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CONVERSION OF ARTEMISININ TO ARTEMISITENE

FAROUK S. EL-FERALY,* ABIDIN AYALP, MOHAMMED A. AL-YAHYA,

Department of Pharmacognosy and Medicinal, Aromatic and Poisonous Plants Research Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

DONALD R. MCPHAIL, and ANDREW T. MCPHAIL*

Department of Chemistry, Paul M. Gross Chemical Laboratory, Duke University, Durham, North Carolina 27706

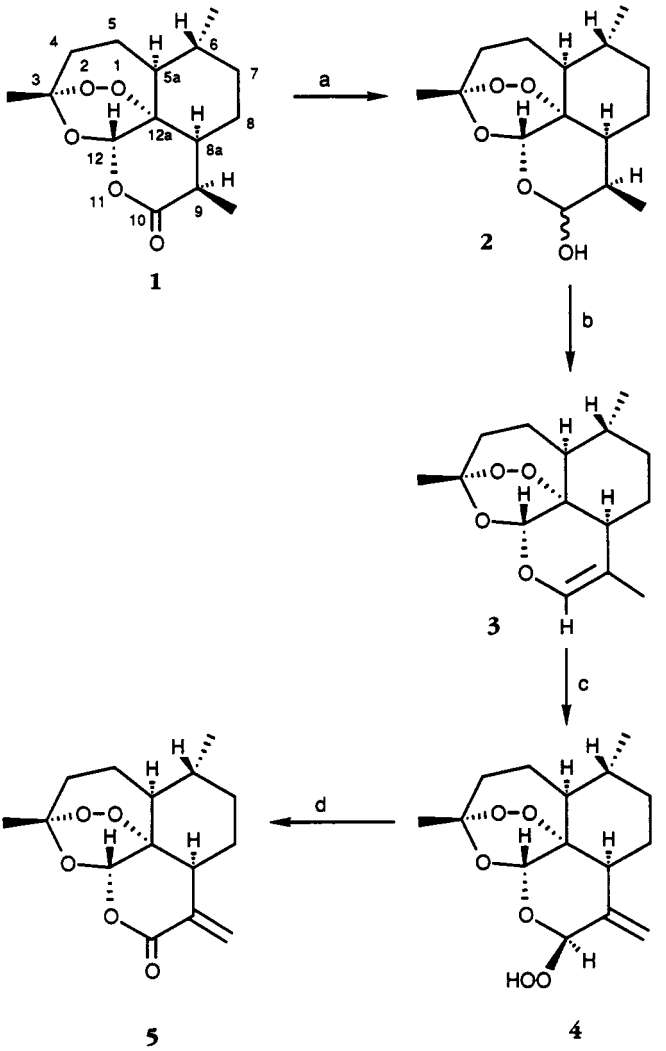
ABSTRACT.—Artemisinin [**1**], a potent antimalarial sesquiterpene endoperoxide, was converted via a photochemical route to its dehydro analogue, artemisitene [**5**]. The structure and stereochemistry of the hydroperoxide intermediate **4**, produced by the action of $^1O_2$ on the enol ether **3**, were established by X-ray crystallographic analysis.

Artemisinin (qinghaosu) [**1**] is a potent antimalarial sesquiterpene endoperoxide isolated (1) from the Chinese weed *Artemisia annua* L. (Compositae). The dehydro analogue, artemisitene [**5**], was reported (2) to occur in an American variant of the same plant but in much smaller yields. There is a current interest in artemisitene [**5**] as it can serve as a precursor for labeled (2,3) artemisinin derivatives that are much needed for metabolic studies. The present paper describes a high-yielding route for converting artemisinin [**1**] to artemisitene [**5**] using a photochemical approach.

RESULTS AND DISCUSSION

The chemistry involved in the conversion of **1** to **5** is summarized in Scheme 1. The enol ether, anhydrodihydroartemisinin [**3**], is the key compound used in the transformation. It was obtained by dehydrating dihydroartemisinin [**2**] (4) using dicyclohexylcarbodiimide under conditions similar to those of Moffatt oxidation (5). It should be noted that **3** was previously reported in the Chinese literature (1,6) as a product of dehydrating **2** by use of boron trifluoride etherate.

Methylene-blue-sensitized photo-oxygenation (7) of an EtOH solution of **3** at 25° provided a mixture of **4** and **6**, which was separated by flash chromatography (8) on Si gel using *n*-hexane–Et_2O (3:2) as solvent. The major product **4** (70% yield) was derived from the ene-reaction of singlet oxygen ($^1O_2$) with the less hindered β face of **3**. The nmr spectral data for **4** (see Experimental) were consistent with its assigned structure and indicated that it existed in solution as a single entity. The relative stereochemistry at the newly created chiral center at C-10 was determined unambiguously by an X-ray crystal structure analysis; the absolute configuration follows from that of the starting material, artemisinin [**1**]. The crystal structure was solved by direct methods (MULTAN11/82). Refinement of atomic positional and thermal parameters (anisotropic C, O; fixed H contributions) converged at $R = \Sigma||F_o|-|F_c||/\Sigma|F_o| = 0.038$, $R_w = [\Sigma w(|F_o|-|F_c|)^2/\Sigma w|F_o|^2]^{1/2} = 0.052$, $GOF = [\Sigma w(|F_o|-|F_c|)^2/(N_{observations}-N_{parameters})]^{1/2} = 1.5$) over 1977 reflections with $I > 3.0\sigma(I)$. Fractional coordinates of the carbon and oxygen atoms are provided in Table 1. The asymmetric unit comprises a pair of hydrogen-bonded [0–18 . . . 0–11′ = 2.827 (4) Å; 0–18′ . . . 0-11 = 2.914 (5) Å] molecules of **4** which have essentially the same conformation (maximum difference between corresponding torsion angles = 6.5°; mean difference = 2.1°). A view of one of these molecules is presented in Figure 1. Corresponding lengths and angles in each of the molecules agree well and are close to expected values (9). The tetrahydropyran ring has a flattened chair conformation with an axial β-oriented hydroperoxy group at C-10. Endocyclic torsion angles ($\omega_{ij} \pm 0.4$–$0.6°$) about the bond between atoms *i* and *j*, which



SCHEME 1. Conversion of artemisinin [**1**] to artemisitene [**5**]: (a) $NaBH_4$ in MeOH at −5° (79%) [see Brossi *et al.* (4)], (b) dicyclohexylcarbodiimide/DMSO/H_3PO_4 (96%), (c) O_2/methylene blue/650 W incandescent light at 25° (70%), (d) Ac_2O/pyridine (100%).

characterize the ring conformation in one of the molecules, with corresponding values in the other crystallographically independent molecule in parentheses, are: $\omega_{8a,9}$ −46.2 (−49.5), $\omega_{9,10}$ 46.0 (46.7), $\omega_{10,11}$ −50.0 (−48.4), $\omega_{11,12}$ 55.3 (53.1), $\omega_{12,12a}$ −52.2 (−52.3), $\omega_{12,8a}$ 47.5 (50.8).

Dehydration (7) of **4** by treatment with Ac_2O in pyridine proceeded smoothly and quantitatively to yield artemisitene [**5**], mp 160–161° [lit. (2) 161–162°], $[\alpha]^{22}D$ +130° (c = 0.05, $CHCl_3$) [lit. (2) +134°], with spectral data indistinguishable from those reported (2).

The minor photo-oxygenation product, the formate ester **6**, is presumably produced from the cleavage of an intermediate dioxetane formed by the addition of $^1O_2$ to the double bond (10). Its spectral data are in full agreement (see Experimental) with the proposed structure.

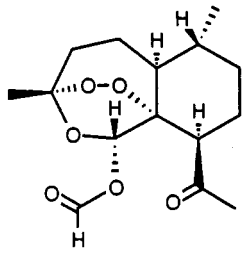


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TABLE 1. Fractional Coordinates and Equivalent Isotropic Thermal Parameters for the Non-hydrogen Atoms in the Two Crystallographically Independent Molecules of Compound **4**, With Estimated Standard Deviations in Parentheses.

Atom	*x*	*y*	*z*	B_{eq} (Å^2)
O-1	0.5138(2)	−0.1115(1)	0.0432(3)	4.6(1)
O-2	0.5740(2)	−0.0860(1)	−0.0641(3)	5.2(1)
C-3	0.6318(2)	−0.0467(2)	0.0112(5)	5.1(1)
C-4	0.6662(2)	−0.0771(2)	0.1478(6)	6.1(1)
C-5	0.6217(3)	−0.0599(2)	0.2866(5)	5.8(1)
C-5a	0.5266(3)	−0.0744(2)	0.2885(4)	4.9(1)
C-6	0.4842(3)	−0.0418(2)	0.4178(5)	6.9(1)
C-7	0.3907(4)	−0.0569(3)	0.4211(6)	9.3(1)
C-8	0.3490(3)	−0.0397(2)	0.2779(6)	7.8(1)
C-8a	0.3889(2)	−0.0733(2)	0.1473(6)	5.6(1)
C-9	0.3479(2)	−0.0558(2)	0.0080(6)	6.7(1)
C-10	0.3728(3)	0.0054(2)	−0.0586(6)	6.4(1)
O-11	0.4615(2)	0.0133(1)	−0.0583(3)	5.5(1)
C-12	0.5033(2)	0.0026(2)	0.0778(4)	4.2(1)
C-12a	0.4842(2)	−0.0626(1)	0.1408(4)	3.9(1)
C-13	0.6969(3)	−0.0294(3)	−0.1007(6)	8.2(1)
C-14	0.5248(5)	−0.0599(3)	0.5621(5)	10.9(2)
C-15	0.2918(3)	−0.0923(2)	−0.0606(8)	9.1(2)
O-16	0.5883(1)	0.0117(1)	0.0484(3)	4.9(1)
O-17	0.3343(2)	0.0544(1)	0.0217(4)	7.5(1)
O-18	0.3436(2)	0.1129(1)	−0.0605(5)	9.7(1)
O-1′	0.5432(2)	0.2754(1)	0.1257(3)	4.9(1)
O-2′	0.4626(2)	0.2471(1)	0.1701(3)	5.7(1)
C-3′	0.4790(3)	0.1976(2)	0.2701(4)	5.7(1)
C-4′	0.5374(4)	0.2167(2)	0.3905(5)	6.9(1)
C-5′	0.6284(4)	0.2017(2)	0.3651(5)	7.6(1)
C-5a′	0.6683(3)	0.2291(2)	0.2262(5)	6.3(1)
C-6′	0.7533(3)	0.1999(2)	0.1956(8)	9.1(2)
C-7′	0.7917(4)	0.2223(2)	0.0532(9)	10.5(2)
C-8′	0.7299(3)	0.2141(2)	−0.0762(7)	8.6(1)
C-8a′	0.6480(3)	0.2494(2)	−0.0465(5)	5.9(1)
C-9′	0.5864(3)	0.2464(2)	−0.1683(5)	6.6(1)
C-10′	0.5389(4)	0.1852(2)	−0.1836(4)	6.8(1)
O-11′	0.5074(2)	0.1646(1)	−0.0481(3)	5.9(1)
C-12′	0.5629(3)	0.1637(2)	0.0725(4)	4.8(1)
C-12a′	0.6062(3)	0.2272(2)	0.0951(4)	4.7(1)
C-13′	0.3915(4)	0.1768(3)	0.3185(6)	8.5(1)
C-14′	0.8184(4)	0.2129(3)	0.3156(11)	13.3(2)
C-15′	0.5696(4)	0.2945(2)	−0.2595(5)	8.3(1)
O-16′	0.5146(2)	0.1449(1)	0.1909(3)	5.2(1)
O-17′	0.5933(3)	0.1396(1)	−0.2434(3)	8.9(1)
O-18′	0.5426(3)	0.0860(2)	−0.2879(4)	10.9(1)

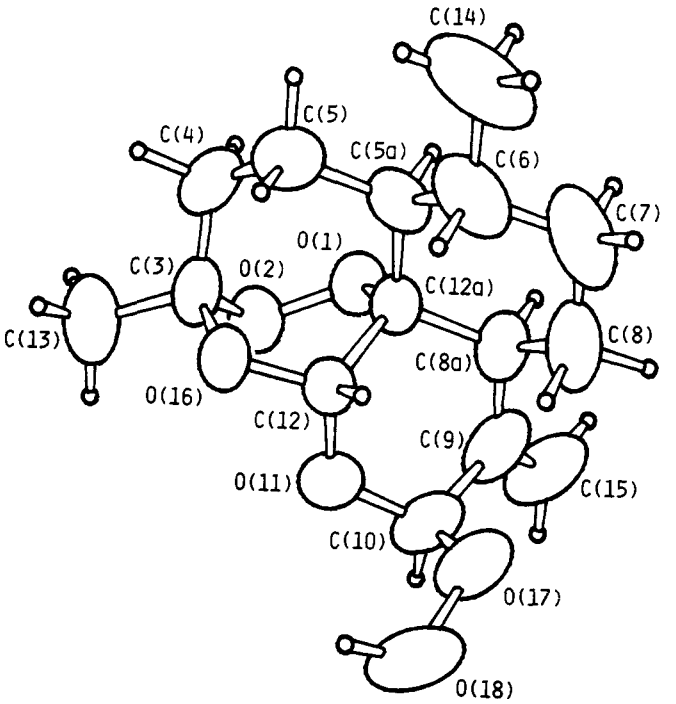

FIGURE 1. Atom numbering scheme and solid-state conformation of one of the molecules of compound **4** in the asymmetric crystal unit. Small circles represent hydrogen atoms.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Ir spectra and specific rotations were obtained on Perkin-Elmer 580 IR and 241 MC instruments, respectively. Nmr spectra were determined on a Varian XL 200 spectrometer or a Varian VSR-300 spectrometer at 300 and 75 MHz for ^{1}H nmr and ^{13}C nmr, respectively, and chemical shift values are given in δ (ppm) with TMS as internal standard. Standard Varian pulse sequences were used for DEPTGL, APT, and HETCOR spectra, which aided nmr assignments. Low-resolution electron impact mass spectra were obtained using an E.I. Finnigan model 3200 (70 eV ionization potential) with INCOS data system or an E.I. Finnigan model 4600 quadrupole system. X-ray crystallographic measurements were made on an Enraf-Nonius CAD-4 diffractometer (Cu-*K*α radiation, λ = 1.5418 Å; incident-beam graphite monochromator). Tlc was performed on Si gel G plates using Et_2O–*n*-hexane (2:3) as solvent, unless otherwise specified, and visualized either under short wavelength uv light or by spraying with anisaldehyde spray reagent (11). *A. annua* was grown at the local (Riyadh, Saudi Arabia) medicinal plant garden. Greenhouse-grown plants were planted in early November 1987. Leaves were picked at the pre-flowering stage in early April 1988. A voucher specimen is preserved at the herbarium of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Artemisinin [**1**] was isolated from the plant material using a literature procedure (12).

DEHYDRATION OF DIHYDROARTEMISININ [**2**] TO **3**.—Dihydroartemisinin [**2**] (1.0 g), obtained from artemisinin [**1**] in 79% yield by reduction with $NaBH_4$ in MeOH at −5° as previously described (4), was dissolved in 1.5 ml of DMSO and 12 ml of C_6H_6. Dicyclohexylcarbodiimide (2.2 g) was stirred in the solution along with 0.1 g of anhydrous H_3PO_4. After 21 h, 50 ml of 5% aqueous oxalic acid solution was added, and the solution was stirred for an additional 30 min. H_2O (100 ml) was added and the mixture was extracted with 4 × 500 ml portions of Et_2O. The Et_2O extract was washed with H_2O, dried (anhydrous Na_2SO_4), and evaporated. The residue was dissolved in CH_2Cl_2 (100 ml), filtered to remove the insoluble dicyclohexylurea, concentrated, and flash chromatographed (8) on Si gel, using $CHCl_3$–*n*-hexane (7:3) as solvent, to give 0.90 g (96%) of the enol ether **3** which was obtained from *n*-hexane–Et_2O as colorless needles: $C_{15}H_{22}O_4$, mp 94–95° [lit. (6) 94–95°], $[\alpha]^{22}D$ + 105° (*c* = 0.05, $CHCl_3$); ir (KBr) (cm^{-1}) 1677 (C=C) with neither OH nor CO absorption bands; ^{1}H nmr ($CDCl_3$) δ 6.19 (1H, d, *J* = 1.5 Hz, H-10),

5.54 (1H, s, H-12), 1.58 (3H, d, J = 1.5 Hz, 9-Me), 1.43 (3H, s, 3-Me), 0.98 (3H, d, J = 6.0 Hz, 6-Me); ^{13}C nmr ($CDCl_3$) δ 135.01 (C-10), 108.09 (C-9), 104.53 (C-3), 89.69 (C-12), 78.96 (C-12a), 28.87 (3-Me), 20.28 (6-Me), 16.18 (9-Me), three methine signals at δ 51.46, 44.46, and 37.49 (C-5a, C-6, and C-8a, not assigned), four methylene signals at δ 36.25, 34.13, 30.0, and 24.42 (C-4, C-5, C-7, and C-8, not assigned); eims m/z (rel. int.) $[M]^+$ 266 (77); calcd for $C_{15}H_{22}O_4$, C 67.74, H 8.33, found C 67.77, H 8.31.

PHOTO-OXYGENATION OF **3** TO **4** AND **6**.—The enol ether **3** (0.25 g) was dissolved in absolute EtOH (20 ml) containing about 1.5 mg of methylene blue. The solution was subjected to 650 W incandescent light while a stream of O_2 was bubbled gently through it, and its temperature was maintained at 25° by cooling. The setup has been described previously (7). After 90 min, tlc showed the disappearance of **3** and the presence of two spots, R_f 0.22 (major, due to **4**) and R_f 0.42 (minor, due to **6**). The solution was evaporated in vacuo at 40°. The residue was dissolved in CH_2Cl_2 and filtered over a short Si gel column to remove the dye. Flash chromatography (8) on Si gel using n-hexane–Et_2O (3:2) as solvent provided pure **6** followed by **4**.

Compound **6** (0.045 g, 16%) was obtained from n-hexane–Et_2O as prisms: mp 103–104°; $[\alpha]^{22}D$ −59° (c = 0.05, $CHCl_3$); ir (KBr) (cm^{-1}) ν max 1704 and 1728 (Me*CO* and H*COO*) with no OH absorption bands; ^{1}H nmr ($CDCl_3$) δ 7.94 (1H, s, H-10), 6.58 (1H, s, H-12), 2.52 (3H, s, 9-Me), 1.48 (3H, s, 3-Me), 1.05 (3H, d, J = 6.2 Hz, 6-Me); ^{13}C nmr ($CDCl_3$) δ 208.18 (C-9), 159.41 (C-10),[1] 105.34 (C-3), 86.63 (C-12), 85.0 (C-12a), 32.29 (9-Me), 25.30 (3-Me), 19.93 (6-Me), three methine signals at δ 57.11, 52.72, and 37.45 (C-5a, C-6, and C-8a, unassigned), four methylene signals at δ 35.74, 33.62, 24.85, and 23.75 (C-4, C-5, C-7, and C-8, unassigned); eims m/z (rel. int.) $[M]^+$ 298 (17); calcd for $C_{15}H_{22}O_6$, C 60.39, H 7.43, found C 60.37, H 7.37.

Compound **4** (0.196 g, 70%) was obtained from CH_2Cl_2/Et_2O/n-hexane as prisms: mp 149–150°; $[\alpha]^{22}D$ +161° (c = 0.05, $CHCl_3$); ir (KBr) (cm^{-1}) ν max 3350 (OH) and no CO bands; ^{1}H nmr ($CDCl_3$) δ 10.18 (1H, s, exchangeable, O*OH*), 5.88 (1H, s, H-12), 5.76 (1H, s, H-10), 5.33 and 5.20 (1H each, s, 9-CH_2), 1.48 (3H, s, 3-Me), 0.99 (3H, d, J = 5.6 Hz, 6-Me); ^{13}C nmr ($CDCl_3$) δ 138.60 (C-9), 119.78 (9-CH_2), 105.58 (C-10), 104.73 (C-3), 88.23 (C-12), 80.68 (C-12a), 25.82 (3-Me), 20.17 (6-Me), three methine signals at δ 51.87, 47.52, and 37.56 (C-5a, C-6, and C-8a, unassigned); four methylene signals at δ 36.27, 34.03, 31.30, and 24.62 (C-4, C-5, C-7, and C-8, unassigned); eims m/z (rel. int.) $[M]^+$ 298 (0.5); calcd for $C_{15}H_{22}O_6$, C 60.39, H 7.43, found C 60.27, H 7.57.

DEHYDRATION OF **4** TO ARTEMISITENE [**5**].—The hydroperoxide **4** (0.3 g) was dissolved in Ac_2O (2 ml) containing 0.1 ml of pyridine. The solution was stirred at room temperature for 50 min and then worked up in the usual manner (7) to give 0.28 g (100%) of crystalline artemisitene [**5**]. Recrystallization from n-hexane–Et_2O gave colorless crystals, mp 160–161° [lit. (2) 161–162°], $[\alpha]^{22}D$ +130° (c = 0.05, $CHCl_3$) [lit. (2) +134°], with spectral data indistinguishable from those previously reported (2).

X-RAY CRYSTAL STRUCTURE ANALYSIS OF COMPOUND **4**.[2]—Crystal data: $C_{15}H_{22}O_6$, MW = 298.34, orthorhombic, space group $P2_12_12_1$, a = 15.902 (1), b = 21.111 (2), c = 9.211 (1) Å (from 25 orientation reflections, 39° < θ < 48°), V = 3092.2 (8) Å^3, Z = 8, D_c = 1.282 g·cm^{-3}, μ (CuKα radiation, λ = 1.5418 Å) = 7.9 cm^{-1}. Crystal dimensions: 0.16 × 0.18 × 0.40 mm. One octant of intensity data was recorded on an Enraf-Nonius CAD-4 diffractometer [CuKα radiation, ω–2θ scans, θ max = 67°, scan width (1.00 + 0.14 tan θ)°]. From a total of 3103 measurements, those 1977 reflections with $I > 3.0\sigma(I)$ were retained for the analysis, and the usual Lorentz and polarization corrections were applied.

The crystal structure was solved by direct methods (MULTAN11/82). Initial non-hydrogen atom coordinates were derived from an E-map. Several rounds of full-matrix least-squares adjustment of positional and anisotropic temperature factor parameters were followed by evaluation of a difference Fourier synthesis which revealed positive regions at positions calculated for hydrogen atoms. Hydrogen atoms were included at their idealized positions in all subsequent least-squares iterations. An extinction correction was included as a variable in the final least-squares cycles which converged at R = 0.038 (R_w = 0.052, GOF = 1.5).

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[1]While the off-resonance spectrum clearly showed this carbon signal to be a doublet, the APT spectrum run at the normal τ value of 7 msec exhibited a positive signal for this carbon instead of the expected negative one. This phenomenon was attributed to the large $^1J_{C\text{-}H}$ value of 233 Hz for this signal. When the APT spectrum was re-recorded at 5 msec, a negative signal was observed. Likewise, the appropriate parameters needed to be adjusted accordingly in order for the signal due to this carbon to be observed in the DEPTGL and HETCOR spectra.

[2]Atomic coordinates for this compound have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

Crystallographic calculations were performed on PDP11/44 and MicroVAX computers by use of the Enraf-Nonius Structure Determination Package. For all structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from International Tables for X-Ray Crystallography (13). In the least-squares iterations, $\Sigma w\Delta^2$ [$w = 1/\sigma^2(|F_o|)$, $\Delta = (|F_o| - |F_c|)$] was minimized.

ACKNOWLEDGMENTS

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