according to HPLC and NMR analyses, but crystallization was successful with neither. The less polar compound was characterized as **methyl 3β,7β,12β-trihydroxycholanate (8a)** [IR 1718 (C=O), 1024, 1000, 955 cm^{-1} (OH); NMR δ 0.76 (3 H, s, C-18 Me), 0.98 (3 H, s, C-19 Me), 3.42 (2 H, br m, C-7 and C-12 CHOH), 3.63 (3 H, s, COOMe), 4.07 (1 H, m, C-3 CHOH).

Anal. Calcd for $C_{25}H_{42}O_5 \cdot 0.5CH_3OH$: C, 69.82; H, 10.11. Found: C, 69.72; H, 10.09], and the more polar was characterized as **methyl 3β,7β,12α-trihydroxycholanate (7a)**: IR 1724 (C=O), 1024, 952 cm^{-1} (OH); NMR δ 0.71 (3 H, s, C-18 Me), 0.96 (3 H, s, C-19 Me), 3.58 (1 H, br m, C-7, CHOH), 3.64 (3 H, s, COOMe), 3.99 (1 H, m, C-12 CHOH), 4.06 (1 H, m, C-3 CHOH).

Anal. Calcd for $C_{25}H_{42}O_5 \cdot 0.5CH_3OH$: C, 69.82; H, 10.11. Found: C, 69.90; H, 10.18.

(b) With *tert*-Butylamine–Borane. Ester **31a** (0.50 g), reduced with the amine–borane complex and processed as in part 1b, yielded 0.46 g of oil which showed by HPLC the same two compounds, **8a** and **7a**, as in part **2a** in the 12β/12α ratio (estimated) of 1.9. Similar column chromatographic separation afforded 0.31 g of **8a** and 0.12 g of **7a**; each was identical with the corresponding product prepared in part 2a according to HPLC and NMR comparisons.

3β,7β,12β-Trihydroxycholanic Acid (8). The trihydroxy ester **8a**, hydrolyzed by the usual method, crystallized from EtOAc containing a small proportion of MeOH as small prisms: mp 164.5–167.0 °C; IR (KBr) 1695 C=O), 3448, 3333, 1020, 1004, 957 cm^{-1} (OH); NMR ($CDCl_3$ + 10% Me_2SO-d_6 + D_2O) δ 0.70 (3 H, s, C-18 Me), 0.94 (3 H, s, C-19 Me), 3.40 (1 H, br m, C-7 and C-12 CHOH), 3.93 (1 H, m, C-3 CHOH).

Anal. Calcd for $C_{24}H_{40}O_5 \cdot 0.5EtOAc$: C, 68.99; H, 9.80. Found: C, 68.88; H, 9.88.

3β,7β,12α-Trihydroxycholanic Acid (7).[23] The trihydroxy ester **7a** was hydrolyzed as above and crystallized from EtOAc: mp 164.5–165.5 °C [lit.[23] mp 154–157 °C (from EtOAc–heptane)]; IR (KBr) 1730 C=O); 3636, 3509, 1031, 1020, 990, 954 cm^{-1} (OH); NMR ($CDCl_3$ + 10% Me_2SO-d_6 + D_2O) δ 0.70 (3 H, s, C-18 Me), 0.94 (3 H, s, C-19 Me), 3.56 (1 H, br m, C-7 CHOH), 3.96 (2 H, m, C-3 and C-12 CHOH).

Anal. Calcd for $C_{24}H_{40}O_5 \cdot 0.5EtOAc$: C, 68.99; H, 9.80. Found: C, 68.99; H, 9.99.

(3) Methyl 3β,7α,12β- (4a)[5] and 3β,7α,12α-Trihydroxycholanate (3a)[5] with *tert*-Butylamine–Borane. The keto ester **36a** (1.0 g), reduced as in parts 1b and 2b, yielded an oil (1.0 g) which HPLC indicated to be a mixture of **4a** and **3a** in the 12β/12α ratio (estimated) of 5.0. The oil, on standing in a small volume of EtOAc, gradually gave minute prisms of **3a** (0.12 g) found to be identical by melting point, HPLC, and NMR comparisons with authentic 3β,7α,12α-trihydroxy ester **3a**.[5] The mother liquor contained the major product which by column chromatography with alumina as in parts 1b and 2b or with Florisil[5] yielded in the CH_2Cl_2–MeOH (98:2) cleanly eluted fractions 0.75 g of homogeneous (HPLC, NMR) but amorphous material identical with the previously prepared and characterized ester **4a**,[5] according to TLC, HPLC, and NMR. Further elution with CH_2Cl_2–MeOH (95:5) gave a negligible amount of **3a**. As contrasted with the finding in the Raney nickel reduction[5] of ester **36a**, no 3α-hydroxy analogues were detected (HPLC).

Acknowledgment. This work was supported in part by a grant from the National Large Bowel Cancer Project. We thank the Chemical Research Institute of Non-Aqueous Solutions, Tohoku University, Japan, for several elemental analyses. We are indebted to Ms. Susan Brannan for valuable technical help.

Registry No. **1a**, 1448-36-8; **2a**, 71883-63-1; **3a**, 28050-54-6; **4a**, 71883-65-3; **5**, 2955-27-3; **5a**, 28050-56-8; **6**, 81938-67-2; **6a**, 81702-92-3; **7**, 10322-18-6; **7a**, 81702-94-5; **8**, 81873-90-7; **8a**, 81702-93-4; **16a**, 21059-36-9; **18a**, 81847-01-0; **19a**, 10538-64-4; **20a**, 81847-02-1; **21a**, 54852-57-2; **22a**, 81847-03-2; **23a**, 81847-04-3; **24**, 81873-91-8; **24a**, 81655-85-8; **25a**, 81847-05-4; **26a**, 81847-06-5; **27**, 81847-07-6; **27a**, 81847-08-7; **28a**, 71837-86-0; **29a**, 81847-09-8; **30a**, 81847-10-1; **31**, 81873-92-9; **31a**, 81644-37-3; **33**, 79732-73-3; **33a**, 81644-40-8; **33a** 3-formate, 81847-11-2; **34a**, 7727-82-4; **36a**, 81847-12-3; **36a** formate, 81847-13-4; **37a**, 42921-40-4; methyl 7β-hydroxy-12-oxo-Δ^3-cholenate, 81847-14-5; methyl 7β-hydroxy-12-oxo-Δ^2-cholenate, 81847-15-6.

(23) The 3β,7β,12α compound (7) obtained by Na–propanol reduction of methyl 3,7-dioxo-12α-hydroxycholanate in low yield was characterized by melting point and α_D (P. Eneroth, B. Gordon, and J. Sjovall, *J. Lipid Res.* 7, 524 (1966).

Solid-State Photooxidation of 21-Cortisol *tert*-Butylacetate to 21-Cortisone *tert*-Butylacetate

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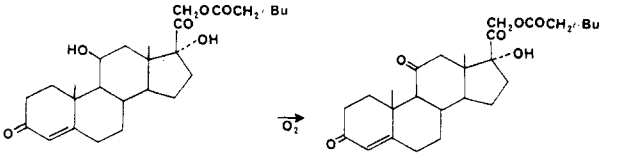
Five polymorphs of 21-cortisol *tert*-butylacetate have been prepared. Two of these forms are reactive. The hexagonal form (form I) that crystallizes as a nonstoichiometric solvate from ethanol oxidizes to 21-cortisone *tert*-butylacetate when exposed to ultraviolet light in air. A second form that crystallizes from pyridine is also reactive. Three other polymorphs are unreactive upon exposure to UV light and air. The crystal structure of the hexagonal form has been determined. The crystals belong to space group $P6_1$ with $a = b = 17.485$ Å, $c = 15.376$ Å, $\alpha = \beta = 90°$, $\gamma = 120°$. The crystal packing of this form has steroid molecules held together by hydrogen bonding arranged in a helix along the 6_1 axis. A channel runs through the center of this helix along the 6_1 axis. The oxidation of crystals of form I is hypothesized to occur because of easy penetration of oxygen into the crystal along this channel. In addition, the crystals of the hexagonal form containing ethanol lose solvent without extensive disordering of the crystal or transformation to a new crystal form.

Introduction

The factors that render crystalline drugs reactive or unreactive are of great importance both from a fundamental and a practical standpoint.[1-4] Knowledge of these factors could lead to an improvement in our understanding of how chemical reactions occur[1-3] and to the development

Scheme I



(1) I. C. Paul and D. Y. Curtin, *Science*, **187**, 19 (1975).
(2) M. D. Cohen and B. S. Green, *Chem. Br.*, 490 (1973).
(3) J. M. Thomas, *Pure Appl. Chem.*, **51**, 1065 (1979).
(4) S. R. Byrn, *J. Pharm. Sci.*, **65**, 1 (1976).

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Table I. Different Crystal Forms of 21-Cortisol *tert*-Butylacetate

crystal form	solvent of crystallization	ethanol content	X-ray data	mp,[a] °C	UV oxidation
I	ethanol, propanol, *n*-amyl alcohol, acetonitrile	0.9[d]	hexagonal, $P6_1$, $a = b = 17.485$ Å, $c = 15.376$ Å, $\alpha = \beta = 90°$, $\gamma = 120°$, $Z = 6$, $V = 4070.9$ Å^3	170-180	rctn
II	ethanol	1.0	monoclinic, $P2_1$, $a = 12.440$ Å, $b = 7.710$ Å, $c = 14.724$ Å, $\alpha = \beta = 90°$, $\gamma = 88.7°$, $Z = 2$, $V = 1415.3$ Å^3	110-120[b]	no rctn
III	ethanol, *tert*-butyl alcohol		triclinic, $a = 23.0$ Å, $b = 12.5$ Å, $c = 29.0$ Å, $\alpha = 74°$, $\beta = 147°$, $\gamma = 74°$, $Z = 2$, $V = 1094.8$ Å^3	123-126[c]	no rctn
IV	pyridine		unstable		rctn
V	heat forms I, II, or III			234-238	no rctn

[a] The exact melting temperature may vary from one crystal to another. [b] Opaque at this temperature range with final melting at 234-238 °C. [c] After melting, the melt resolidifed as the temperature was rising and finally remelted at 234-238 °C. [d] When crystallized from ethanol, form I contains ethanol; when crystallized from the other solvents, no solvent of crystallization can be detected by elemental analysis.

of rational approaches to the stabilization of drugs.[4]

In this paper we report an investigation of the solid-state light-induced oxidation of the polymorphs of cortisol *tert*-butylacetate (Scheme I). This reaction was reported over 10 years ago[5,6] and provides an excellent example of how different crystal polymorphs exhibit different reactivity.

The crystal structure of a reactive polymorph of cortisol *tert*-butylacetate is reported in this paper along with studies of the reactivity of four other polymorphs.

Experimental Section

Melting points were measured on a Kofler hot stage and are uncorrected. NMR spectra were measured on a Varian EM-360 or FT-80 spectrometer. X-ray powder patterns were measured with a Debye-Scherrer powder camera using Cu Kα radiation. The single-crystal X-ray studies were performed with a Nicolet (Syntex) P3 diffractometer equipped with a monochromator and a copper X-ray tube. Measurements of the ethanol content of crystals were conducted with a Varian flame-ionization gas chromatograph. Irradiation experiments were performed with a UVS-11 mineralight with a listed output of 240 μW/cm^2 at 254 nm at 6 in. All solvents used were of reagent or spectroscopy grade.

Preparation of Cortisol *tert*-Butylacetate and Its Crystal Forms. Cortisol *tert*-butylacetate was prepared following published procedures.[7] Crystallization from absolute ethanol either at room temperature or in the refrigerator gave mixtures of forms I, II, and III (see Table I). Crystallization from pyridine gave form IV. Single-crystal X-ray diffraction and/or powder diffraction showed that these forms had different crystal structures. Elemental analysis of fresh crystals of form I from ethanol gave the following data: C, 68.93; H, 9.00, (calcd for $C_{27}H_{40}O_6 \cdot 0.9C_2H_5OH$: C,68.97, H, 9.05). Form I could also be formed by crystallization from propanol, *n*-amyl alcohol, and acetonitrile. Crystals of form I from *n*-amyl alcohol gave the following data: C, 70.60; H, 8.81 (calcd for $C_{27}H_{40}O_6$: C, 70.43; H, 8.69). The crystallographic data for forms I–IV and other parameters are shown in Table I.

Crystallographic Data for Form I. Crystals of form I belong to the hexagonal crystal system with $a = b = 17.485$ (6) Å $c = 15.376$ (7) Å, $\alpha = \beta = 90°$, $\gamma = 120°$, $V = 4070.9$ Å^3, $Z = 6$, $F(000) = 1500$, μ(Cu Kα) = 5.57, ρ_{calcd} for $C_{27}H_{40}O_6 = 1.13$ g/cm^3; systematic absences $000l$ ($l = 6n$).

Data Collection for Form I. Crystallographic data were collected with the θ–2θ scan technique out to a 2θ of 116.0°. A variable scan rate was used with a maximum of 29.30°/min and a minimum of 2.0°/min. The scan range was from 1.2° less than Kα_1 to 1.2° more than Kα_2, and backgrounds were counted at each end of the scan range. The length of time the background was counted was equivalent to the length of time required for the scan. Three standard reflections were measured every 50 reflections. The data were corrected for decay even though the standards only decayed 2% during data collection. A linear (zero order) rate of decay was assumed. A total of 1923 reflections were measured.

Table II. Comparison of the Percent Desolvation and Oxidation of Crystalline 21-Cortisol *tert*-Butylacetate–0.9 Ethanol upon Exposure to UV Light

days	% cortisone formed	% EtOH lost
1	20.0	43.3
2	38.9	75.6
3	50.0	83.3
6	52.9	88.9
10	56.3	93.3
14	66.7	95.6
21	71.4	96.7

Structure Analysis for Form I. The structure of form I was solved assuming space group $P6_1$ by using the MULTAN 78 program. The original MULTAN run revealed the positions of 32 of the 33 nonhydrogen atoms. The remaining atom was located on a difference map. The refinement proceeded smoothly, using first isotropic and then anisotropic temperature factors for the nonhydrogen atoms to a final R factor of 0.102.[9] Attempts to locate the hydrogen atoms on difference maps failed. Thus, during the final stages of the refinement, the hydrogen atoms were included in calculated positions. A final difference map revealed no peaks greater than 0.8 e/Å^3.

Loss of Ethanol from Form I·0.9EtOH. As indicated in Table I, form I can be obtained from ethanol, propanol, *n*-amyl alcohol, and acetonitrile. The fresh crystals from ethanol gave an elemental analysis consistent with the presence of 0.9 mol of ethanol.

A fresh crystal of form I from ethanol was mounted on the diffractometer and a set of data collected over an approximately 2-week period. The structure refined smoothly to an R factor of 0.18. A difference map revealed no peaks greater than 1 e/Å^3. The three largest peaks on this difference map had heights of between 0.8 and 0.98 e/Å^3 and coordinates of (0.081, –0.026, 0.910), (0.057, 0.002, 0.801), and (0.071, –0.005, 0.850). These peaks were near the 6_1 axis and were separated by the following distances: peaks 1–2, 1.75 Å; peaks 2–3, 0.76 Å. Attempts to refine these peaks as a CH_3CH_2OH group failed.

(5) E. E. Brenner, F. E. Roberts, A. Hoinowski, J. Budavari, B. Powell, D. Hinkley, and E. Schoenewaldt, *Angew. Chem., Int. Ed. Engl.*, 8, 975 (1969).
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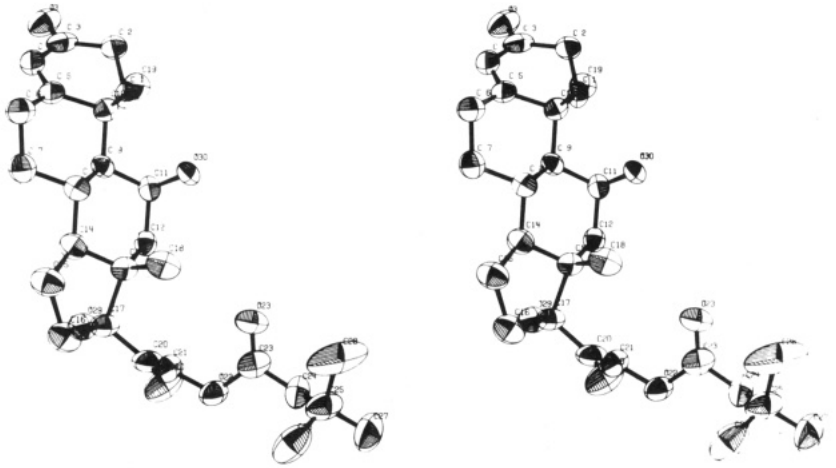

Figure 1. Stereoscopic drawing of cortisol *tert*-butylacetate showing the numbering scheme used in this structure determination.[10]

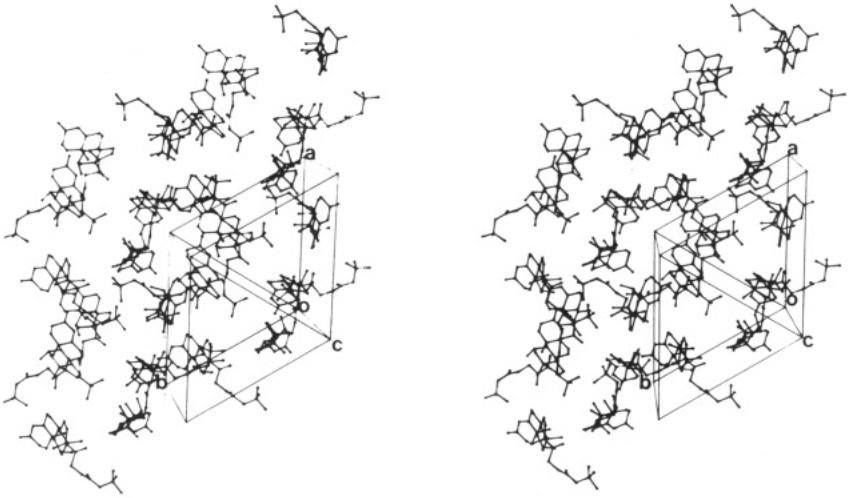

Figure 2. Stereoscopic view of the crystal packing of cortisol *tert*-butylacetate.[10]

The change of the shape of several diffraction peaks from a fresh crystal was followed in the θ–2θ scan mode on the Syntex diffractometer. Figure 3 shows a typical example.

Gas chromatography was used to investigate the ethanol content of a batch of crystals of form I·0.9C_2H_5OH. These experiments are summarized in Table II.

Oxidation of Cortisol *tert*-Butylacetate. Crystals of cortisol *tert*-butylacetate were placed on a microscope slide and exposed to light from a UVS-11 mineralight. The oxidation was studied by dissolving the crystals in $CDCl_3$ and measuring the ratio of the methyl signal in cortisol *tert*-butylacetate to that in cortisone *tert*-butylacetate. Control experiments showed cortisol *tert*-butylacetate did not oxidize in $CDCl_3$ under conditions of the NMR experiment. For comparative rate studies, crystals of approximately the same size were used.

Crystallographic Data for Form II. Crystals of form II belong to the monoclinic crystal system with a = 12.440 (6) Å, b = 7.710 (3) Å, c = 14.724 (7) Å, β = 88.70°, V = 1415.23 Å^3, Z = 2, $F(000)$ = 556.0, μ(Cu Kα) = 5.95. The data for form II was collected in exactly the same way as that for form I. Numerous attempts were made to solve the structure of form II by using the MULTAN 78 program.[8] The program was run with up to 500 E's but in all cases the resulting E maps did not yield refinable atomic positions. It was noticed that the reflections at the bottom of the E map had their k index divisible by 3. Thus these reflections were separately normalized to an average E^2 value of 1.0. The renormalization factor was 1.07. Unfortunately this approach still did not yield a solution.

Results and Discussion

Cortisol *tert*-butylacetate appears to be typical of many steroids that crystallize in several polymorphs.[11,12] As indicated in Table I, cortisol *tert*-butylacetate crystallizes in five polymorphs. Form I appears to be the most common and crystallizes as a nonstoichiometric solvate from ethanol.[5] Fresh crystals from ethanol contain 0.9 mol of C_2H_5OH. Form I also crystallizes from propanol, *n*-amyl alcohol, and acetonitrile. Crystals of form I from *n*-amyl alcohol gave an elemental analysis that indicates they did not contain solvent of crystallization. Form I also appears to be the only form that reacts upon exposure to ultraviolet light in air.

The crystal structure of the reactive polymorph, form I, has been determined by using single-crystal X-ray techniques. Form I belongs to the hexagonal crystal system. Systematic absences indicate that it belongs to the enantimorphous space groups $P6_1$ or $P6_5$. The structure solution was conducted in $P6_1$ and indeed the correct absolute configuration resulted. The structure of crystals of form I from *n*-amyl alcohol was refined to a final R factor of 0.102. Figure 1 shows a stereoscopic drawing of cortisol *tert*-butyl acetate and the numbering scheme used and Table 2 of the supplemental material lists the atomic parameters. Tables of bond lengths, bond angles, and intermolecular contacts also appear in the supplemental material. The crystal structure of this form is consistent

(10) The ORTEP program was used: C. K. Johnson, ORNL-3794, 1965.
(11) C. M. Weeks and W. L. Duax, *Acta Crystallogr.,Sect. B*, **32**, 2819 (1976).
(12) M. Kuhnert-Brandstatter, "Thermomicroscopy in the Analysis of Pharmaceutical", Pergamon Press, New York, 1971, p 41.

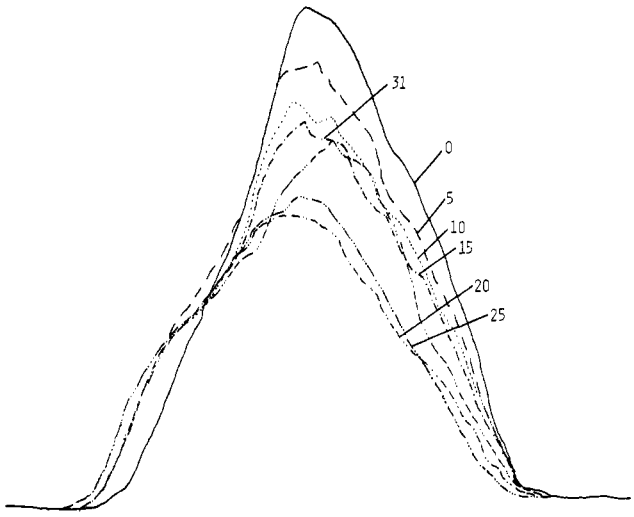

Figure 3. The shape of the 220 reflection of a fresh crystal of hydrocortisone *tert*-butylacetate at various times (days) after mounting.

with structures of other cortisol derivatives. The bond lengths and angles are within 2 SD of the corresponding value for 9α-fluoro-2α-methylcortisol and 9α-fluoro-11β-hydroxy-2α-methylprogesterone.[11] The crystal packing of cortisol *tert*-butylacetate that is shown in Figure 2 is different from that in these hydrocortisone derivatives.[11,14] The steroid molecules in cortisol *tert*-butylacetate are arranged in helices linked together by $O_{29}H\cdots O_{23}{=}C$ hydrogen bonds (2.75 Å) between the carbonyl oxygen of the *tert*-butylacetoxy group and the OH attached to C_{17}. A helical packing arrangement has been observed for 9α-bromocortisol.[13] The molecules in 9α-bromocortisol are held together by hydrogen bonds between the C_3-keto group and the OH attached to C_{17}.

Crystals of form I can be obtained from a variety of solvents, indicating that this form is very stable. The crystals of form I from ethanol contain 0.9 mol of ethanol of crystallization that is lost without completely disordering the crystal. A single crystal of the ethanol solvate gives a single crystal of the unsolvated form. Photographs of a crystal of this form during reaction indicate that solvent is lost without extensively disordering the crystal. The crystals remain clear and are apparently unchanged even though up to 96% of the ethanol of crystallization is lost according to analysis by gas chromatography. This observation is consistent with the change in shape of several diffraction peaks of a crystal of form I·0.9·C_2H_5OH as shown in Figure 3. The diffraction peaks of this particular crystal broadened and decreased in intensity with time up to about 25 days and then began to increase. This is a rare occurence since most desolvation reactions result in disordering of the crystal and often a product with a different crystal structure is produced.[4,15,16] These results also indicate that the crystal packing of this form is stable and is not disrupted by solvent loss.

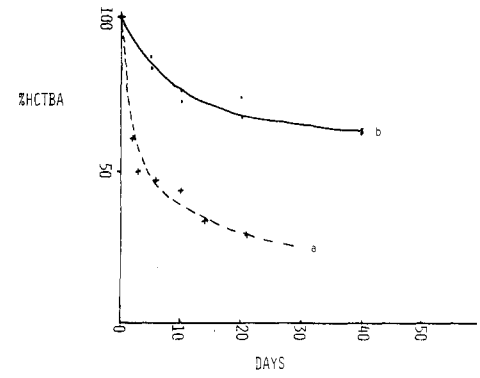

Figure 4. Solid-state oxidation of crystals of form I of cortisol *tert*-butylacetate: (a) crystals from C_2H_5OH, (b) crystals from *n*-amyl alcohol. The figure shows the combined results of two separate experiments.

Form I is reactive upon exposure to oxygen and UV light. It is hypothesized that this form is reactive because oxygen penetrates the crystal along the 6_1 helix axis and oxidizes the C_{11} atom, which lines this channel. Examination of Figure 2 shows that there is a channel running through the crystal along the 6_1 helix axis. The smallest cross sectional area of this channel is 3.5 Å^2. This area was calculated by projecting the crystal structure on the *ab* plane. Then the channel boundaries were delimited by drawing a circle with the proper atomic radius (H, 1.17 Å; C, 1.36 Å; and O, 1.80 Å.[17] The cross sectional area is then determined by integration. This projection shows that no other channels are running through the crystal. The cross sectional area of this channel compares favorably with tunnels in crystal hydrates. For example, the smallest cross sectional areas of the water tunnels in thymine hydrate and cytosine hydrate are 12.48 and 2.49 Å^2, respectively.[15]

Table II shows results that indicate that the rate of loss of ethanol of crystallization is faster than that of oxidation but does not completely precede oxidation. In addition, crystals of form I that have lost ethanol of crystallization are slightly more reactive than crystals of form I from *n*-amyl alcohol (Figure 4), which were shown by elemental analysis to contain only steroid molecules. This order of reactivity is consistent with the observation that the diffraction peaks of the ethanol solvate show some broadening and weakening during desolvation. Perhaps the crystals that have lost solvent of crystallization while single crystals are more disordered and contain more nucleation sites than crystals from other solvents that did not crystallize with solvent of crystallization.

In conclusion, it is hypothesized that the crystal packing of form I that allows facile loss of ethanol of crystallization is related to the solid-state reactivity of this form. This hypothesis is consistent with the results presented in this paper.

Registry No. 21-Cortisol *tert*-butylacetate, 508-96-3; 21-cortisone *tert*-butylacetate, 81741-06-2.

Supplementary Material Available: Tables listing temperature factors for cortisol *tert*-butylacetate, atomic parameters, bond lengths and angles, and intermolecular contacts for cortisol *tert*-butylacetate (5 pages). Ordering information is given on any current masthead page.

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(15) P. Perrier and S. R. Byrn, unpublished results.
(16) S. R. Byrn and C. T. Lin, *J. Am. Chem. Soc.*, **98**, 4004 (1976).
(17) A. I. Kitaigorodskii, "Organic Chemical Crystallography", Consultants Bureau, New York, 1961, p 7.