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CRYSTAL STRUCTURE OF ARTEMISINIC ACID: A POSSIBLE BIOGENETIC PRECURSOR OF ANTIMALARIAL ARTEMISININ FROM *ARTEMISIA ANNUA*[1]

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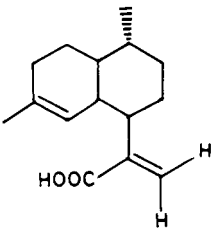
ABSTRACT.—Artemisinic acid [**1**], a possible biogenetic precursor of the antimalarial artemisinin [**2**], was isolated from the hexane extract of *Artemisia annua*. X-ray crystallography of the dimer of artemisinic acid shows that the cyclization during intermolecular hydrogen bonding occurs by the opposite orientation of the α,β-methylene group in each molecule. Complete spectroscopic data of **1** are also given.

During the last decade there has been intense interest in the Chinese antimalarial plant *Artemisia annua* L. (Asteraceae) because of the isolation of a novel sesquiterpene peroxide, artemisinin [**2**] (1,2). We have introduced this herb in India for large scale cultivation and the isolation and chemical transformation of artemisinin into more active derivatives (3). During the course of isolation we found a high concentration of a sesquiterpene acid known as artemisinic acid or qinghao acid [**1**] in its hexane extract. It has been earlier reported (4) that *A. annua* contains 8–10 times more **1** than **2**, thereby attracting several research groups for its possible chemical transformation into biologically active compounds. It was later suggested (5) that **1** is a possible biogenetic precursor of **2** which further encouraged some groups to pursue the microbial transformation of **1** into **2** and its prototypes (6). A survey of the literature shows that limited numbers of sesquiterpene acids have been subjected to crystallographic analysis and very little is known about the stereochemistry of dimerization during intermolecular hydrogen bonding of such acids. Keeping this fact in view, and the importance of artemisinic acid as one of the major constituents of *A. annua*, we have studied the crystals of its hydrogen-bonded dimer by X-ray. The spectroscopic data along with X-ray crystallographic results of **1** are discussed in this paper.

The ir, eims, and cims were consistent with the structure of a sesquiterpene acid (see Experimental). ^{13}C-nmr values have also been given in the Experimental. High resolution ^{1}H nmr showed characteristic signals for an α,β-unsaturated acid with the singlets at δ 6.47 and 5.56 for methylene protons. Singlets at δ 1.59 (H-15) and 4.98



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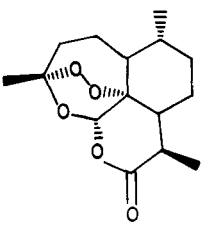

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[1]CIMAP Approval No. 92-18J.

(H-5) along with other signals were in agreement with structure **1**. Although some spectroscopic data of **1** were available in the literature, the structure of its crystalline dimer had not been studied. The ORTEP drawing with the carbon numbering system has been given in Figure 1. The structure determination summary has been incorporated in Tables 1–3[2].

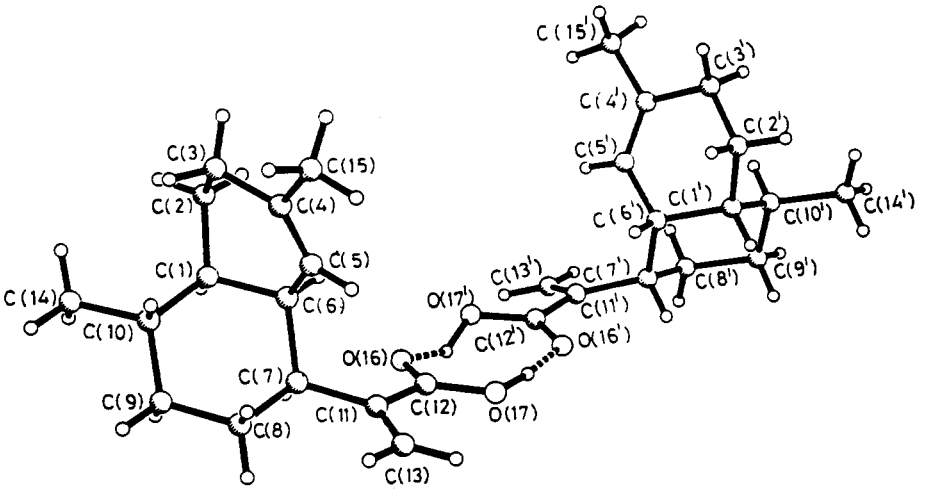

FIGURE 1. ORTEP drawing of the hydrogen-bonded dimer of **1**; carbon and oxygen atoms appear as shaded and dotted circles, respectively; H-bond has been shown by dotted lines.

The crystal structure shows that the two molecules of **1** lie approximately parallel to each other while undergoing cyclization during intermolecular hydrogen bonding. The opposite orientation of exomethylene groups in the dimer causes slight distortion of the rings along the plane. The deviation in the bond length of OH in the dimer is about 0.21 Å which is normal for H-bonded molecules. An interesting point is that the bond

TABLE 1. Crystal Data

Empirical formula	$C_{15}H_{22}O_2$
Color; Habit	Colorless cube
Crystal size (mm)	0.20 × 0.25 × 0.40
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	$a = 9.558(2)$ Å
	$b = 9.717(2)$ Å
	$c = 30.645(6)$ Å
Volume	2846.260 (0) Å^3
Z	8
Formula weight	234.3
Density (calcd)	1.094 Mg/m^3
Absorption coefficient	0.554 mm^{-1}
F (000)	1024

[2]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK.

TABLE 2. Data Collection

Diffractometer used	Siemens R3m/V
Radiation	CuKα (λ = 1.54178 Å)
Temperature (k)	296
Monochromator	Balanced filters
2θ Range	0.0 to 112.0°
Scan type	ω
Scan speed	Variable; 3.00 to 29.30°/min in ω
Scan range (ω)	2.00°
Background measurement	Stationary crystal and stationary counter at beginning and end of scan, each for 50.0% of total scan time
Standard reflections	2 measured every 80 reflections
Index ranges	$0 \leq h \leq 10$, $0 \leq k \leq 10$ $0 \leq l \leq 32$
Reflections collected	2151
Independent reflections	2151 (R_{int} = 0.00%)
Observed reflections	1638 ($F > 4.0\sigma(F)$)
Absorption correction	Empirical methods applied

lengths of the two H bonds are not equal, one being longer by 0.14 Å. However, the bond lengths of C=O in both molecules are exactly the same (1.22 Å).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded on Bruker AM-360 MHz and JEOL 100.4 MHz spectrometers, respectively. Eims and cims were recorded on MS 80 RFA, Kratos, and Ft-ir on Perkin-Elmer 1710B instruments. X-ray crystallographic data was obtained on the Diffractometer: Nicolet/Siemens R3m.

PLANT MATERIAL.—Large scale cultivation of *A. annua* commenced at our experimental farm at Bonera, Kashmir in 1985 for the extraction and isolation of artemisinin. We have taken material from the 1988 cultivation for the chemical investigation of a portion of the fraction obtained after cc. The specimen voucher has been deposited in our Institute's herbarium.

EXTRACTION AND ISOLATION OF ARTEMISINIC ACID [**1**].—A pilot plant extraction of *A. annua* was carried out using *n*-hexane. After removal of the solvent, a slurry with Si gel was loaded over a stainless steel Si gel column (330 × 30 cm). Mobile phase was adjusted by increasing the polarity with EtOAc in hexane. A portion of the fraction with EtOAc–*n*-hexane (1:19) was defatted with MeOH at low temperature. After further cc on Si gel using EtOAc–*n*-hexane (1:19) pure crystals of **1** (5 g) were obtained.

TABLE 3. Solution and Refinement

System used	Siemens SHELXTL PLUS (PC Version)
Solution	Direct Methods
Refinement method	Full-Matrix Least-Squares
Quantity minimized	$\Sigma w(F_o - F_c)^2$
Absolute structure	N/A
Extinction correction	N/A
Hydrogen atoms	Riding model, fixed isotropic U
Weighting scheme	$w^{-1} = \sigma^2(F) + 99.0000F^2$
Number of parameters refined	307
Final R indices (obs. data)	R = 5.99%, wR = 5.78%
R indices (all data)	R = 8.23%, wR = 7.21%
Goodness-of-fit	1.34
Largest and mean Δ/σ	0.019, 0.003
Data-to-parameter ratio	5.3:1
Largest difference peak	0.17 eÅ^{-3}
Largest difference hole	−0.18 eÅ^{-3}

Artemisinic acid (qinghao acid) [**1**].—Colorless cubes: mp 131°; ir ν max (KBr) 3400–2600, 1682, 1620, 1440, 1275, 1155, 940, 660 cm^{-1}; cims *m/z* (rel. int.) $[M+NH_4]^+$ 252 (72), $[M+H]^+$ 235 (67), $[M]^+$ 234 (33), 121 (40), 35 $[N_2H_7]^+$ (100); eims *m/z* (rel. int.) $[M]^+$ 234 (85), $[M-Me]^+$ 219 (14), $[M-COOH]^+$ 189 (32), $[M-C_3H_4O_2]^+$ 162 (12), 136 (58), 121 (100), 119 (78), 93 (75), 79 (62); ^1H nmr see Table 4; ^{13}C nmr see Table 5.

TABLE 4. ^1H-nmr Data (360 MHz, δ ppm) of **1**.

H-1	1.40	m	—
H-2	1.87	dd	13.5, 4
H-2′, H-3, H-3′	1.30–1.45	m	—
H-5	4.98	br s	—
H-6	1.94	ddd	10, 4, 4
H-7	2.69	ddd	11.5, 4, 4
H-8, H-8′	1.40	m	—
H-9	1.7–1.8	m	—
H-9′	1.07	br dddd	12, 12, 12, 6
H-10	1.55	m	—
H-13	6.47	s	—
H-13′	5.56	s	—
H-14	0.90	d	6
H-15	1.59	s	—
OH	2.60	br s	—

The complete structure of artemisinic acid was determined by X-ray analysis. Slow evaporation of a hexane-EtOAc (19:1) solution yielded colorless transparent crystals.

Crystal data: 0.2 × 0.3 × 0.4 mm; orthorhombic space group *P2(1)2(1)2(1)*, $a=9.558$ (2) Å, $b=9.717$ (2) Å, $c=30.645$ (6) Å; $Z=8$; $d=1.094$ g/cm^3; 2151 unique reflexions measured with CuK_α radiation, 1638 observed with $F>4\sigma(F)$. Absorption correction was applied. The structure was solved by direct methods using SHELXTL (7). The hydrogen atoms were calculated from the positions of the carbons to which they are bound except those in the hydroxy groups which were located from a difference map. Refinement converged at R = 5.78% (with unit weights).

TABLE 5. ^{13}C-nmr Data (100 MHz, δ ppm) of **1**.

Carbon	ppm
C-1	41.3
C-2	25.5
C-3	26.3
C-4	134.9
C-5	120.1
C-6	37.8
C-7	42.0
C-8	25.9
C-9	35.2
C-10	27.5
C-11	142.5
C-12	172.3
C-13	126.6
C-14	19.9
C-15	23.7

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