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DETECTION OF METABOLITES OF QINGHAOSU IN NANOMOLAR QUANTITIES : DADF-ESTERS OF DIHYDROQINGHAOSU AND ITS EPOXY ANALOG. THE CHEMISTRY OF DRUGS. V[1]

XUAN-DE LUO,[2] HERMAN J. C. YEH, and ARNOLD BROSSI
National Institutes of Health, National Institute of Arthritis, Diabetes, and
Digestive and Kidney Diseases, Bethesda, Maryland 20205

Abstract —— Esters of dihydroqinghaosu (2) and its 4,6-epoxy analog 4 were prepared with diacetyldihydrofluorescein (DADF). Both esters 3 and 5 can be separated by tlc and made visible on plates after exposure to ammonia and iodine vapors as deep red colored spots.

The Chinese antimalarial qinghaosu (1, QHS) represents a new type which holds considerable promise for the treatment of malaria resistent to classical drugs.[1,2] A metabolite isolated from the urine of patients treated with QHS was shown to be the epoxy analog 4 of dihydroqinghaosu 2.[3] We now describe the esters 3 and 5, obtained from 2 and 4 by esterification with diacetyldihydrofluorescein (DADF), and their detection on tlc-plates after exposure to ammonia and iodine vapors as deep red colored spots.[4] The fully characterized alcohol 2 was obtained from 1 by reduction with sodium-borohydride,[5] and the epoxy analog 4 from 2 by reduction in methanol over $Pd/CaCO_3$ catalyst.[5] The alcohol 4 in $CDCl_3$ solution exists as mixture of isomers in a proportion of 7:3, indicated by the presence of the H-C(12) proton at δ 4.80 and 5.33, with a coupling constant $J_{11,12}$ of 6.4 and 5.3 Hz respectively. The esterification of 2 and 4 with DADF was carried out as described,[4] affording esters 3 and 5 in crystalline form. A solution of 3 in $CDCl_3$ showed the H-C(12) proton at δ 6.10 with a coupling constant $J_{11,12}$ of 9.7 Hz and 5 showed the H-C(12) proton at 6.07 with a coupling constant $J_{11,12}$ of 6.3 Hz, assigning both to the α-series of compounds.[6] Bands to be assigned to the peroxide group can be seen in the IR-spectra of 2 and 3 between 906-840 cm^{-1} when measured in $CHCl_3$-solution

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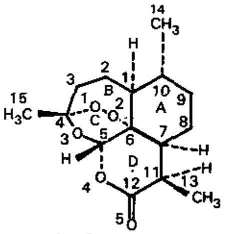
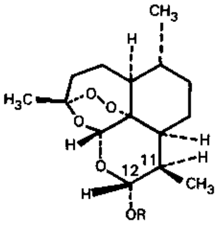
1) See lit. ref. 6.
2) Guest worker from the Institute of Chinese Materia Medica, Academy of Traditional Chinese Medicine, Beijing, China, on a program sponsored by the Special Program for Research and Training in Tropical Diseases by WHO, Geneva.

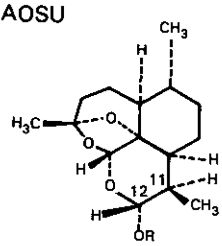
and are lacking in the 4,6-epoxy analogs 4 and 5, were instead bands assigned to the epoxy group occurred between 1066-1058 cm^{-1}.



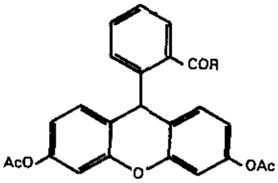
1. QINGHAOSU



2 R=H
3 R=DADF



4 R=H
5 R=DADF



DADF, R=OH

EXPERIMENTAL SECTION

Melting points were taken on a Fisher-Johns apparatus and uncorrected. Silica gel 60 (particle size 0.040-0.063 mm or 0.015-0.040 mm) from Merck was used for column chromatography with a Flask-chromatography column (Aldrich Chemical Co.). Silica gel GF plate from Analtech, Inc. was used for thin layer chromatography with the solvent system of petroleum ether/ethyl acetate 7:3. Then the silica plate after drying was exposed to ammonia vapors for 15 min and to iodine vapors for 5 min to get a red spot. Optical rotations were measured with a Perkin-Elmer Model 241 MC polarimeter in $CHCl_3$ and concentrations specified. UV. spectra (λ max) were measured in $CHCl_3$ solution with a Hewlett Packard 8450 A spectrophotometer. IR spectra (in cm^{-1}) were obtained on a Beckman 4230 instrument as KBr tablet or chloroform solution between sodium chloride plates. Chemical ionization (CI) mass spectra (m/z) were obtained by using a Finnigan 1015 D spectrometer. ^1H-NMR spectra were obtained using JEOL FX-100 and Varian XL-300 spectrometers with Me_4Si as an internal reference (δ in ppm, J in Hz).

Diacetyldihydrofluoresceyl dihydroqinghaosu (3):

A mixture of 2[5] (142 mg, 0.5 mmol), DADF[4] (209 mg, 0.5 mmol) and 4-dimethylaminopyridine (DMAP, 10 mg, catalytic quantity) in dry CH_2Cl_2 (10 ml) was stirred in an ice bath. To the solution was added dicyclohexylcarbodiimide (DCC, 103 mg, 0.5 mmol) and was stirred at room temperature overnight. The finishing of the reaction was detected with tlc. The mixture was filtered. The filtrate was washed with 2N HCl (2 x 2 ml), water (2 x 2 ml), 10% aq. $NaHCO_3$ (2 x 2 ml) and water (2 x 2 ml), dried with Na_2SO_4, filtered and evaporated to get a white powder which was chromatographed on a silica gel column with petroleum ether/ethyl acetate 7:3 to give a white solid after evaporation of the solvent. Recrystallization from isopropyl alcohol afforded 3 (237.6 mg, 69.4%): mp 136.5-138.5°C; $[\alpha]_D^{31}$ + 2.89° (c, 1.02, $CHCl_3$); IR ($CHCl_3$): 1714 (AcO), 1615 (arom.) and 855, 876, 906 (-O-O-); UV: 241 nm (log ε, 3.9); CI-MS (NH_3): 685 (M^+ +1); ^1H-NMR ($CDCl_3$): 0.99 (d, 6H, J = 6.5, C_{13}-CH_3 and C_{14}-CH_3), 1.44 (s, 3H, C_{15}-CH_3), 2.78 (m, 1H, C_{11}-H), 5.57 (s, 1H, C_5-H), 6.10 (d, 1H, J = 9.7, C_{12}-H), 2.30 (s, 6H, 2AcO), 6.74 (s, 1H, DADF-CH), 6.73-8.00 (m, 10H, 10 arom. H).

The 4,6-epoxy analog diacetyldihydrofluoresceyl dihydroqinghaosu (5): The compound (5) was similarly prepared from 4[5]: mp 106-108°C (Hexane); $[\alpha]_D^{29}$ -68.19° (c, I.0, $CHCl_3$); IR ($CHCl_3$): 1754 (AcO), 1615 (arom.), 1056 (-O-); UV: 241 nm (log ε, 4.2); CI-MS (NH_3): 669 (M^+ +1); ^1H-NMR ($CDCl_3$): 0.92 (d, 3H, J = 5.1, C_{14}-CH_3), 1.11 (d, 3H, J = 7.3, C_{13}-CH_3), 2.78 (m, 1H, C_{11}-H), 5.44 (s, 1H, C_5-H), 6.07 (d, 1H, J = 6.3, C_{12}-H), 2.30 (s, 6H, 2AcO), 6.44 (s, 1H, DADF-CH), 6.72-7.89 (m, 10H, 10 arom. H).

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