Some Crystalline Modifications of the *tert*-Butylacetates of Prednisolone and Hydrocortisone

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Four phases of each of the two steroid esters have been identified. The phases have been characterized by using optical crystallography, X-ray powder diffraction, and infrared spectrometry. The optical crystallographic properties, melting points, densities, molar refractions, infrared spectra, and *d* distances are presented.

THE EFFECT of polymorphism on the activity of a drug has been emphasized in the recent literature. McCrone has stated (1) that the thermal stability of a drug depends on the polymorphic form and that "often the performance of a given compound can be improved through change to a different polymorphic form." Frederick said that occasionally a polymorph would be found whose properties were such that it would stand out in clinical effectiveness. He cautioned, however, that such a favorable polymorph might not be isolated for every compound studied (2). Higuchi, in discussing the physical chemical analysis of the percutaneous absorption process, stated that the highest thermodynamic potential possible for the penetrating agent must be used to obtain the maximum rate of penetration. Different crystalline modifications may exist having different free energies—thus different thermodynamic activities (3).

Two polymorphs of 6-α-methylprednisolone have been isolated (4). Ballard and Nelson prepared pellets of each of the two polymorphs and found that Form II of the steroid was absorbed after implants in rats 1.7 times as fast as Form I. They also reported that Form II was 1.2 times more soluble than Form I (5). There is evidence of four polymorphs of hydrocortisone alcohol, two of hydrocortisone acetate, and six crystalline modifications of cortisone acetate (6–8). Smakula and co-workers have reported one amorphous form and four polymorphs of 17-β-estradiol (9). Progesterone and ethynylestradiol are reported to be polymorphic (10, 11). Dickson, Page, and Rogers summarized the polymorphism of some additional steroids (12).

Cooper has stated that prednisolone *tert*-butylacetate (TBA) was at least trimorphic, and that this polymorphism presented a problem in formulation procedures (13). Rogers and Conbere reported that hydrocortisone TBA was dimorphic, while Williams reported that this ester was trimorphic (6, 14). The purpose of this communication is to report the crystalline modifications of the TBA of prednisolone and hydrocortisone. Each phase has been characterized by using three different tools. It was found that each tool could be successful in identifying the phase, but that the combination provided a powerful technique for identification and isolation of each of the crystalline modifications.

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EXPERIMENTAL

Crystallization of the Steroid Esters.—The two steroid esters were crystallized from various organic solvents at various temperatures. The crystals were collected following crystallization and dried. The crystals obtained were heated on the Kofler melting point block to determine phase changes and the melting point.

Hydrocortisone TBA was crystallized from various water-alcohol mixtures. Phase I, *i.e.*, the first modification obtained, was isolated from 20% ethanol. When the 90% alcoholic solution saturated with the steroid was allowed to evaporate at room temperature, Phase II was isolated. These methods of isolating the two phases have been described previously (14). A glass was isolated when hydrocortisone TBA was crystallized from chloroform. A white crystalline powder was obtained on scratching the glass. This was found to be a new crystalline modification and was designated as Phase III. A phase transition occurred when phase III was heated to 170°. In this manner Phase IV was isolated. It was also determined that when Phase I was heated on the Kofler block, a phase change occurred at approximately 140°. The phase obtained was identical with Phase IV.

The prednisolone TBA was crystallized from water-alcohol mixtures also. Phase I was isolated from 20% ethanol. Phase II was isolated from 90% ethanol. When the prednisolone ester was crystallized from 50% ethanol, three separate phases were distinguished by optical crystallographic procedures. Two of the modifications corresponded to Phases I and II. The third modification was termed Phase III; however, this phase could not be isolated in a

pure form. A fourth modification of the steroid was isolated when crystallized from acetone and termed Phase IV.

Determination of Solvates.—The amount of solvent bound to the steroid ester was determined by molar absorptivities and weight loss studies.

Determination of Physical Properties.—The procedures for the determination of the optical crystallographic properties, densities, and molar refractions have been described elsewhere (15, 16). The optical crystallographic properties of the modifications are recorded in Table I. The melting points, densities, and molar refractions of the phases are recorded in Table II. Photomicrographs of the phases are

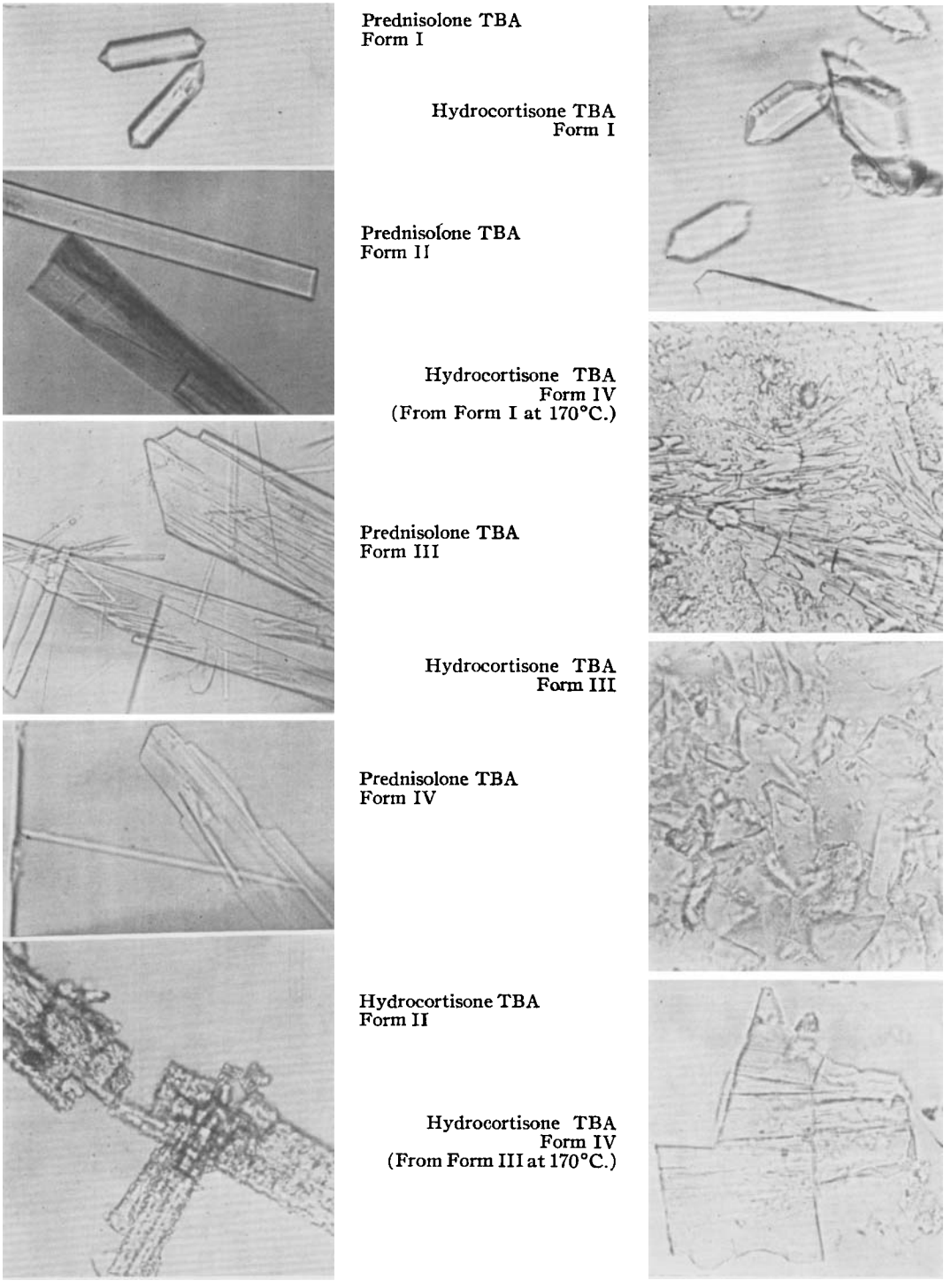
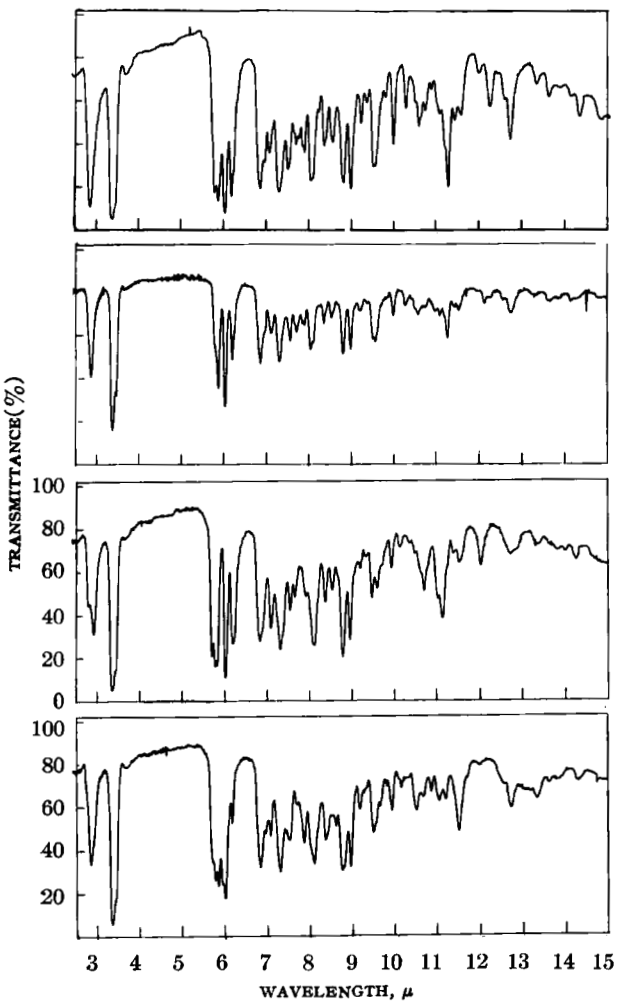


Fig. 1.—Photomicrographs showing the crystal modifications of TBA of prednisolone and hydrocortisone.



The infrared absorption spectra of prednisolone TBA crystallized from 20% ethanol. (Nujol mull). Phase I. ←

The infrared absorption spectra of hydrocortisone TBA crystallized from 95% ethanol. (Nujol mull). Phase II. →

The infrared absorptlon spectra of prednisolne TBA crystallized from 90% ethanol. (Nujol mull). Phase II. ←

The infrared absorption spectra of hydrocortisone TBA crystallized from chloroform. (Nujol mull). Phase III. →

The infrared absorption spectra of prednisolone crystallized from acetone. (Nujol mull). Phase IV. ←

The infrared absorption spectra of hydrocortisone TBA crystallized from chloroform and dried at 175°C. (Nujol mull). Phase IV. →

The infrared absorption spectra of hydrocortisone TBA crystallized from 20% ethanol. (Nujol mull). Phase I. ←

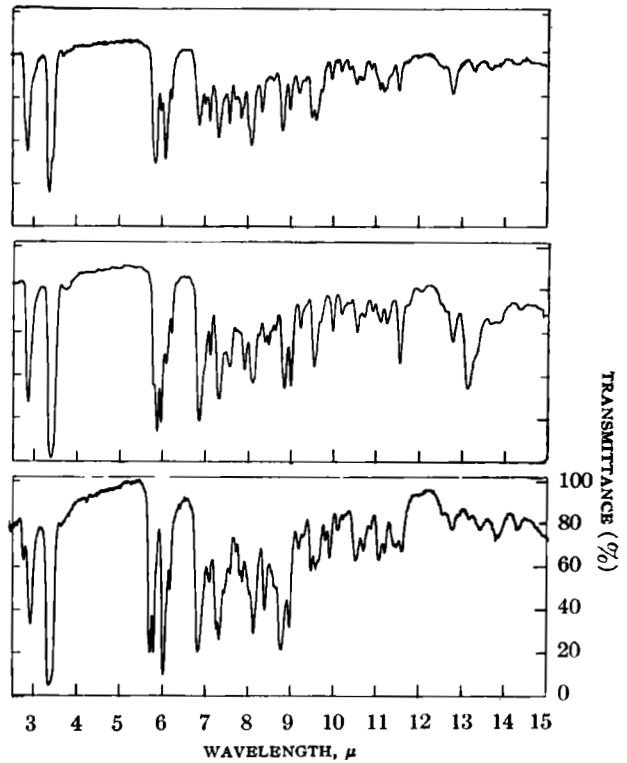


Fig. 2.—The infrared spectra of the crystal modifications of the TBA of prednisolone and hydrocortisone.

shown in Fig. 1. The photomicrographic techniques have been described previously (17).

Nujol mulls were prepared and the infrared absorption spectra were obtained using the Perkin-Elmer infracord spectrophotometer. The spectra of the crystalline modifications are illustrated in Fig. 2.

The X-ray powder analysis was made using a General Electric XRD-5 spectrometer.[1] The diffracted copper radiation (filtered by nickel) was detected by a quantum counter and was automatically recorded. The intensity of radiation was read as 2θ. The 2θ values were converted to d distances using the Bragg equation. The d distances are recorded in Table III.

RESULTS AND DISCUSSION

Four separate phases of the TBA of prednisolone and hydrocortisone have been isolated. One phase of prednisolone TBA was anhydrous; a second phase was isolated from 95% ethanol and found to be a monoethanol solvate. A third phase was identified but not isolated when crystallized from 50% ethanol. A fourth phase, crystallized from acetone, was found to be the hemisolvate form of the ester. When Phases II and IV of prednisolone TBA were dried in the Abderhalden at 140°, Phase I was obtained. The phase transition can also be observed on the Kofler block. Identification of the phases were determined by infrared and X-ray diffraction powder analyses and optical crystallographic analysis.

Phases I and II of hydrocortisone TBA were isolated by crystallizing from ethanol. Phase I was isolated when crystallized rapidly from ethanol, while Phase II was isolated on slow evaporation of the solvent. Both phases were found to be monoethanol solvates. Phase III of hydrocortisone TBA was isolated from a chloroformic solution. The phase was determined to be a hemichloroform solvate. When Phases I, II, and III were heated in the Abderhalden apparatus and on the Kofler block Phase IV was isolated. The latter phase is anhydrous.

The physical properties of all phases were studied. The melting points (uncorrected), densities, and molar refractions are reported in Table II. The optical crystallographic properties of the various phases are recorded in Table I. An outstanding feature of optical crystallography is the identification and characterization of crystalline substances in heterogeneous systems. Only with this method was Phase III of the prednisolone ester studied extensively.

The photomicrographs of the phases are shown in Fig. 1. The photomicrograph of Phase II of hydrocortisone TBA shows that the crystal solvate dissolved rapidly in the immersion oil. The same oil was used to immerse Phase I of the same ester. Thus, the rates of dissolution of the dimorphic phases in a mineral oil–bromonaphthalene immersion oil are different. Similar cleavage planes were observed of Phase IV of hydrocortisone TBA when Phases I or III were heated to 170°. The melting point of Phase I and resolidification were observed

[1] The infrared and X-ray spectra were determined in the Pharmacy Research and Development Laboratories, The Upjohn Co., Kalamazoo, Mich.

TABLE I.- OPTICAL CRYSTALLOGRAPHIC PROPERTIES OF THE CRYSTAL MODIFICATIONS

Crystal Modification	System	Crystal Habit	Optic Sign	Axial Angle	Optic Orientation[a]	Dispersion	Refractive Indexes $\alpha(\omega)$	$\beta(\epsilon)$	γ
Prednisolone TBA									
Phase I (Anhydrous phase)	Hexagonal	Columnar	+	0°	$\omega \parallel$ a $\epsilon \parallel$ c	None	1.552	1.572	...
Phase II (Monoethanol solvate)	Monoclinic	Columnar	+	56°	YY $\parallel$ b XX $\wedge$ c 27°	r>v	1.516	1.542	1.645
Phase III	Orthorhombic	Tabular	+	65°	XX $\parallel$ a YY $\parallel$ b ZZ $\parallel$ c	None	1.502	1.532	1.616
Phase IV (Hemiacetone solvate)	Orthorhombic	Tabular	+	31°	XX $\parallel$ a YY $\parallel$ c ZZ $\parallel$ b	v>r Strong	1.535	1.542	1.644
Hydrocortisone TBA									
Phase I (Monoethanol solvate)	Hexagonal	Columnar	+	0°	$\omega \parallel$ a $\epsilon \parallel$ c	None	1.541	1.565	...
Phase II (Monoethanol solvate)	Orthorhombic	Lamellar or prismatic	+	...	XX $\parallel$ a YY $\parallel$ c ZZ $\parallel$ b	r>v	1.505	1.526	...[b]
Phase III (Hemichloroform solvate)	....[c]	Tabular fragments	−	0°	...[c]	None	1.574	1.545	...
Phase IV (Anhydrous)	....[c]	Tabular fragments	+	37°	...[c]	r>v	1.525	1.534	1.623

[a] Crystallographic axes determined from crystal habit; a<b<c. [b] Could not determine because of phase change and solubility in oils. [c] Could not grow suitable crystals to determine system or optic orientation.

TABLE II.—MELTING POINT, DENSITY, AND MOLAR REFRACTIONS FOR CRYSTAL MODIFICATIONS

Crystal Modification	M. p., °C.[a]	Density	$\sqrt[3]{\alpha\beta\gamma}$ or $\sqrt[3]{\omega^2\epsilon}$	Exptl. M_{RD}	Calcd. M_{RD}
		Prednisolone TBA			
Phase I	244–249	1.210	1.559	122.40	121.76
Phase II	145[b]	1.239	1.566	132.87	134.54
Phase III	240–248	...[c]	1.549	...[c]	...[c]
Phase IV	226–230	1.254	1.573	130.55	129.83
		Hydrocortisone TBA			
Phase I	170[d]	1.194	1.549	135.18	135.04
Phase II	140[d]	...[e]	...[e]	...[e]	135.04
Phase III	145[d]	1.239	1.564	136.58	132.97
Phase IV	216–219	1.213	1.560	122.77	122.26

[a] Uncorrected. [b] Crystals darkened; possible phase change. Darkened crystals melted at 248–260°C. [c] Could not isolate to determine values. [d] Phase change. [e] Metastable phase. Could not determine all values.

under the microscope when using the Kofler block. No melting was observed when Phase III was converted to Phase IV by heating to 170°.

The X-ray powder patterns for the seven phases of the two steroid esters isolated were obtained using an XRD-5 spectrometer. The *d* distances are recorded in Table III. The first reflection for all phases, with one exception, corresponded to a distance of 14 + Å. Often this distance is indicative of the longest length of the unit cell. The first reflection which appeared for Phase III of the hydrocortisone ester corresponded to a distance of 22.64 Å.

TABLE III.—*d* DISTANCES FOR CRYSTAL MODIFICATIONS USING CU K-α RADIATION[a]

Prednisolone TBA Phase I	Prednisolone TBA Phase II	Prednisolone TBA Phase IV
14.97	14.97(s)[b]	14.80(s)
10.69	12.10	11.78(s)
8.65(s)	9.44(s)	10.16
7.50(s)	7.50	9.40
6.75	6.85	8.58
5.65(s)	6.01	7.56
5.32(s)	5.64	7.30
4.77	5.37	6.19
4.37	4.98	6.07
4.18	4.74(s)	5.90
4.02	4.63	5.78(s)
3.76	4.38	5.65
3.64	4.17	5.57
3.37	3.95	5.33
3.21	3.75	5.06(s)
3.02	3.58	4.39
...	3.22	4.29
...	...	4.23
...	...	3.90
...	...	3.87

Hydrocortisone TBA Phase I	Hydrocortisone TBA Phase II	Hydrocortisone TBA Phase III	Hydrocortisone TBA Phase IV
14.97	14.48	22.64(s)	14.24(s)
10.69	12.32	11.47(s)	12.62
9.64	9.50(s)	10.04(m)	11.23
8.61(s)	7.30	7.58	9.64
7.50(s)	6.75	7.19	7.72
6.36	5.95	5.89	6.60
5.65(s)	5.72	5.58	6.14
5.30(s)	5.28	5.50(m)	5.71(s)
4.76	4.77(s)	5.01(m)	5.48(s)
4.52	4.57(s)	4.67	5.24(s)
4.37	3.99	4.65	4.69
4.18	3.88	4.46	4.47
4.03	3.63	4.37	4.38
3.76	3.55	4.29	4.22
3.64	...	4.23	3.97
3.49	...	4.02	3.77
3.37	...	...	3.41
3.21	...	...	3.24
3.01	...	...	...

[a] Wavelength of 1.54051 Å. [b] (s) is strong and (m) is medium.

The infrared spectra for the phases are presented in Fig. 2. Since Phase III of the prednisolone ester could not be isolated in pure form, no spectra are submitted. The infrared spectra of the various phases are different and are of value for phase identification. The author observed that a given phase could be identified more easily using the XRD-5 spectrometer than using the infrared spectrometer. Gaebler, Parsons, and Beher have stated that X-ray diffraction methods are more effective than infrared methods in distinguishing compounds from mixtures (18). The hydroxyl stretching frequencies for the crystal phases varied between 3413 cm.$^{-1}$ for Phase IV of the prednisolone ester and 3534 cm.$^{-1}$ for Phase II of the hydrocortisone ester. Two peaks were observed for Phase IV of the hydrocortisone ester. One vibration was recorded at 3636 cm.$^{-1}$ which was indicative of a free hydroxyl group. The other vibration was recorded at 3448 cm.$^{-1}$, indicative of rather strong hydrogen bonding.

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