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POLYMORPHISM OF ARTEMISININ FROM *ARTEMISIA ANNUA*

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Key Word Index—*Artemisia annua*; Asteraceae; triclinic and orthorhombic polymorphs; X-ray and physicochemical evidences; artemisinin.

Abstract—X-ray crystallography studies have confirmed the discovery of a new polymorphic artemisinin crystal belonging to the triclinic space group, P1. The physicochemical properties of the new polymorph have been compared with those of the previously known orthorhombic $P2_12_12_1$ crystal by microscopic examination, density measurements, differential scanning calorimetry, infrared spectroscopy and dissolution studies.

INTRODUCTION

The polymorphic state of a drug can have a significant influence on its therapeutic efficacies especially when the rate of dissolution is the rate determining step for its absorption in the gastrointestinal tract. Any variation in solubility, dissolution, density, flow properties and crystal shape may affect the absorption and hence the bioavailability of a drug [1–4].

Artemisinin, the antimalarial drug isolated from the plant *Artemisia annua*, is a sesquiterpene lactone with an endoperoxide function and is currently recommended for acute treatment of multidrug resistant malaria from *Plasmodium falciparum* especially cerebral malaria. Artemisinin has been previously reported to be insoluble in water and oil but soluble in most aprotic organic solvents [5, 6]. The absolute configuration of artemisinin was determined by Chinese scientists using X-ray diffraction analysis on crystals obtained from 50% aqueous ethanol and an orthorhombic $P2_12_12_1$ unit cell was confirmed [7]. Hitherto, no other crystal form of artemisinin has been reported even though the drug has been obtained by recrystallization from various solvents [6]. In this paper, we present X-ray crystallographic evidence for the existence of triclinic crystals of artemisinin and compare their physicochemical properties with those of the orthorhombic crystals.

RESULTS AND DISCUSSION

The X-ray single crystal diffraction studies showed that the crystal recrystallised from cyclohexane has four independent molecules closely packed in a unit cell with dimensions of $a = 9.891(4)$ Å; $b = 15.343(2)$ Å; $c = 9.881(2)$ Å; $V = 1458(1)$ Å^3; $\alpha = 90.92(1)^\circ$; $\beta = 102.99(2)^\circ$ and $\gamma = 93.28(2)^\circ$ contributing to a triclinic system of a space group P1. The crystal obtained from 50% aqueous ethanol contains similar number of molecules but they are arranged further apart from one another (Fig. 1). Its cell constants of $a = 9.450(3)$ Å; $b = 24.090(3)$ Å; $c = 6.364(2)$ Å; $V = 1449(1)$ Å^3 corresponded to an orthorhombic system of $P2_12_12_1$ space group, and these values were identical to those previously described [7]

For $z = 4$ and $F.W. = 282.34$, the calculated density for the triclinic crystals was 1.286 g ml^{-1} whereas for the orthorhombic type, a value of 1.294 g ml^{-1} was obtained. These values were near to the measured densities of 1.293 ± 0.003 g ml^{-1} for triclinic and 1.300 ± 0.001 g ml^{-1} for orthorhombic, obtained by the volume measurements of accurately weighed artemisinin crystals using a nitrogen gas multipycnometer. The calculated and measured densities of 1.296 g ml^{-1} and 1.30 g ml^{-1}, respectively, for the orthorhombic crystals as reported by previous authors [7] were also similar to our present findings.

The conformation of both artemisinin molecules (Fig. 2) and their bond lengths appeared similar. However, there are differences in bond angles and torsion angles between the two polymorphs as shown by the asterisk values in Tables 1–3 (Fig. 3).

The *in vitro* dissolution profiles in Fig. 4 suggest that the triclinic crystal has a faster dissolution rate than the orthorhombic crystal. This may be attributed to the higher solubility of the former. The triclinic crystals produced a higher maximum aqueous concentration of 48.0 μg ml^{-1} ± 0 SD ($n = 4$) after 4 hr at

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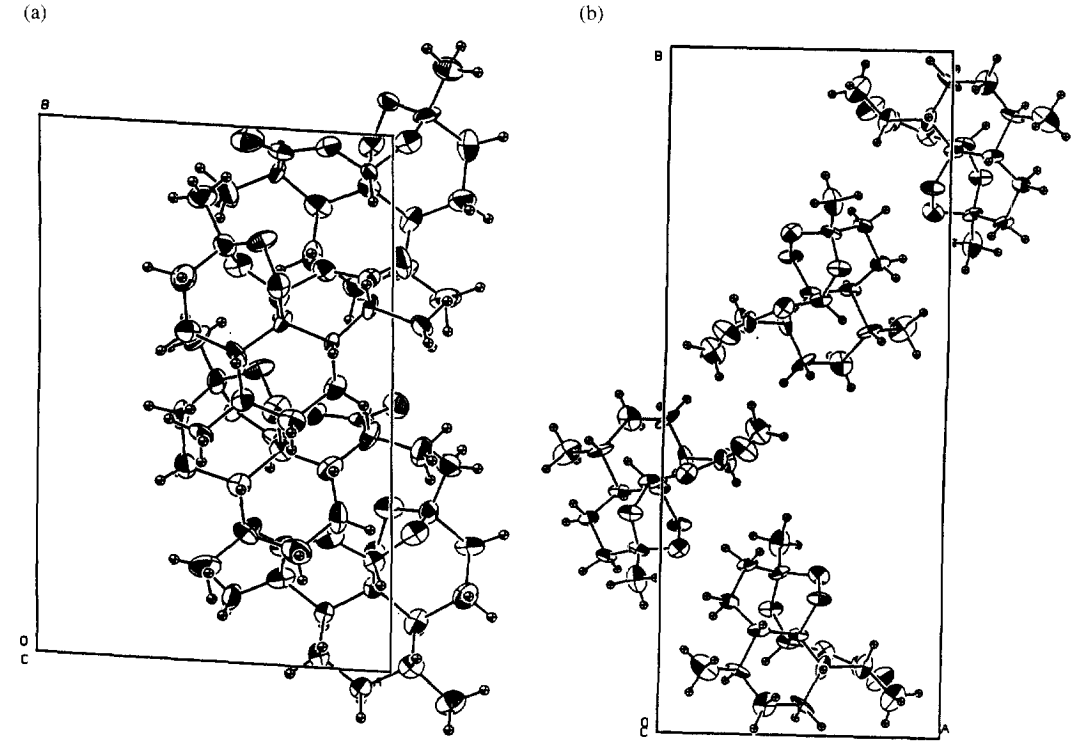

Fig. 1. Molecular packing of the (a) triclinic and (b) orthorhombic artemisinin crystal unit cell as viewed along the *z* axis.

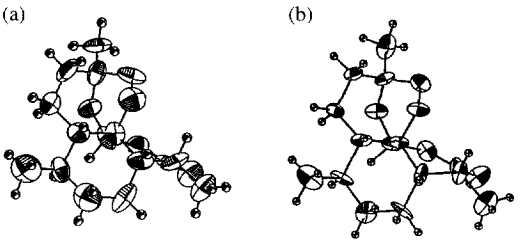

Fig. 2. ORTEP drawings of (a) triclinic and (b) orthorhombic artemisinin as viewed along the *z* axis.

37°, whereas only 20.0 μg ml^{-1} $\pm$0.1 SD (n = 4) of the orthorhombic crystals were in solution after 18 hr at the same temperature. In addition, the two polymorphic crystals of similar particle size range also showed obvious morphological differences as shown in Fig. 5. The orthorhombic crystal had a more dense and thicker appearance of rods and prisms compared to the triclinic which was mainly of thin transparent blades and plates. The latter crystal having greater surface area may contribute partly to its higher solubility and dissolution than the thicker variety.

Differential scanning calorimetry studies (DSC) showed that the triclinic crystal produced one melting endotherm (T_m) of 155.00 $\pm$ 0.03°, presumably a more stable form when compared to the orthorhombic crystal. In contrast, the latter showed two melting endotherms, a major of T_m = 154.88 $\pm$ 0.2°, containing more of the less stable polymorphs and a minor identical to that of the triclinic crystal. A lower enthalpy value of 80.76 $\pm$ 2.37 *J* g^{-1} (n = 3) was observed for the melting of the triclinic crystal when compared to the higher value of 82.91 $\pm$ 5.95 *J* g^{-1} (n = 3) for the orthorhombic crystal.

The infrared spectra of both crystals were virtually identical except that significantly broader absorptions are observed for triclinic than orthorhombic crystals at the regions between 2845–3000 cm^{-1} and 1300–1500 cm^{-1}, due to the stretching and bending vibrations, respectively, of the saturated CH hydrocarbons. Similarly, at 1738 cm^{-1}, the stretching vibrations of the C=O due to the lactone for triclinic showed broader absorptions than that of orthorhombic.

EXPERIMENTAL

Extraction from plants. Dried *Artemisia annua* leaves, purchased from a trading company in Hanoi, Viet Nam, were powdered before extraction with petrol (40–60°) (Merck) and subsequent flash column chromatography (silica gel 60, particle size: 0.040–0.063 mm) (Merck) of the extract was performed using petrol–EtOAc mixt. (4:1). Frs rich with artemisinin were pooled together, concd and then recrystallized several times either from cyclohexane to yield triclinic crystals (0.39%) or from 50% aq. EtOH to produce orthorhombic crystals (0.24%). The structure of artemisinin was confirmed by comparison of its TLC, HPLC, MS, ^{1}H and ^{13}C NMR data with that of an authentic sample (Aldrich).

Microscopic examination. Samples of the two artemisinin crystals were sieved through a laboratory test sieve (Endecotts Ltd., England) of range between 300–

Table 1. Intramolecular bond angles [°±(SD)] involving nonhydrogen atoms of triclinic and orthorhombic artemisinin

Atom	Atom	Atom	Triclinic	Orthorhombic
O3	C6	O5	106(2)	107(3)
O3	C6	C7	110(2)	115(3)*
O3	C6	C14	107(2)	105(4)
O5	C6	C7	106(2)	108(4)
O5	C6	C14	107(2)	112(3)*
C7	C6	C14	120(3)	110(4)*
C6	C7	C8	119(3)	112(3)*
C7	C8	C9	114(2)	116(3)
C8	C9	C10	109(2)	110(4)
C8	C9	C4	112(2)	115(4)
C10	C9	C4	112(2)	110(4)
C9	C10	C11	110(2)	117(3)*
C9	C10	C15	111(2)	109(4)
C11	C10	C15	112(3)	109(4)
C10	C11	C12	114(3)	111(3)
C11	C12	C3	113(3)	114(4)
C12	C3	C4	112(2)	116(4)
C12	C3	C2	111(3)	117(4)*
C4	C3	C2	112(3)	111(4)
O4	C4	C9	105(2)	106(3)
O4	C4	C3	104(2)	110(4)*
O4	C4	C5	113(2)	111(3)
C9	C4	C3	112(2)	115(4)
C9	C4	C5	109(2)	108(4)
C3	C4	C5	113(2)	107(3)*
O5	C5	O1	104(2)	111(4)*
O5	C5	C4	116(2)	109(3)*
O1	C5	C4	113(2)	110(4)
C3	C2	C1	112(2)	109(3)
C3	C2	C13	116(3)	117(4)
C1	C2	C13	113(3)	108(4)*
O1	C1	O2	122(3)	117(6)
O1	C1	C2	118(3)	118(4)
O2	C1	C2	119(3)	125(5)*

* Values of triclinic different from that of orthorhombic.

Table 2. Intramolecular bond angles (°) involving hydrogen atoms of triclinic and orthorhombic artemisinin

Atom	Atom	Atom	Triclinic	Orthorhombic
C6	C7	H4	111.69	111.20
C6	C7	H5	107.68	108.40
C8	C7	H4	109.62	108.89
C8	C7	H5	101.13	109.22*
C6	C7	H5	106.46	108.40
C7	C8	H6	109.62	110.86
C7	C8	H7	104.72	112.17*
C9	C8	H6	111.78	107.08*
C9	C8	H7	110.07	110.28
H6	C8	H7	106.52	99.21*
C8	C9	H8	108.61	110.33
C10	C9	H8	104.83	97.54*
C4	C9	H8	109.99	112.07
C9	C10	H9	109.66	113.75
C11	C10	H9	107.66	101.16
C15	C10	H9	107.11	106.31
C10	C11	H10	107.98	119.32*
C10	C11	H11	112.83	105.17*
C12	C11	H10	105.77	114.18*
C12	C11	H11	109.21	102.07*
H10	C11	H11	106.24	102.26*
C11	C12	H12	111.06	105.41*
C11	C12	H13	109.40	106.46*
C3	C12	H12	110.57	118.32*
C3	C12	H13	106.39	110.30*
H12	C12	H13	106.49	100.01*
C12	C3	H2	104.82	89.98*
C4	C3	H2	109.15	110.89
C2	C3	H2	106.86	116.65*
O5	C5	H3	105.46	102.89
O1	C5	H3	109.30	116.65*
C4	C5	H3	108.81	107.26
C3	C2	H1	100.42	99.11
C1	C2	H1	114.15	117.91
C13	C2	H1	99.48	106.10*
C2	C13	H14	121.98	113.50*
C2	C13	H15	110.77	108.02
C2	C13	H16	113.41	120.70*
H14	C13	H15	102.73	99.64
H14	C13	H16	103.94	107.61
H15	C13	H16	101.69	104.97
C10	C15	H20	116.51	114.35
C10	C15	H21	107.70	114.90*
C10	C15	H22	112.22	111.95
H20	C15	H21	104.71	104.87
H20	C15	H22	113.06	107.74*
H21	C15	H22	100.96	102.00
C6	C14	H17	111.48	111.36
C6	C14	H18	106.79	118.07*
C6	C14	H19	111.81	118.73*
H17	C14	H18	107.13	104.92
H17	C14	H19	109.97	99.92*
H18	C14	H19	109.49	102.44*

* Values of triclinic different from that or orthorhombic.

710 μm. They were then observed and photographed at 10×40 magnification using a light microscope (Model EC, Olympus, Japan), fitted with a 35 mm camera (Model C-35, Olympus, Japan).

Density measurement. Artemisinin crystals of approximately 4 g were accurately weighed and their corresponding vols were measured using a N_2 gas multipycnometer (Quantachrome Corporation, U.S.A.). The densities of the crystals were calculated by dividing the wt with the vol. obtained. Five measurements were performed for each crystal to obtain the average density.

In vitro *dissolution studies.* The *in vitro* artemisinin crystals dissolution profile was determined under non-sink conditions, using the paddle method of the USP 23 dissolution test apparatus (Model AT7, Sotax CH-4008, Basel, Switzerland). Artemisinin crystals were sieved with laboratory test sieve (Endecotts Ltd.) to a size range of 300–710 nm. Below 250 nm, the crystals were found to aggregate and difficult to disperse in the dissolution medium resulting in a reduced rate of dissolution. The tests were conducted with 150 mg of artemisinin crystals in 500 ml of distilled H_2O as the dissolution medium and was maintained at $37.0\pm0.5^\circ$

Table 3. Torsion or conformation angles [° ±(SD)]† of triclinic and orthorhombic artemisinin

Atom 1	Atom 2	Atom 3	Atom 4	Triclinic	Orthorhombic
O4	O3	C6	O5	−74(2)	−74(3)
O4	O3	C6	O5	41(3)	46(4)
O4	O3	C6	C14	172(2)	167(3)*
O4	C4	C9	C8	69(2)	69(4)
O4	C4	C9	C10	−168(2)	−166(3)
O4	C4	C3	C12	166(2)	170(3)
O4	C4	C3	C2	−68(2)	−53(4)*
O4	C4	C5	O5	−47(3)	−50(6)
O4	C4	C5	O1	73(3)	72(5)
O3	O4	C4	C9	−110(2)	−106(3)
O3	O4	C4	C3	132(2)	129(3)
O3	O4	C4	C5	10(3)	11(5)
O3	C6	O5	C5	34(3)	32(5)
O3	C6	C7	C8	−94(3)	−94(4)
O5	C6	C7	C8	20(3)	26(4)*
O5	C5	O1	C1	158(2)	145(5)*
O5	C5	C4	C9	70(3)	66(4)
O5	C5	C4	C3	−164(2)	−170(5)
O1	C5	O5	C6	−101(2)	−94(4)*
O1	C5	C4	C9	−170(2)	−172(3)
O1	C5	C4	C3	−44(3)	−48(4)
O1	C1	C2	C3	34(3)	29(7)
O1	C1	C2	C13	168(2)	157(5)*
O2	C1	O1	C5	165(2)	166(4)
O2	C1	C2	C3	−158(3)	−153(6)
O2	C1	C2	C13	−24(4)	−24(7)
C6	O3	O4	C4	50(3)	49(4)
C6	O5	C5	C4	23(3)	27(5)
C6	C7	C8	C9	60(3)	57(4)
C7	C6	C8	C9	−83(3)	−92(4)*
C7	C8	C9	C10	−161(2)	−165(2)
C7	C8	C9	C4	−36(3)	−39(5)
C8	C7	C6	C14	141(3)	148(4)*
C8	C9	C10	C11	179(2)	171(3)*
C8	C9	C10	C15	−58(3)	−65(4)*
C8	C9	C4	C3	−179(2)	−169(4)*
C8	C9	C4	C5	−53(3)	−50(5)
C9	C10	C11	C12	−52(3)	−46(4)
C9	C4	C3	C12	53(3)	50(4)
C9	C4	C3	C2	179(2)	173(3)*
C10	C9	C4	C3	−56(3)	−44(4)*
C10	C9	C4	C5	70(3)	75(4)
C10	C11	C12	C3	50(4)	48(4)
C11	C10	C9	C4	54(3)	43(4)*
C11	C12	C3	C4	−50(3)	−53(4)
C11	C12	C3	C2	−177(3)	−174(3)
C12	C11	C10	C15	−175(3)	−170(3)
C12	C3	C4	C5	−71(3)	−70(4)
C12	C3	C2	C1	79(3)	79(5)
C12	C3	C2	C13	−54(3)	−44(5)*
C4	C9	C10	C15	178(2)	167(3)*
C4	C3	C2	C1	−48(3)	−57(5)*
C4	C3	C2	C13	180(2)	180(3)
C4	C5	O1	C1	32(3)	24(6)
C5	O5	C6	C14	148(2)	147(4)
C5	O1	C1	C2	−27(3)	−15(8)*
C5	C4	C3	C2	55(3)	67(4)*

* Values of orthorhombic different from that of triclinic.

† The sign is positive if when looking from atom 2 to atom 3 a clockwise motion of atom 1 would superimpose it on atom 4.

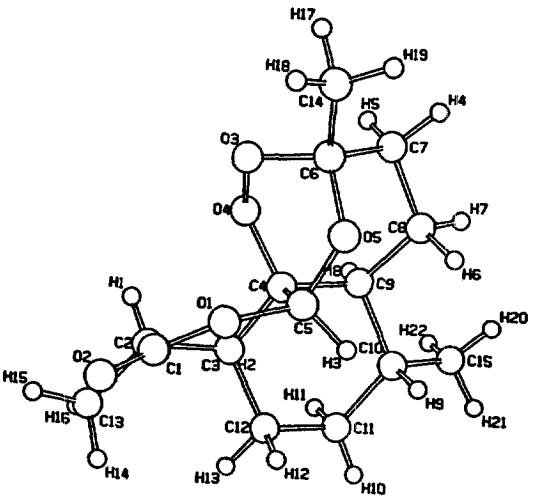

Fig. 3. Atom label of artemisinin molecule.

with a paddle rotation speed of 100 rpm. Samples of 1 ml were collected at various intervals using an automated fr. collector (Model CY7-50, Sotax), over a 24 hr period. The drug concns were measured by HPLC using an electrochemical detector at reductive mode after appropriate dilutions [8]. Each test was repeated ×4 and the average concn of artemisinin in soln *vs* time was calcd and plotted.

Thermal analysis. The differential scanning calorimetry instrument used was a Perkin-Elmer DSC-4 calibrated with indium. Artemisinin crystals of approximately 2 mg were accurately weighed into standard aluminium pans (Perkin-Elmer) and scanned from 50 to 170° at 10° min^{-1}. The scans were performed in triplicates for each type of crystals. The peak temp. of the major endotherm (T_m) and the total enthalpy for the melting of crystals were determined in triplicates.

Infrared analysis. Artemisinin crystals diluted to 1% with KBr (99% pure, Aldrich) were finely grounded and mixed thoroughly using an agate pestle and mortar. Adequate sample mixt. was transferred between two stainless disc dies and then compressed at 9 tons with a hydraulic press to form a disc. The infrared spectrum of the disc sample was obtained by irradiation with an infrared beam from a Glowbar light source at 20 scans and 4.00 cm^{-1} resolution in a Bomen Fourier Transform Infared (FTIR), Model MB 100 containing a DTG 2 mm detector.

X-ray Diffraction analysis. A colourless plate crystal of $C_{15}H_{22}O_5$ having approximate dimensions of 0.2 × 0.300 × 0.100 mm for triclinic and 0.200 × 0.400 × 0.100 mm for orthorhombic crystals, was mounted on a glass fibre. All measurements were made on a Rigaku AFC5S diffractometer with graphite monochromated Cu K_α radiation (μ = 7.52 cm^{-1} for triclinic and 7.57 cm^{-1} for orthorhombic) and a 12 kW rotating anode generator. The cell parameters were determined by the least square method. For triclinic, a total of 5162 reflections were collected and 4847 were unique (R_{int} = 0.094), whereas for ortho-

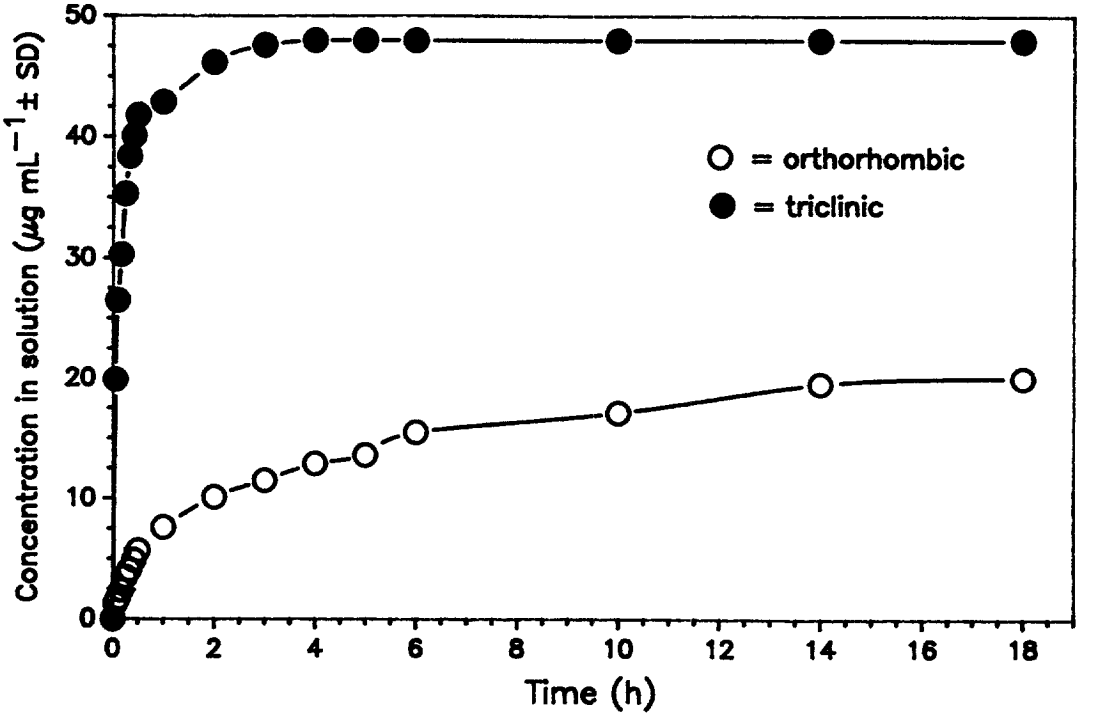

Fig. 4. Dissolution profiles of triclinic and orthorhombic crystals under non-sink condition ($n = 4$).

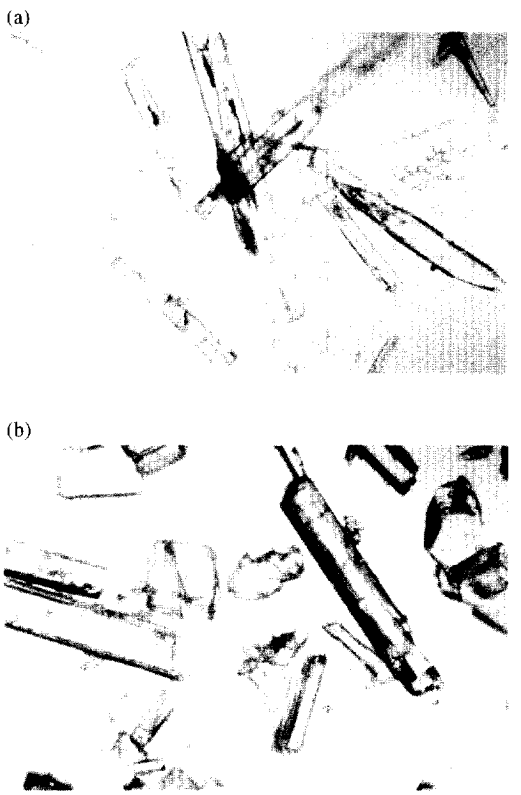

Fig. 5. Microscopic observation (magnification: 10×40) of artemisinin polymorphs: (a) triclinic crystals; (b) orthorhombic crystals.

rhombic only 1449 reflections were obtained. The intensities of the three representative reflections which were measured after every 150 reflections remained constant throughout data collection indicating crystal and electronic stability (no decay correction was applied). The data were corrected for Lorentz and polarization effects, at a temperature of $23 \pm 1°$ using the ω-2θ scan technique to a maximum 2θ value of 135.2° (triclinic) and 135.1° (orthorhombic). Omega scans of several intense reflections, made prior to data collection, had an average width at half-height of 0.24° (triclinic) and 0.29° (orthorhombic) with a take-off angle of 6.0°. Scans of $1.37 \pm 0.30 \tan\theta$° for triclinic and $1.52 \pm 0.30 \tan\theta$° for orthorhombic were made at a speed of 8.0° min^{-1} (in omega). The weak reflections [$I < 10.0\,\sigma(I)$] were rescanned (maximum of 1 rescan) and the counts were acummulated to assure good counting statistics. Stationary background counts were recorded on each side of the reflection. The ratio of peak counting time to background counting time was 2:1. The diameter of the incident beam collimator was 0.5 mm and the crystal to detector distance was 25.8 cm. The structure was solved by direct method [9]. The non-hydrogen atoms were refined anisotropically. The final cycle of full-matrix least-squares refinement for triclinic was based on 1722 observed reflections [$I > 3.00\,\sigma(I)$] and 718 variable parameters and converged (largest parameter shift was 3.02 times its esd) with unweighted and weighed agreement factors of: $R = \Sigma(|[F_o] - [F_c]|)/\Sigma[F_o] = 0.071$ and $R_w = \{\Sigma w(|[F_o] -$

$[F_c])^2/\Sigma w F_o^2\}^{1/2} = 0.052$, respectively. For orthorhombic, it was based on 373 observed reflections [$I > 3.00\ \sigma\ (I)$] and 181 variable parameters and converged (largest parameter shift was 2.69 times its esd) with unweighted and weighed agreement factors of: $R = \Sigma([F_o]-[F_c])/\Sigma[F_o] = 0.084$ and $R_w = \{\Sigma w([F_o]-[F_c])^2/\Sigma w F_o^2\}^{1/2} = 0.056$, respectively. The standard deviation of an observation of unit weight was 2.95 for triclinic and 3.55 for orthorhombic. The weighing scheme was based on counting statistics and included a factor ($p = 0.01$) to downweigh the intense reflections. Plots of $\Sigma w([F_o]-[F_c])^2$ vs $[F_o]$, reflection order in data collection, $\sin\theta/\lambda$, and various classes of indices showed no unusual trends. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.28 and $-0.25\ e^-/Å^3$, respectively, for triclinic, whereas for orthorhombic, they corresponded to 0.30 and $-0.25\ e^-/Å^3$, respectively. Neutral atom scattering factors were taken from Cromer and Waber [10]. Anomalous dispersion effects were included in Fcalc [11]; the values for $\Delta f'$ and $\Delta f''$ were those of Cromer [12]. All calculations were performed using the TEXSAN crystallographic software package of Molecular Structure Corporation [13].

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