Confirmation of the Structure of (3*S*)-3-Hydroxyquinine: Synthesis and X-ray Crystal Structure of Its 9-Aceto Analogue

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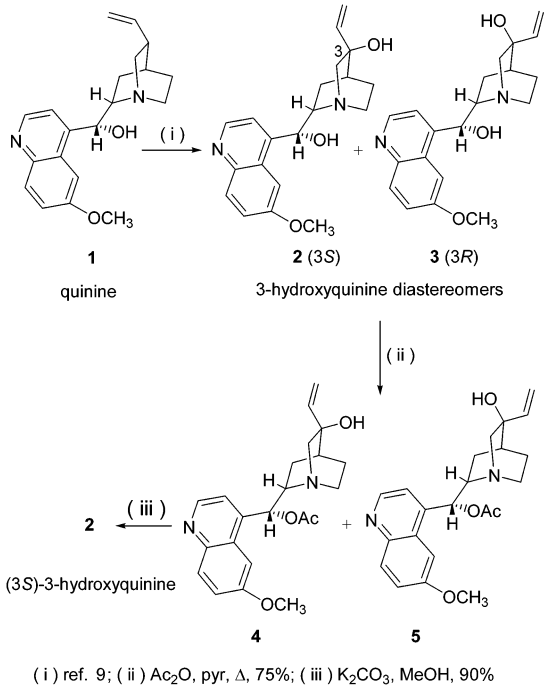
3(*S*)-3-Hydroxyquinine (**2**) has been separated from its epimeric mixture at C-3 by conversion into the 9-aceto analogue followed by chromatography. The molecular structure of the acetate was determined through single-crystal X-ray analysis, and this confirms the structure of (3*S*)-3-hydroxyquinine (**2**), the major metabolite of quinine (**1**).

Quinine (**1**) still remains the drug of choice for the treatment of severe and complicated malaria in most parts of the world.[1,2] Knowledge of the biotransformation of quinine (**1**) in humans is incomplete. Although several quinine (**1**) metabolites have been isolated and characterized, neither the quantitative importance of these metabolites on the elimination of quinine nor the metabolic pathways have been fully elucidated.[3,4] Quinine (**1**) and its diastereomer quinidine are metabolized in a very similar fashion in man. The metabolites that result are the products of oxidation reactions taking place on either the quinoline or the quinuclidine moieties. In vitro studies with human liver microsomes have shown that quinidine oxidation to the (3*S*)-3-hydroxy and *N*-oxide products appears to be catalyzed primarily by cytochrome *P*450-3A4.[5−7]

The major biotransformation product of the metabolism of quinidine has been isolated from human urine and was found to be (3*S*)-3-hydroxyquinidine.[8] On the basis of this finding, 3-hydroxyquinine has been postulated to be the major metabolite of quinine (**1**); however, only NMR evidence in regard to the assignment[9] of the configuration at C-3 has been reported. Quinine and its metabolites have been analyzed in plasma and urine using HPLC and GC-MS methods, and 3-hydroxyquinine was indeed found as the major metabolite of quinine.[10−12] However, only one epimer of 3-hydroxyquinine was reported as a major metabolite, and it was proposed as the 3(*S*) isomer. In this context, it was of importance to establish, unequivocally, the structures of both epimers of 3-hydroxyquinine. Herein, we wish to report the synthesis and the X-ray crystal structure of the 9-aceto analogue of 3(*S*)-3-hydroxyquinine and its conversion into 3(*S*)-3-hydroxyquinine (**2**). The absolute configuration of 3(*S*)-3-hydroxyquinine (**2**) has, therefore, been determined, and the comparison of NMR spectra to those of previous reports[9,13] can now be made.

The 3-hydroxyquinine was synthesized from quinine following the scheme reported previously by Diaz-Arauzo et al.[9,13] The epimeric mixture of **2** and **3**, obtained in a 4:1 ratio, could not be separated by flash chromatography on either silica gel or alumina. It appears that the isomers **2** and **3** undergo equilibration on chromatography. Consequently, the mixture of **2** and **3** was partially acylated to generate the epimeric mixture of 9-aceto analogues of 3-hydroxyquinine **4** and **5** (see Scheme 1). This epimeric mixture was rapidly and conveniently separated by column chromatography on silica gel.

Scheme 1. Synthesis of 3(*S*)-3-Hydroxyquinine (**2**)



(i) ref. 9; (ii) Ac_2O, pyr, Δ, 75%; (iii) K_2CO_3, MeOH, 90%

The major isomer was crystallized from CH_2Cl_2−hexane, and its absolute configuration was established by single-crystal X-ray analysis, the structure of which is depicted in Figure 2 (see Experimental Section for details). This confirmed the configuration of the major diastereomer **4** as 3(*S*). Hydrolysis of **4** under basic conditions with K_2CO_3 in methanol yielded pure 3(*S*)-3-hydroxyquinine (**2**). The structure of **2** was further established using 2D-NMR spectroscopy (see Figure 1 for the NOE observed for 3(*S*)-3-hydroxyquinine) and correlated with spectra from earlier NMR work.[9] This makes available the correct structures of both metabolites (3-hydroxyquinine) and can be employed to clear the confusion in the literature as regards the major metabolite **2** of quinine (**1**). In addition, the pure *R* and *S* isomers of 3-hydroxyquinine as well as the related 9-aceto analogues are now available for biological screening.

Experimental Section

General Experimental Procedures. The experimental protocols were carried out as previously reported.[14]

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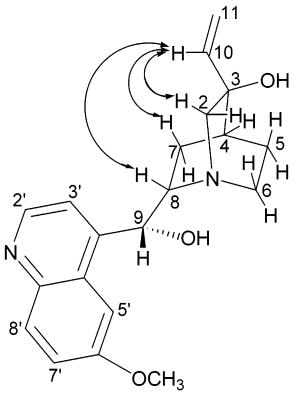

Figure 1. NOE observed for (3*S*)-3-hydroxyquinine (**2**).

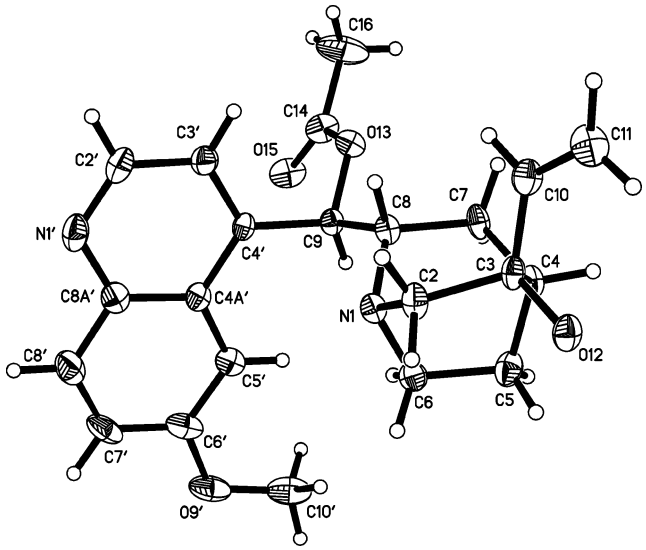

Figure 2. Structure of the 9-aceto analogue of 3(*S*)-3-hydroxyquinine (**4**) as determined by X-ray crystallography with displacement ellipsoids shown at the 50% level.

(3*S*)-3-Hydroxyquinine 9-acetate (4). To a round-bottomed flask (50 mL) that contained a solution of the epimeric mixture of 3-hydroxyquinines **2** and **3** (800 mg, 2.35 mmol) and pyridine (8 mL) was added acetic anhydride (0.34 mL, 3.53 mmol), and the contents were heated at 100 °C for 3 h. The reaction mixture was cooled and the pyridine was removed under reduced pressure. The residue was dissolved in MeOH (10 mL), followed by the addition of a cold saturated solution of methanolic hydrogen chloride (10 mL). The solvent was removed under reduced pressure, and the solid was dissolved in water and extracted with chloroform (3 × 50 mL). The organic layer was discarded, the aqueous phase was brought to pH 8−9 with Na_2CO_3, and the solution was extracted with chloroform (3 × 100 mL). The organic layer was washed with water and brine and dried (Na_2SO_4). The solvent was removed under reduced pressure, and the two epimers were separated by flash chromatography [silica gel, $CHCl_3$ (98%)−MeOH (2%)]. **4**: mp 146−148 °C; R_f = 0.35 [silica gel, $CHCl_3$ (98%)−MeOH (2%)]; $[\alpha]^{25}_D$ −57.7° (*c* 0.60, MeOH); IR (KBr) ν_{max} 3283, 2936, 1732, 1623, 1033 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.78 (1H, d, J = 4.5 Hz, H-2′), 8.08 (1H, d, J = 9.2 Hz, H-8′), 7.49 (1H, d, J = 2.15 Hz, H-5′), 7.43 (1H, dd, J = 2.65, 9.15 Hz, H-7′), 7.39 (1H, d, J = 4.5 Hz, H-3′), 6.60 (1H, br d, J = 5.85 Hz, H-9), 6.11 (1H, dd, J = 10.8, 17.25 Hz, H-10), 5.34 (1H, d, J = 17.85 Hz, H-11t), 5.18 (1H, d, J = 10.8 Hz, H-11c), 4.02 (3H, s, OCH_3), 3.30 (1H, br q, J = 16 Hz, H-8), 3.16 (1H, m, H-6en), 3.02 (1H, br d, J = 14.25 Hz, H-2c), 2.87−2.84 (2H, br m, H-2t, H-6ex), 2.23−2.19 (1H, br m, H-5ex), 2.19 (3H, s, $COCH_3$), 1.92−1.85 (2H, br m, H-4, H-7ex), 1.77−1.74 (1H, br m, H-7en), 1.57 (1H, br m, H-5en); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 21.5 (CH_2 and CH_3, C-5, $COCH_3$), 26.4 (CH_2, C-7), 34.7 (CH, C-4), 42.8 (CH_2, C-6), 56.2 (CH_3, OCH_3), 58.9 (CH, C-8), 65.1 (CH_2, C-2), 72.1 (C, C-3), 73.9 (CH, C-9), 101.9 (CH, C-5′), 113.6 (CH_2, C-11), 119.2 (CH, C-3′), 122.3 (CH, C-7′), 127.3 (C, C-4A′), 132.3 (CH, C-8′), 143.9 (C and CH, C-4′, C-10), 145.2 (C, C-8A′), 147.8 (CH, C-2′), 158.5 (C, C-6′), 170.3 (C, *C*OCH_3); EIMS *m/z* 382 [M$^+$], 231 (40), 189 (25), 152 (100); *anal*. C 57.67%, H 6.07%, N 5.58%, calcd for $C_{22}H_{26}N_2O_4 \cdot 1.2CH_2Cl_2$, C 57.53%, H 5.91%, N 5.78%.

3(*R*)-3-Hydroxyquinine 9-acetate (5): mp 166−168 °C; R_f = 0.40 [silica gel, $CHCl_3$ (98%)−MeOH (2%)]; IR (KBr) ν_{max} 3280, 2925, 1729, 1033 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.73 (1H, d, J = 4.53 Hz), 8.02 (1H, d, J = 9.2 Hz), 7.47 (1H, d, J = 2.62 Hz), 7.39−7.37 (1H, m), 7.35 (1H, d, J = 2.66 Hz), 6.54 (1H, d, J = 7.56 Hz), 6.11 (1H, dd, J = 10.77, 17.31 Hz), 5.28 (1H, d, J = 17.28 Hz), 5.15 (1H, d, J = 11.01 Hz), 3.97 (3H, s), 3.70 (1H, q, J = 16.60 Hz), 3.19−3.10 (1H, m), 2.89 (1H, d, J = 14.33 Hz), 2.74 (1H, dd, J = 1.25, 14.40 Hz), 2.64−2.55 (1H, m), 2.27−2.14 (1H, m), 2.14 (3H, s), 1.93−1.91 (2H, m), 1.67−1.63 (2H, m), 1.53−1.42 (1H, m); ^{13}C NMR ($CDCl_3$, 75 MHz) 21.0, 23.1, 24.5, 33.5, 41.9, 55.6, 58.1, 64.3, 72.1, 73.4, 101.5, 113.3, 118.9, 121.8, 127.1, 131.6, 142.6, 143.7, 144.6, 147.3, 157.8, 170.0; EIMS *m/z* 382 [M$^+$], 231 (40), 189 (25), 152 (100).

3(*S*)-3-Hydroxyquinine (2). Anhydrous K_2CO_3 (108 mg, 0.78 mmol) was added to a solution of **4** (100 mg, 0.26 mmol) in methanol (5 mL). The mixture that resulted was stirred at room temperature for 3 h. It was then diluted with water and extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4), and filtered, and the solvent was removed under reduced pressure. The residue that resulted was washed with ether to yield pure **2** (80 mg, 90%): mp 148−150 °C [lit.[15] mp 147−149 °C]; R_f = 0.15 [silica gel, $CHCl_3$ (98%)−MeOH (2%)]; $[\alpha]^{25}_D$ −196.80° (*c* 0.50, MeOH); IR (KBr) ν_{max} 3350, 1615, 1500, 1240 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 8.67 (1H, d, J = 4.55 Hz, H-2′), 7.96 (1H, d, J = 9.15 Hz, H-8′), 7.70 (1H, d, J = 4.55 Hz, H-3′), 7.46−7.42 (2H, m, H-5′ and H-7′), 5.98 (1H, dd, J = 10.85, 17.25 Hz, H-10), 5.60 (1H, d, J = 3.35 Hz, H-9), 5.22 (1H, d, J = 17.2 Hz, H-11t), 5.02 (1H, d, J = 10.85 Hz, H-11c), 3.99 (3H, s, OCH_3), 3.63 (1H, br t, J = 10.5 Hz, H-6en), 3.07−3.00 (2H, m, H-8 and H-2c), 2.83−2.77 (2H, m, H-6ex and H-2t), 2.14−2.10 (1H, m, H-5ex), 2.06−2.01 (1H, m, H-7en), 1.82 (1H, br s, H-4), 1.67−1.62 (1H, m, H-5en), 1.48−1.43 (1H, m, H-7ex); ^{13}C NMR (CD_3OD, 125 MHz) δ 20.8 (CH_2, C-5), 22.8 (CH_2, C-7), 34.3 (CH, C-4), 43.0 (CH_2, C-6), 55.5 (CH_3, OCH_3), 59.5 (CH, C-8), 64.3 (CH_2, C-2), 71.1 (C, C-9), 71.3 (CH, C-3), 101.5 (CH, C-5′), 112.0 (CH_2, C-11), 119.1 (CH, C-3′), 122.3 (CH, C-7′), 127.2 (C, C-4A′), 130.4 (CH, C-8′), 143.6 (CH, C-10), 143.8 (C, C-8A′), 147.2 (CH, C-2′), 149.7 (C, C-4′), 158.7 (C, C-6′); EIMS *m/z* no M$^+$ peak observed 189 (95), 152 (100); *anal*. C 67.94%, H 7.04%, N 7.70%, calcd for $C_{20}H_{24}N_2O_3 \cdot 0.8CH_3OH$, C 68.24%, H 7.49%, N 7.65%. The spectral data for **2** were in excellent agreement with the literature.[9,13]

3(*R*)-3-Hydroxyquinine (3): mp 157 °C; R_f = 0.18 [silica gel, $CHCl_3$ (98%)−MeOH (2%)]; IR (KBr) ν_{max} 3350, 1610, 1500, 1248 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.67 (1H, d, J = 4.5 Hz), 7.94 (1H, d, J = 9.1 Hz), 7.70 (1H, d, J = 4.5 Hz), 7.44−7.39 (2H, m), 6.14 (1H, dd, J = 10.8, 17.3 Hz), 5.67 (1H, d, J = 2.88 Hz), 5.28 (1H, d, J = 17.3 Hz), 5.12 (1H, d, J = 10.77 Hz), 3.98 (3H, s), 3.83−3.73 (1H, m), 3.47−3.42 (1H, m), 3.36−3.32 (1H, m), 3.00−2.96 (1H, m), 2.82−2.78 (1H, m), 2.71−2.62 (1H, m), 1.92−1.62 (4H, m); ^{13}C NMR (CD_3OD, 75 MHz) δ 158.2, 149.1, 146.6, 143.3, 142.4, 129.8, 126.6, 121.9, 118.5, 112.0, 101.0, 71.2, 70.8, 63.5, 58.5, 55.0, 42.3, 33.4, 22.0, 20.4.

X-ray Crystal Structure of 3(*S*)-3-Hydroxyquinine 9-acetate (4). Single-crystal X-ray diffraction data on compound **4** were collected at 103 K using Mo Kα radiation and a Bruker SMART 1000 CCD area detector. A 0.40 × 0.35 × 0.28 mm^3 crystal was prepared for data collection by coating with high-viscosity microscope oil (Paratone-N, Hampton Research). The oil-coated crystal was mounted on a glass rod and transferred immediately to the cold stream (−170 °C) on the diffractometer. The crystal was tetragonal in space group $P4_12_12$ with unit cell dimensions $a = b$ = 10.8089(12) Å, c = 42.926(7) Å. Corrections were applied for Lorentz polarization

and absorption effects. Data were 95.4% complete to 28.31° θ (approximately 0.75 Å resolution) with an average redundancy of 5.5. The structure was solved by direct methods and refined by full-matrix least squares on F^2 values using the programs found in the SHELXTL suite.[17] Parameters refined included atomic coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms on carbons were included using a riding model [coordinate shifts of C applied to H atoms] with C−H distance set at 0.96 Å. The absolute configuration was set based on the known configuration of the optically pure quinine (one of the starting materials for the synthesis). The asymmetric unit contains one molecule plus two molecules of solvent (dichloromethane). Both solvent molecules were disordered; one of these sits on a special position at half-occupancy. Atomic coordinates for compound **4** have been deposited with the Cambridge Crystallographic Data Centre (deposition number 260728). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk

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Supporting Information Available: Crystal data and structural refinement for **4**. Coordinates are also available from Cambridge Crystallographic Database.[16] This material is available free of charge via the Internet at http://pubs.acs.org.

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