dichloride, dried over anhydrous magnesium sulfate, and distilled under reduced pressure to yield crystalline residue (5, 4 g) upon cooling. Without further purification this residue was dissolved in Me_2SO (10 mL) and treated with DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) (1.5 g). After stirring for 15 h, the mixture was added to 50 mL of ice–water and the resulting oil was extracted with methylene dichloride, washed with HCl, water, sodium bicarbonate, and water, dried over anhydrous magnesium sulfate, and evaporated in vacuo to furnish a residue. Recrystallization from ether–hexane gave 6 as colorless crystals in 25% yield. Compound 6: mp 148 °C; TLC R_f = 0.54 [silica gel, petroleum ether–ether (3:1)]; IR (KBr) 3030 (C=C), 1770 (lactone C=O) cm^{-1}; NMR ($CDCl_3$) δ 1.05 (3 H, s, Me-10), 1.25 (3 H, d, J = 7.5 Hz, Me-11), 1.97 (3 H, d, J = 2.0 Hz, Me-4), 2.28 (1 H, m, H-11), 4.51 (1 H, d, J = 11.0 Hz, H-6), 5.72 (2 H, m, H-2 and H-3). Anal. ($C_{15}H_{20}O_2$) C, H; calcd m/z 232.1462. Found m/z 232.1467. After the isolation of 6, the mother liquor was purified by preparative TLC (silica gel–benzene) to afford 53% yield of 8: mp 70–73 °C; TLC R_f = 0.6 (silica gel–benzene); IR ($CHCl_3$) 3020, 1670 (C=C), 1790 (γ-lactone C=O) cm^{-1}; NMR ($CDCl_3$) δ 1.03 (3 H, s, Me-10), 1.29 (3 H, d, J = 7.5 Hz, Me-11), 2.01 (3 H, d, J = 2.0 Hz, Me-4), 5.43 (1 H, br s, H-3). Anal. ($C_{15}H_{20}O_2$) C, H; calcd m/z 232.1462. Found m/z 232.1461. The purity of 6 and 8 was substantiated by TLC in three solvent systems.

2,5α-Peroxy-6,11βH-eudesm-3-en-6,13-olide (7). A solution of 6 (300 mg) and eosine Y (20 mg) in pure benzene (300 mL) was irradiated with a 260-W photoflood lamp while a finely dispersed stream of purified oxygen was bubbled through the reaction mixture. After 30 h of irradiation, benzene was distilled under reduced pressure and the resulting oil was column chromatographed on silica gel (30 g). The benzene–ethyl acetate (1:1) eluate yielded 200 mg (60% yield) of the desired peroxied 7 as colorless prisms after recrystallization from ether. Compound 7: mp 143–145 °C; IR (KBr) 3030 (C=C), 1760 (lactone C=O), 1115, 895, and 820 (O–O)[18] cm^{-1}; NMR ($CDCl_3$) δ 0.99 (3 H, s, Me-10), 1.24 (3 H, d, J = 7.5 Hz, Me-11), 2.07 (3 H, d, J = 2.0 Hz, Me-4), 4.35 (1 H, d, J = 11.0 Hz, H-6), 4.58 (1 H, m, H-2), 6.31 (1 H, dd, J = 7.5 and 2.0 Hz, H-3), 2.32 (1 H, m, H-11). Anal. ($C_{15}H_{20}O_4$) C, H; calcd m/z 264.1360. Found m/z 264.1360.

Acknowledgment. This investigation was supported by the U.S. Army Medical Research and Development Command under Research Contracts DAMD 17-83-C-3098 and DAMD 17-85-C-5010 (K.H.L.). This is Contribution No. 1754 to the Army Research Program on Antiparasitic Drugs. We thank Dr. David L. Harris, Department of Chemistry, The University of North Carolina at Chapel Hill, for NMR spectra and Andria Dietrich, School of Public Health, The University of North Carolina at Chapel Hill, for mass spectral data.

(12) Lee, K. H.; Huang, H. C.; Huang, E. S.; Furukawa, H. *J. Pharm. Sci.* **1972**, *61*, 629.
(13) Greene, A. E.; Muller, J.; Ourisson, G. *J. Org. Chem.* **1974**, *39*, 186 and literatures cited therein.
(14) Corey, E. J.; Hortmann, A. G. *J. Am. Chem. Soc.* **1965**, *87*, 5736.
(15) Barrett, H. C.; Büchi, G. *J. Am. Chem. Soc.* **1967**, *89*, 5665.
(16) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710.
(17) Osdene, T. S.; Russell, P. B.; Rane, L. *J. Med. Chem.* **1967**, *10*, 431.
(18) Liu, J. M.; Ni, M. Y.; Fan, Y. F.; Tu, Y. Y.; Wu, Z. H.; Wu, Y. L.; Chou, W. S. *Huaxue Xuebao* **1979**, *37*, 129.
(19) Unpublished data. These nine compounds were tested by the Department of Medicinal Chemistry, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.
(20) The in vitro and in vivo antimalarial assays were carried out by the Department of Medicinal Chemistry, Division of Experimental Therapeutics, Walter Reed Institute of Research, Washington, DC.