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A NEW METHOD FOR THE PREPARATION OF ARTEETHER AND ITS C-9 EPIMER

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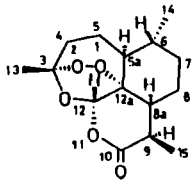
ABSTRACT.—Arteether [**4**] was obtained by treating anhydrodihydroartemisinin [**3**] with absolute EtOH in the presence of *p*-toluenesulfonic acid as a catalyst. 9-*epi*-Arteether [**6**] was obtained as the minor product. The use of CH_2Cl_2 as a solvent yielded the latter as the major product. Its structure and absolute stereochemistry were unambiguously established from its spectral data and by X-ray crystallographic analysis. This procedure permitted the preparation of C-9 deuterated arteether [**5**], which may be needed for metabolic studies.

Artemisinin (qinghaosu) [**1**] is the active constituent of *Artemisia annua* L. (Compositae) that has long been used for the treatment of malaria in China. It is a saturated sesquiterpene lactone with a hindered endoperoxide group that serves a pharmacophore (1). Because the pharmacokinetic properties of artemisinin [**1**] are inadequate, a search for analogues with increased potency and better solubility was initiated in China and elsewhere, focusing on esters and ethers of dihydroartemisinin [**2**] (2). The β-ethyl ether isomer of **2**, now called arteether [**4**], has been chosen by the World Health Organization (WHO) as a new drug candidate useful for treatment of the erythrocytic stages of chloroquine-resistant *Plasmodium falciparum* and for cerebral malaria (3).

To date, the only reported method for preparing arteether [**4**] depends on treating dihydroartemisinin [**2**] with EtOH and boron trifluoride etherate (3), followed by fractional crystallization or chromatography to remove the α epimer. This paper describes an alternative route to arteether [**4**] that may prove to be valuable for the preparation of specifically labeled material for metabolic studies.

Anhydrodihydroartemisinin [**3**], prepared from artemisinin [**1**] as previously described (2,4), was stirred with absolute EtOH in the presence of *p*-toluenesulfonic acid as a catalyst. Upon workup, the product (91%) was found to be a mixture of arteether [**4**] and its C-9 epimer in the ratio of 3:1. Since this mixture was only partially resolved on Si gel G plates, analysis was performed by hplc (see Experimental). Separation of the two compounds was accomplished, nearly quantitatively, using preparative hplc to yield arteether [**4**] (3) and 9-*epi*-arteether [**6**], mp 73.3–73.5°, $[\alpha]^{22}D + 156°$.

9-*epi*-Arteether [**6**], a new compound, was found to have spectral data (see Experimental) that were similar, but not identical, to those of arteether [**4**]. Thus, the ^1H-nmr signals of **6** were virtually the same as those for **4**, except for the expected (5) upfield shift of the H-9 signal to δ 1.49, versus 2.59 for arteether [**4**] (6), and the downfield shift of the doublet due to H-15 to δ 1.17 from 0.88, due to deshielding by the



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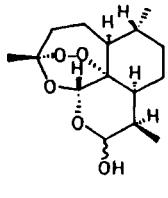

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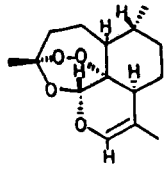

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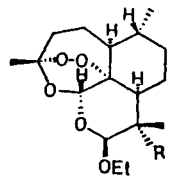

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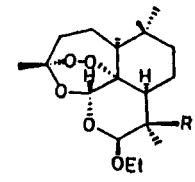

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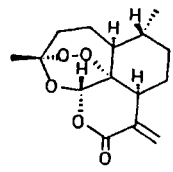
7 R=D



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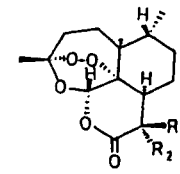

9 R_1=CH_2D, R_2=D
10 R_1=D, R_2=CH_2D

TABLE 1. Fractional Atomic Coordinates[a] for Compound **6**, with Estimated Standard Deviations in Parentheses.

Atom	10^4x	10^4y	10^5z	Atom	10^4x	10^4y	10^5z
O-1	1182(1)	3541(2)	18099(6)	C-10	1969(3)	1285(2)	10531(7)
O-2	1147(2)	2720(2)	22752(6)	O-11	2410(2)	1692(2)	15781(5)
C-3	2509(3)	3464(3)	25205(7)	C-12	3242(2)	3186(2)	16798(7)
C-4	3024(3)	5063(3)	25943(9)	C-12a	2502(2)	4014(2)	15120(7)
C-5	4022(3)	6016(3)	21642(9)	C-13	2282(4)	2632(3)	30256(8)
C-5a	3409(2)	5664(2)	16105(8)	C-14	5337(3)	8114(3)	12942(13)
C-6	4592(3)	6485(2)	12084(10)	C-15	−373(3)	1237(3)	9900(10)
C-7	3996(3)	6111(3)	6595(10)	O-16	3527(2)	3287(2)	22182(5)
C-8	3263(3)	4507(2)	5666(8)	O-17	3150(2)	1554(2)	7355(5)
C-8a	1999(2)	3661(2)	9403(8)	C-18	3767(3)	670(3)	8652(10)
C-9	1207(2)	2027(3)	8260(8)	C-19	4606(3)	647(3)	4107(13)
Atom	**10^3x**	**10^3y**	**10^3z**	**Atom**	**10^3x**	**10^3y**	**10^3z**
H-4A	355(3)	534(3)	293(1)	H-13A	167(3)	139(3)	294(1)
H-4B	220(3)	511(3)	265(1)	H-13B	324(3)	297(3)	319(1)
H-5A	499(3)	593(3)	218(1)	H-13C	174(3)	283(3)	326(1)
H-5B	427(2)	700(2)	226(1)	H-14A	571(3)	838(3)	162(1)
H-5a	268(2)	600(2)	157(1)	H-14B	604(3)	856(3)	103(1)
H-6	533(2)	615(2)	123(1)	H-14C	461(3)	848(3)	129(1)
H-7A	476(3)	664(2)	41(1)	H-15A	−95(3)	22(3)	87(1)
H-7B	338(3)	656(2)	60(1)	H-15B	−44(3)	126(3)	137(1)
H-8A	401(2)	417(3)	61(1)	H-15C	−92(3)	182(3)	86(1)
H-8B	295(3)	426(2)	22(1)	H-18A	286(3)	−40(3)	97(1)
H-8a	124(2)	400(2)	91(1)	H-18B	437(3)	102(3)	117(1)
H-9	122(2)	200(2)	45(1)	H-19A	498(4)	1(4)	49(1)
H-10	124(2)	21(2)	106(9)	H-19B	364(3)	−12(3)	21(1)
H-12	421(2)	362(2)	151(1)	H-19C	538(4)	178(4)	31(2)

[a]Hydrogen atoms bear the same labels as the atoms to which they are bonded.

peroxide group. The ^{13}C-nmr of **6** was similar to that of **4**, the major difference being that the C-15 peak was shifted from 13.1 in **4** to 19.4 in **6**, which was expected (5).

X-ray crystallographic analysis verified the structure and relative stereochemistry of **6**; the absolute stereochemistry followed from the method of synthesis. Fractional atomic coordinates are listed in Table 1. A view of the solid-state conformation, which clearly shows the α configuration and axial orientation of the methyl group at C-9, is presented in Figure 1. Bond lengths are in accord with expectations (7).

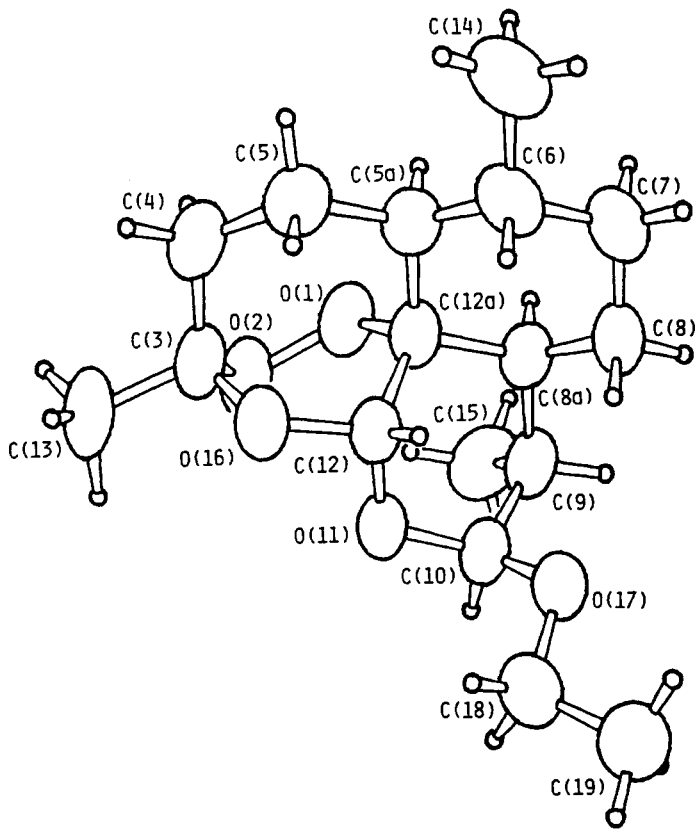

FIGURE 1. Atom numbering scheme and solid-state conformation of compound **6**; small circles represent hydrogen atoms.

It should be noted that the ratio of 9-*epi*-arteether [**6**] to arteether [**4**] was reversed to 3:1 simply by using CH_2Cl_2 as a solvent. This modification would be of value when **6** is the desired product. Also, the use of EtOH saturated with HCl, previously employed (8) for the formation of 9-*epi*-deoxyarteether by etherification of deoxydihydroartemisinin, led to extensive decomposition, producing neither **4** nor **6**.

The formation of arteether [**4**] using the above-mentioned procedure made it possible to prepare, for the first time, its C-9 deutero analogue [**5**], which may be needed for metabolic studies (9) (see Experimental). The ^{1}H-nmr data of this compound are identical to those reported (6) for arteether [**4**], except for the total absence of the signal at δ 2.59 due to H-9, while the ^{13}C nmr showed a very low intensity triplet at δ 31.0 for the now deuterated C-9. The nmr spectra of the C-9 deutero analogue of **6**, namely **7**, indicated the absence of both the H-9 signal at δ 1.49 and the carbon signal at δ 39.9 due to

C-9. Also, the signals due to H-10 and H-15 resonated as singlets, instead of doublets, in both **5** and **7**.

The previously reported (10) C-15 monodeuterium-labeled artemisinin, obtained by a circuitous route, can serve as a precursor for making arteether labeled at this site. Artemisinin labeled with deuterium at both C-9 and C-15, and its C-9 epimer, **9** and **10**, respectively, can be obtained (see Experimental) by $NaBD_4$ MeOH-*d* reduction of artemisitene [**8**], now readily available by chemical conversion from artemisinin [**1**] (4). Since the carbonyl group undergoes partial reduction to a lactol group under the same conditions (3), it was deemed necessary to oxidize the crude reaction mixture using Jones reagent (11). The two products **9** and **10** were separated by flash chromatography. Unfortunately, the major product in this case was the epimer **10** because of the favored deuteride attack from the β face of the molecule (4).

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 VXR-300 spectrometer at 300 and 75 MHz for ^1H 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 DEPT, APT, and HETCOR spectra, which aided nmr assignments. Low-resolution eims (70 eV) were obtained using Finnigan model 4600 quadrupole system, while cims were obtained using a Vestec Model 201 thermospray system with NH_3 as ionizing gas. Tlc was preformed on Si gel G plates using EtOAc–*n*-hexane (1:19), unless otherwise specified, and visualized by spraying with anisaldehyde reagent (12). Hplc was performed using a Waters 600E solvent delivery system, equipped with a U_6K injector, 746 data module, and a uv detector, model 486. A Waters C_{18} Resolve® 5 μm column, 30 cm × 7.8 mm, was used both analytically and preparatively. The solvent system used was 10% aqueous MeCN at a flow rate of 3.0 ml per minute. The detector wavelength was set at 215 nm. *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**] used in preparing **3** was isolated from the plant material using a literature procedure (13).

PREPARATION OF ARTEETHER [**4**], 9-*epi*-ARTEETHER [**6**], AND THEIR DEUTERATED ANALOGUES **5** AND **7**.—The enol ether **3** (100 mg), prepared from artemisinin [**1**] as previously reported (2,4), was stirred for 22 h at room temperature with 2 ml of absolute EtOH containing 60 mg of *p*-toluenesulfonic acid. Evaporation in vacuo yielded a glassy residue that was dissolved in $CHCl_3$ (100 ml) and washed with 10 ml of 5% aqueous $NaHCO_3$ solution. Drying (anhydrous Na_2SO_4) and evaporation of the $CHCl_3$ phase yielded 124 mg of a crystalline mixture of **4** and **6** that was decolorized by dissolving in EtOAc–*n*-hexane (1:19) and filtered over a short bed of Si gel 60. The product (107 mg, 91%) gave two partially overlapping tlc spots, R_f value 0.33. Hplc showed two peaks, Rt 6.87 and 7.65 min, in the ratio of 1:3, due to **6** and **4**, respectively. The mixture was separated using preparative hplc to give 24 mg of **6** and 70 mg of **4**. The identity of **4** as arteether was established by comparing its physical and spectral data with those of the literature (3,6) and by direct comparison with an authentic sample prepared by a literature method (3). Compound **6**, 9-*epi*-arteether, was obtained as colorless prisms: mp 73.3–73.5°; $[\alpha]^{22}D$ +156° (c = 0.1, $CHCl_3$); ir (KBr) no absorption bands for OH, CO or C=C; ^1H nmr ($CDCl_3$) δ 5.45 (1H, s, H-12), 5.00 (1H, d, J = 5.3 Hz, H-10), two dq at 3.92 (J = 7.1, 9.8 Hz) and 3.57 (J = 7.1, 9.8 Hz) (-O-CH_2-CH_3), 2.30 (1H, ddd, J = 3.9, 13.6, 14.6 Hz, H-4α), 1.41 (3H, s, H-13), 1.23 (3H, t, J = 7.1 Hz, -O-CH_2CH_3), 1.17 (3H, d, J = 7.2 Hz, H-15), 0.94 (3H, d, J = 5.9, H-14); ^{13}C nmr ($CDCl_3$) δ 102.9 (s, C-3), 102.4 (d, C-10), 89.2 (d, C-12), 81.7 (s, 12a), 64.2 (t, -O-CH_2-CH_3), 51.8 (d, C-5a), 46.6 (d, C-8a), 39.9 (d, C-9), 37.3 (d, C-6), 36.5 (t, C-4), 34.4 (t, C-7), 31.6 (t, C-8), 25.9 (q, C-13), 24.7 (t, C-5), 20.0 (q, C-14), 19.3 (q, C-15), 15.2 (q, O-CH_2CH_3); cims *m/z* (rel. int.) $[M + NH_4]^+$ 330 (28). Calcd for $C_{17}H_{28}O_5$, C 65.36, H 9.03; found C 65.56, H 9.11.

For a higher yield of **6**, **3** (100 mg) was dissolved in CH_2Cl_2 (10 ml) and absolute EtOH (2 ml), and *p*-toluenesulfonic acid (100 mg) was added. The mixture was refluxed for 12 h and worked up as before. The product (101 mg) was a mixture of **6** and **4** in a 3:1 ratio by hplc.

The deuterated analogues **5** and **7** of arteether and 9-*epi*-arteether, respectively, were prepared and purified as above but by using $MeCH_2OD$ instead of absolute EtOH and deuterated *p*-toluenesulfonic acid. The latter was obtained by exchange with $MeCH_2OD$, then evaporation. The ^1H-nmr spectrum of **5** was similar to that of **4**, but the signals for H-10 and H-15 were singlets. The ^{13}C-nmr spectrum, on the other

hand, showed a low intensity triplet at δ 31.0 due to C-9 (6); eims *m/z* (rel. int.) $[M]^+$ 313 (2). Likewise, the ^{1}H-nmr spectrum of **7** exhibited singlets due to H-10 and H-15, and the intensity of the signal due to C-9 was too low to be observed in the ^{13}C-nmr spectrum; eims *m/z* (rel. int.) $[M]^+$ 313 (2%).

SODIUM BORODEUTERIDE REDUCTION OF ARTEMISITENE [**8**].—Artemisitene [**8**] (100 mg) was dissolved in 2 ml of MeOD. The solution was chilled to −5°, and $NaBD_4$ (25 mg) was added over 2 h and the mixture was stirred for 1 h, maintaining the temperature in the 0 to −5° range. The mixture was neutralized with HOAc (5 drops), concentrated by distilling off the solvent, diluted with H_2O, and extracted with $CHCl_3$. Drying (anhydrous Na_2SO_4) and evaporation of the $CHCl_3$ extract yielded 93 mg of a mixture of products **9** and **10**, and their C-10 lactols. This product was dissolved in 10 ml of dry Me_2CO and stirred for 1 h at 0° to −5° with 2 ml of Jones reagent (prepared by dissolving 26.7 g of CrO_3 in 23 ml of concentrated H_2SO_4 and diluting to 100 ml with H_2O). MeOH (2 ml) was added to quench the reaction, followed by 10 ml of H_2O. The mixture was extracted with $CHCl_3$ (3 × 25 ml), and the combined $CHCl_3$ phases were washed with 1% aqueous $NaHCO_3$ (10 ml). Drying (anhydrous Na_2SO_4) and evaporation yielded a crystalline residue that exhibited two spots on Si gel G tlc plates, using Et_2O–*n*-hexane (3:2), R_f 0.50 (**9**, minor) and R_f 0.40 (**10**, major). Flash chromatography on Si gel using Et_2O–*n*-hexane (1:1) as solvent yielded 24 mg and 64 mg of these products, respectively. Compound **9**, thus obtained, had spectral data similar to the unlabeled substance (14) but the ^{1}H-nmr spectrum showed the H-15 signal as a low intensity (2H) singlet at δ 1.17 with the signal due to H-9 totally absent. The C-15 carbon signal appeared as a low intensity triplet at δ 12.2 (positive in the APT), while the C-9 signal appeared as a much weaker triplet at δ 32.8. Compound **7**, likewise, showed no signal for H-9, while the H-15 signal appeared as a singlet superimposed on the H-13 signal at δ 1.44; the ^{13}C-nmr spectrum showed the C-15 signal as a low intensity triplet at δ 20.4 (positive APT) with the C-9 signal at δ 39.7 (5) appearing as a much weaker triplet.

X-RAY CRYSTAL STRUCTURE ANALYSIS OF COMPOUND **6**[1].—Crystal data: $C_{17}H_{28}O_5$; MW = 312.41, trigonal, space group $P3_221$, $a = b = 10.705$ (1) Å, $c = 25.720$ (3) Å (from 25 orientation reflections, $30° < \theta < 44°$), $V = 2552.6$ (8) Å^3, $Z = 6$, $Dc = 1.219$ g·cm^{-3}, μ(CuKα radiation, $\lambda = 1.5418$ Å) = 6.9 cm^{-1}; crystal dimensions 0.24 × 0.26 × 0.30 mm.

Preliminary unit-cell parameters and space group information were derived from oscillation and Weissenberg photographs. Intensity data ($h \geq 0$, $k \leq 0$, $\ell \geq 0$; 3875 reflections) were recorded on an Enraf-Nonius CAD-4 diffractometer (CuKα radiation, graphite monochromator; ω–2θ scans, scanwidth 1.00 + 0.14 tan θ, $\theta_{max} = 75°$). The intensities of four reference reflections, remeasured every 2 h during data collection, indicated significant crystal decay (−29%). The usual Lorentz and polarization corrections were applied to the intensity data. A linear decay correction was made, and equivalent reflections were averaged ($R_{merge} = 0.023$ on *I*) to give 2041 structure amplitudes out of which those 1099 with $I > 3.0\sigma(I)$ were retained for the analysis.

The crystal structure was solved by direct methods (MULTAN11/82). Initial carbon and oxygen atom coordinates were obtained from an *E* map. Several rounds of full-matrix least-squares adjustment of atomic positional and thermal parameters (at first isotropic, then anisotropic) were followed by evaluation of a difference Fourier synthesis which yielded hydrogen atom positions. Continuation of the least-squares iterations, with hydrogen atom positional and isotropic thermal parameters included as variables, led to convergence (max. shift; ESD = 0.03) at $R = \Sigma||F_o| - |F_c||/\Sigma|F_o| = 0.026$, $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2]^{1/2}$, $GOF = [\Sigma w(|F_o| - |F_c|)^2/(N_{observations} - N_{parameters})]^{1/2} = 0.73$.

Crystallographic calculations were performed on PDP11/44 and MicroVAX computers by use of the Enraf-Nonius Structure Determination Package (SDP). For all structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were from the literature (15). In the least-squares iterations, $\Sigma w\Delta^2$ [$w = 1/\sigma^2(|F_o|)$, $\Delta = (|F_o| - |F_c|)$] was minimized.

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[1]Atomic coordinates for compound **6** have been deposited at the Cambridge Crystallographic Data Centre, and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

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