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THE SOLID STATE REACTIVITY OF THE CRYSTAL FORMS OF HYDROCORTISONE ESTERS

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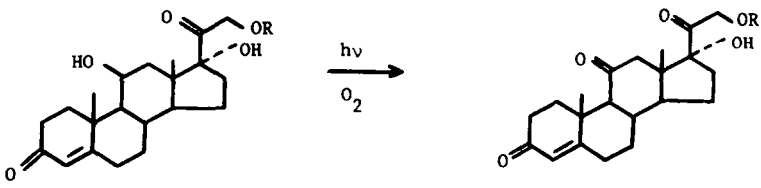
Abstract - The crystal structure of the hexagonal and orthorhombic crystal forms of 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione, the orthorhombic crystal form of 21-oxopropoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione and the hexagonal forms of 21-oxopentoxy-, 21-oxocaproxy-, 21-(3-cyclopentyl-1-oxopropoxy)-, 9-α-fluoro-21-oxobutoxy, and 9-α-fluoro-21-oxopentoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione were solved and refined. The A-ring is in the normal conformation (1α-2β-half chair) in all of the hexagonal crystal forms and in the inverted conformation in the two orthorhombic crystal forms. The hexagonal crystal forms tightly bind solvent in a ratio of about 1:2 solvent:steroid. They also react with oxygen in the presence of UV light to form the corresponding 11-ketone. The orthorhombic forms are not oxygen sensitive. The presence of the free radical scavenger BHT reduces the oxidation of the hexagonal forms. The extent of oxidation is related to surface area. One hypothesis explaining this behavior is that the solvent present in the crystal inhibits oxygen penetration. Preliminary solid state nmr studies of the thermal motion of the solvent indicate that it is not "liquid-like" in its behavior.

INTRODUCTION

Hydrocortisone is the primary glucocorticoid in man. Hydrocortisone esters have been used as drugs for many years because of their antiinflammatory activity and other hydrocortisone-like properties.

Hydrocortisone esters are known to oxidize to the corresponding cortisone ester upon exposure to oxygen. This reaction is accelerated by heat or UV light. In 1969, a group of



researchers at Merck studied the various crystal forms of several hydrocortisone esters.[1] They classified these crystals into three categories. Type A crystals are nonstoichiometric solvates which are oxygen sensitive. Type B crystals are stoichiometric solvates which are not oxygen sensitive and Type C crystals are unsolvated and are also not oxygen sensitive.

For the past few years we have been investigating the molecular basis for the reactivity of these different types of crystals.[2] Recently we have completed a study of the reactivity of different crystal forms of prednisolone esters.[3] This paper reports a similar study on the reactivity of the different crystal forms of a series of hydrocortisone esters.

These studies show that an understanding of the crystal packing provides the molecular basis for understanding the reactivity of Type A, B and C crystals. In addition, these studies are important because they provide knowledge which can lead (1) to preparation of more stable pharmaceuticals, and (2) to an understanding of the dissolution rates and bioavailabilities of different dosage forms.

RESULTS AND DISCUSSION

The crystal structure and reactivity of a series of hydrocortisone esters were determined. Table 1 summarizes the results of these studies. This table lists the crystallographic data, whether solvent of crystallization was located in the crystal and the type of crystal (see above, Type A-reactive, nonstoichiometric solvate; Type C-unreactive, unsolvated crystal form). The structure of both crystal forms of 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione is a good example of these esters.

Crystal Structures of the Hexagonal Form (Form I) of 21-Oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione. Form I was crystallized from 2-propanol and found to be hexagonal, belonging to the space group $P6_1$. It also crystallized in this hexagonal space group from methanol, ethanol, 1-propanol, acetone, ethyl acetate, n-butyl acetate and DMF. Crystals formed from all these solvents show identical X-ray powder diffraction patterns. Figure 1 shows the stereoscopic view of the crystal structure of 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione-I. Figure 2 shows a stereoscopic view of the crystal packing.

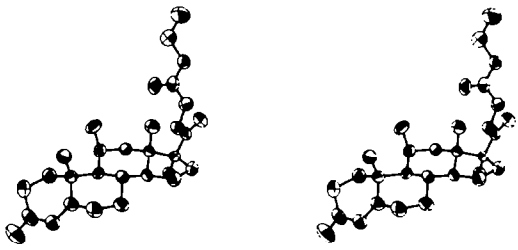


Figure 1. Stereoscopic view of the crystal structure of 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione-I

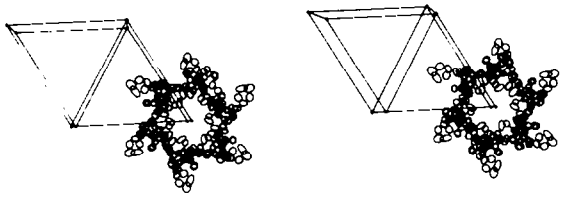
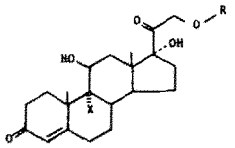


Figure 2. Unit cell of 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione-I, view from the a x b direction

There is nothing unusual about the conformation of 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione in the crystal and the A-ring exists in the normal 1α-2β-half chair conformation. The crystal packing of 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione-I is consistent with that of other hexagonal forms. There are six molecules per unit cell which form a helix parallel to the c-axis. A tunnel is found in the center of the helix. Although from the X-ray data, 2-propanol, the solvent of crystallization, could not be found, the TGA thermogram shows that there is about 5% solvent (by weight) in the crystal. Similar crystal

Table 1. Summary of Cell Parameters and Crystal Data for Different Hydrocortisone Esters.



R	Form	Type	Solvent for Crystallization	a	b	c	α	β	γ	space group	z	Number of Measured Reflections	Number of Unique Reflections	Omit	Number of Reflections Removed due to Extinction	Final R	Largest Peak on Final Difference Map	Crystal Solvate Present and Located
CCH_2CH_3	I	C	2-propanol	8.955(3)	14.018(4)	17.545(4)	90	90	90	$P2_12_12_1$	4	1764	1741	5	24	0.079	0.41	No
$CCH_2CH_2CH_3$	II	C	Toluene	9.053(4)	13.940(4)	18.179(6)	90	90	90	$P2_12_12_1$	4	1821	1798	5	12	0.0842	0.40	No
$CCH_2CH_2CH_3$	I[a]	A	2-propanol	16.807(5)	16.810(4)	15.117(5)	90	90	120	$P6_1$	6	5491	1773	5	50	0.0890	0.30	No
$CCH_2CH_2CH_2CH_3$	I[a]	A	Ethanol	17.011(6)	17.004(3)	15.194(3)	90	90	120	$P6_1$	6	5615	1804	5	39	0.0746	0.29	Yes
$CCH_2CH_2CH_2CH_2CH_3$	I[b]	A	Ethanol	17.704(8)	17.736(10)	15.035(2)	90	90	120	$P6_1$	6	6067	1954	5	15	0.0881	0.24	Yes
CCH_2-cyclopentyl	I[b]	A	Ethanol	17.991(24)	17.980(14)	15.165(8)	90	90	120	$P6_1$	6	6278	2041	5	11	0.0980	0.22	Yes
$CCH_2CH_2CH_3$(X=F)	I[a]	A	Methanol	16.832(8)	16.827(9)	15.135(5)	90	90	120	$P6_1$	6	4922	1766	5	37	0.0565	0.18	Yes
$CCH_2CH_2CH_2CH_3$(X=F)	I[a]	A	Methanol	17.188(11)	17.193(10)	15.141(11)	90	90	120	$P6_1$	6	2971	1765	5	0	0.0690	0.20	Yes

[a]These four compounds are isomorphous

[b]These are unique structures which give unique powder patterns

packing was observed for the hexagonal crystal forms of prednisolone tert-butylacetate and hydrocortisone tert-butylacetate.[3]

Crystal Structure of the orthorhombic form (Form II) of 21-Oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione. Form II crystallized from toluene and it was found to belong to space group $P2_12_12_1$. Figure 3 shows a stereoscopic view of the crystal structure of 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione-II. Figure 4 shows a stereoscopic view of the crystal packing.

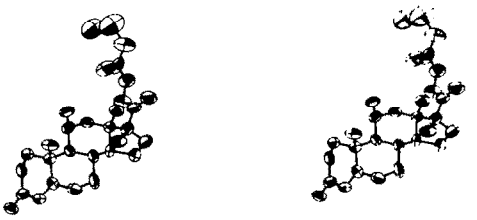


Figure 3. Stereoscopic view of the crystal structure of 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione-II

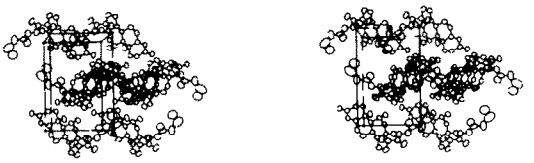


Figure 4. Unit cell of 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione-II, view from the a x b direction

The conformation of the A-ring is unusual. Normally, the A-ring of Δ-4-ene-3-one steroids exists in a 1α,2β-half chair conformation. Carbons 3, 4, 5 and 10 are coplanar, while carbon 1 is below the plane and carbon 2 is above the plane.[4] However, in this orthorhombic crystal form, an inverted A-ring exists. In this new conformation, carbon 1 is above the plane while carbon 2 is below the plane, making the A-ring a 1β,2α-half chair.

Oxidation of Forms I and II of 21-Oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione. Both crystal forms were exposed to 254 nm UV light for 15 days and analyzed for oxidation to the corresponding cortisone-21-ester using both NMR and HPLC. The orthorhombic form (Form II) did not oxidize. In addition, the isomorphous orthorhombic crystal form of 21-oxopropoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione-I did not oxidize. However, analysis showed that the hexagonal crystal form (Form I) oxidized as did the other hexagonal crystal forms listed in Table 1. Thus these forms are all classified as Type A even though these forms are stoichiometric solvates containing a 1:2 ratio of solvent:hydrocortisone ester.

In the proton NMR spectrum, there are several characteristic peaks which can be used to determine whether the oxidation has occurred. The C-18 methyl protons resonate at 0.95 ppm in the hydrocortisone ester and 0.7 ppm in cortisone ester. The 11β-OH at 4.5 ppm disappears in the cortisone ester and a new doublet of doublets appears for the C-21 protons at about 5 ppm. After 15 days of UV exposure, the proton spectrum shows signals characteristic of a mixture of the hydrocortisone ester and the corresponding cortisone ester. The oxidized product was positively identified as 21-oxobutoxy-17β-hydroxypregn-4-ene-3,11,20-trione by separation of the mixture on a chromatotron and analysis of the purified product using NMR and MS.

Initial experiments showed that the maximum amount of oxidation of powdered samples of Form I was about 30% regardless of length of exposure to UV light. This is consistent with studies of hydrocortisone tert-butylacetate. It is hypothesized that this is because oxidation is

limited to molecules near the surface of the solid. In order to test this hypothesis, whole crystals of the hexagonal crystal form were exposed to UV light for 15 days. A few of these crystals were analyzed for their extent of oxidation using HPLC. It was evident that only a small amount of oxidation occurred. This result is consistent with the surface oxidation hypothesis since when crystals were ground into a fine powder exposing a larger surface area more oxidation occurred (30%). For whole crystals, the surface is reduced, resulting in a dramatic decrease in the extent of oxidation.

In a more definitive experiment hexagonal crystals were exposed to UV light and washed with chloroform. Both the washings and the washed crystals remaining were analyzed using HPLC. Oxidation product was found in the washings but not in the residual crystals, indicating that the inner part of these crystals was not oxidized. This result further supports the hypothesis that oxidation is limited to molecules near the surface.

This surface oxidation phenomenon seems to be quite odd for the hexagonal crystal form, especially with the presence of a tunnel through the center of the unit cell and the close proximity of the 11β-OH to the inner surface of this tunnel. One hypothesis which may explain this result is that the tunnel is too small to allow oxygen to penetrate the crystal. To test this hypothesis, the cross sectional area of this tunnel was measured. However, simply measuring this area by measuring the tunnel area on a projection perpendicular to the c-axis is not appropriate due to the helical nature of the crystal packing. In doing so, only a small area would be measured due to the overlapping of atoms from various molecules into the tunnel which zig-zags along the c-axis. In order to obtain a more accurate approximation to the true cross sectional area, the c-axis of the tunnel is cut into 3 layers (Figure 5). From the atomic coordinates the x, y and z value of each atom of each layer was determined using the symmetry operations. Each layer is 5.039 Å deep along the c-axis and consists of different parts of each of the molecules. Hydrogen atoms which face the inner part of the tunnel were also incorporated in calculated positions.

The area was determined by delineating the boundries of the tunnel and determing the area by the cut and weigh method. The cross sectional area of layers 1, 2 and 3 was calculated to be 27.44, 27.93, and 28.04 $Å^2$. This area is obviously large enough for molecular oxygen to pass in and out of the tunnel. Therefore a narrow tunnel is not the cause for the limited oxidation in this hexagonal crystal form.

An alternative hypothesis is that the presence of solvent of crystallization in the tunnel of the hexagonal crystal form prevents internal oxidation. Crystallographic studies show that in all hexagonal crystals studied in our laboratory the solvent of crystallization occupies this tunnel. TGA shows there is about 5% weight loss occurring at 140°C-160°C apparently due to the loss of the solvent, 2-propanol. This 5% weight loss corresponds to a 2:1 ratio of steroid to solvent. Unfortunately, signals for propanol could not be found in the solid state NMR, so its molecular motion could not be studied. However, the solvent of crystallization could be found in the solid state NMR spectra of several other hexagonal crystals.

In order to determine whether the extent of oxidation could be increased by decreasing the amount of solvent in the crystal, several attempts were made to totally desolvate Form I of 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione. First the hexagonal crystals were ground into a fine powder, spread into a thin layer in a petri dish, and placed in a vacuum oven at 60° for 24 hours. At the end of this time, a small sample was analyzed using the TGA. The thermogram showed a 5% weight loss from 140°-160°C due to the solvent of crystallization, 2-propanol. Therefore, heating the sample at 60° in a vacuum oven does not desolvate this hexagonal crystal form.

A fresh sample of Form I of 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione was ground into a powder and placed in the sample pan of the TGA. The sample was heated at 140°C until no further weight loss could be seen. The sample was cooled, and a small amount was removed from the pan for X-ray powder analysis. The sample left in the pan was slowly heated to 250°C, cooled and then reanalyzed. The thermogram obtained showed no weight loss due to solvent. The

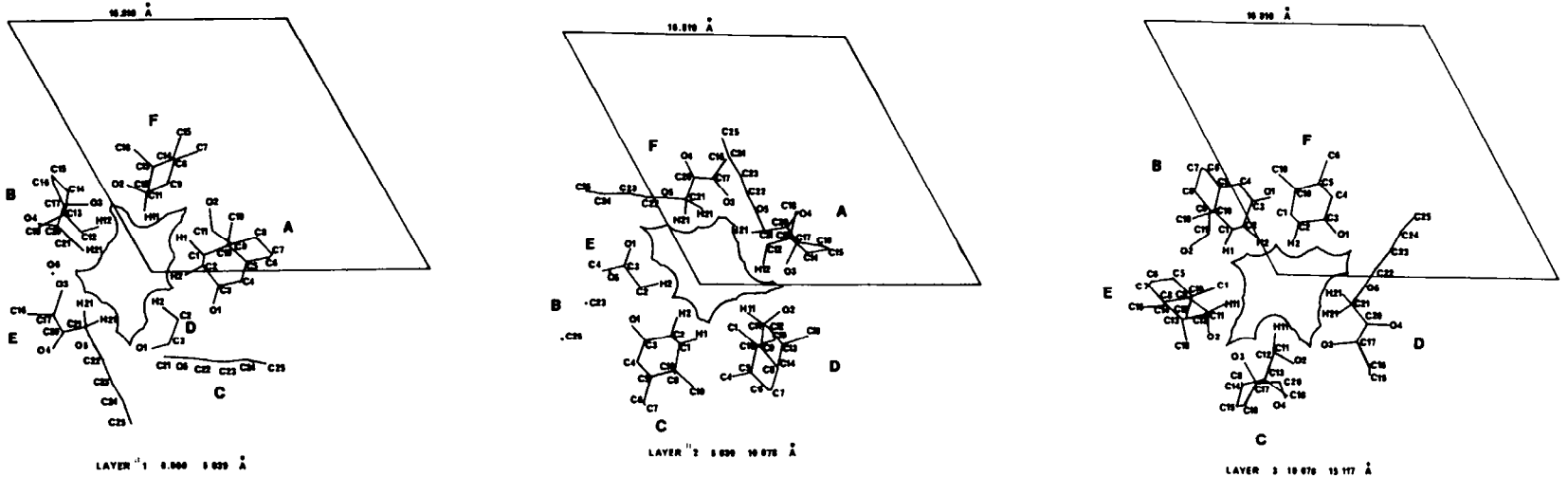


Figure 5. Cross sectional area of the tunnel down the c axis of 21-oxobutoxy-11β,17α-dihydroxy-pregn-4-ene-3,20-dione.

X-ray powder diffraction pattern obtained from this desolvated crystal no longer matched the original powder pattern. Instead, it matched the powder pattern of the orthorhombic crystal form. This indicates that desolvating the hexagonal crystal form causes a transformation into the orthorhombic crystal form.

The next step was to investigate the mechanism of this oxidation process. Several crystals of 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione-I were ground into a fine powder and placed in a quartz cell. After this quartz cell was flushed with dry nitrogen, it was exposed to 254 nm UV light for 15 days. At the end of the 15 days, a ^{1}H NMR spectrum showed none of the new peaks characteristic of the oxidation product. HPLC analysis showed only one peak which belonged to the original starting material. This shows that molecular oxygen is needed for the oxidation process to occur.

When a 1:1 molar ratio of Form I of 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione and butylated hydroxytoluene (BHT) were ground together into a fine powder and exposed to UV light the extent of oxidation was reduced suggesting that the oxidation is a free radical reaction. Oxidation in an O-18 atmostphere and mass spectral analysis showed that O-18 is not incorporated during oxidation. This shows that the 11β-oxygen atom is not exchanged during oxidation. These experiments indicate that the oxidation reaction follows a free radical mechanism.

Other Hydrocortisone-21-Esters

Cystal Structures. 21-Oxopentoxy, 21-oxocaproxy, and 21-(3-cyclopentyl-1-oxopropoxy) hydrocortisone esters were all crystallized from ethanol and found to be hexagonal, belonging to the space group P6$_1$. The molecular packing of all three of these hexagonal crystals are similar to the 21-oxobutoxy hexagonal crystal. There are six molecules per unit cell with a tunnel through the unit cell along the c-axis. The solvent of crystallization, ethanol, was located in all three hexagonal crystals, and it occupies this tunnel. Thermal gravimetric analysis indicates a 5% weight loss due to the solvent. This 5% weight loss corresponds to a 2:1 ratio of steroid to solvent. This is consistent with the electron density of the solvent seen in the Fourier difference maps.

21-Oxobutoxy-9α-fluoro and 21-oxopentoxy-9α-fluoro hydrocortisone esters were crystallized from methanol and found to be hexagonal, belonging to the space group P6$_1$. They also contain 6 molecules per unit cell and exhibit a tunnel through the unit cell along the c-axis. The solvent of crystallization, methanol, was located in the hexagonal crystals and it occupies this tunnel. Thermal gravimetric analysis again indicates a 2:1 ratio of steroid to solvent.

The 2θ values and intensities of 21-oxopentoxy, 21-oxobutoxy-9α-fluoro, and 21-oxopentoxy-9α-fluoro are all similar to those of 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione-I (Table 2). Also, the atomic coordinates of these compounds are quite similar. Therefore, it can be concluded that these hexagonal crystal structures are isomorphous.

The hexagonal crystal form of 21-oxocaproxy-11β,17α-dihydroxypregn-4-ene-3,20-dione-I gives different X-ray powder patterns from the hexagonal form of the 21-oxobutoxy hydrocortisone ester (Table 2). This is surprising since the cell parameters are very similar.

The hexagonal crystal form of 21-(3-cyclopentyl-1-oxopropoxy)-11β,17α-dihydroxypregn-4-ene-3,20-dione gives different X-ray powder patterns from both the 21-oxobutoxy and the 21-oxocaproxy hydrocortisone esters.

Thus, even though the hexagonal cell parameters are similar for all of these esters they exist in three different polymorphs since three different powder patterns are obtained. However, all of the hexagonal forms are oxygen sensitive.

Like the hexagonal crystal of 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione, desolvation of 21-oxopentoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione-I and 21-(3-cyclopentyl-1-oxopropoxy)-11β,17α-dihydroxypregn-4-ene-3,20-dione-I results in the generation of Form II. These desolvations were achieved using the TGA. The X-ray powder diffraction patterns of the new form show a dramatic difference from the original hexagonal crystal form, both in 2θ values and in intensities. However, the crystal structure of 21-oxopentoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione-II and 21-(3-cyclopentyl-1-oxopropoxy)-11β,17α-dihydroxypregn-4-ene-3,20-dione-II

TABLE 2. X-RAY POWDER DIFFRACTION PATTERNS OF VARIOUS HYDROCORTISONE ESTERS

21-Oxopropoxy-11β, 17α-Dihydroxypregn-4-ene-3,20-dione-I		21-Oxobutoxy-11β, 17α-Dihydroxypregn-4-ene-3,20-dione-II		21-Oxobutoxy-11β, 17α-Dihydroxypregn-4-ene-3,20-dione-I		21-Oxopentoxy-11β, 17α-Dihydroxypregn-4-ene-3,20-dione-x	
Observed		Observed		Observed		Observed	
2θ	Intensity[a]	2θ	Intensity[a]	2θ	Intensity[a]	2θ	Intensity[a]
8.00	S	8.00	M	8.35	M	8.40	M
9.95	W	9.70	M	10.45	VS,Br	10.38	S
10.98	M	10.95	W	12.03	VS,Br	11.93	S
11.78	S	11.63	S	13.18	M	13.00	M
12.58	W	12.68	M	16.10	S	15.05	W
13 45	M	13.80	M	17.15	S	15.90	S
14.08	M	15.20	M	18.58	W	16.93	S
15.33	M	16 10	M	19.25	W	18.98	W
16.05	M	16.78	W	19.93	W	19.68	W
16 75	M	17.63	W	20.50	M	20.35	M
19.05	VS,Br	18.78	S	21.75	M,Br	21.55	M
21.13	M	19.48	W	22.88	M	22.55	W
22 03	M	20.53	M	24.15	M	24.03	W
23.30	M	21.83	M	24.95	W	24 78	W
26 00	W			25.75	W	25.63	W
27 00	M					26.83	M

21-Oxobutoxy-9α-Fluoro-11β,17α-Dihydroxypregn-4-ene-3,20-dione-I		21-Oxopentoxy-9α-Fluoro-11β,17α - Dihydroxypregn-4-ene-3,20-dione-I		21-Oxocaproxy-11β, 17α-Dihydroxypregn-4-ene-3,20-dione-I		21-(3-cyclopentyl-1-oxopropoxy)-11β,17α Dihydroxypregn-4-ene-3,20-dione-I	
Observed		Observed		Observed		Observed	
2θ	Intensity[a]	2θ	Intensity[a]	2θ	Intensity[a]	2θ	Intensity[a]
8.33	M	5.88	S	5.60	M	10.15	M
10.38	S	6.88	W	8.43	W	11.18	M
11.90	S	11.93	M	10.03	VS,Br	13.30	M
13.00	M	12 88	M	11.53	M,Br	14.80	M
15.88	M	13.45	M	13.05	W	15.93	S
16 95	M	16.60	VS	14.63	VS	16.80	W
18.33	W	18.03	M	15.45	M	17.78	M
19.05	W	20.85	W	16.40	M	20.13	M
20.30	W	21.55	W	17.23	M	23.15	M
21.50	M	22.18	W	18.15	S,Br	24.23	W
23.95	W	23.20	M	19.53	W	25.43	W
24 75	W	26.60	M	20.30	S	26.73	W
25.55	W			20.93	W		
26.78	M			21.63	W		
				23.45	M		
				24.43	W		

[a]Peak Intensity. VS = very strong, S = strong, M = moderate, W = weak, and Br = broad.

are not known and according to their 2θ values, they are not isomorphous to 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione-I. Like the orthorhombic form of 21-oxobutoxy, they do not oxidize to the corresponding 11-ketone upon exposure to 254 nm UV light. Also, we have been unable to obtain these forms from solution by recrystallization.

It should be noted that the solvent to steroid ratio in all of these hexagonal crystal forms is 1:2 (see discussion above). In these crystals the solvent appears to be tightly bound and high temperatures (>100°) are required for desolvation. Thus, the original suggestion that Type A crystals are reactive, nonstoichiometric solvates should be modified to include these hexagonal forms. A better statement is that Type A crystals belong to the hexagonal crystal system.

Present research is focusing on using solid state NMR to understand more about the thermal motion of the solvent (ethanol or methanol) in these hexagonal crystal forms. It is hoped that these studies will lead to an explanation of the reactivity of these crystals and will also provide a new method for studying the thermal motion of crystalline materials and solids.

Preliminary studies show that the methylene and methyl resonances of ethanol in crystals of the 21-(3-cyclopentyl-1-oxopropoxy) ester survive 71 μs of interrupted decoupling in contrast to most of the resonances of the steroid. This suggests that the ethanol has greater mobility than the steroid. However, a nonspinning CP spectrum showed no clear signals for ethanol indicating that the ethanol does not have "liquid-like" motion. We have also measured the T_1 values of some of the carbon atoms in this crystal and they are consistent with these results. The results of this NMR study will be published shortly.

EXPERIMENTAL

Powder diffraction patterns were measured using CuK_α radiaion and a Debye Scherrer Powder camera. The d spacings were measured from the film.

Thermogravimetric analyses were performed using a Perkin-Elmer TGS-2 thermogravimetric system on samples weighing between 3 and 5 mg under N_2. A heating rate of 5°/min was used.

NMR spectra were measured on a Varian FT-80 spectrometer. Mass spectra were recorded on a Finnigan 4023 or a Kratos MS-50.

HPLC was performed on a Waters 6000A equipped with a UV detector (254 nm) and an Altex C-18 column.

Crystal data were collected using a Nicolet P3 automated diffractometer using CuK_α radiation. The θ-2θ scan technique was used. Data were collected out to a 2θ value of 116.0°. A scan rate of 7°/min was used. Three standard reflections were measured every 50 reflections. Decay of these was less than 2%. The structures were solved using MULTAN 80 and refined using SHELX 76. During the refinement, all of the reflections within 5 standard deviations of background were omitted. The data were plotted using the ORTEP program.

Preparation of Crystals. The 21-esters were dissolved in either individual solvents or a combination of solvents. If there was poor solubility, the solvents were heated. All solutions were cooled to room temperature and filtered before crystallization. Crystallization conditions varied from quick evaporation of the solvent to very slow evaporation by covering the beaker with a watch glass. All crystallizations took place at room temperature.

Oxidation Studies. A small amount of a crystal form was ground into a fine powder. The powder was placed in a well of a spot plate. The spot plate was then placed three inches below a Model UVG-11 short wave UV-254 nm Mineralight Lamp (115 volts) for 15 (or 45) days. Immediately following the exposure, both the ^1H NMR spectrum and an X-ray powder diffraction pattern were measured. A small amount of each sample was dissolved in 0.5 mL acetonitrile and analyzed using high performance liquid chromatography. A 1:1 acetonitrile/water mobile phase was used at a flow rate of 2.0 mL/min. Those samples which showed multiple peaks from the chromatograph were preparatively separated using the chromatotron. A 1 mm silica plate was used with 4:1 cyclohexane/ethyl acetate mobile phase. A pump rate of about 3 mL/min was used. Bands were observed using a 254 UV light. Each band was collected, the solvent rotovaped to dryness, and submitted for MS and ^1H NMR. Each of the UV exposed esters were also co-chromatographed with their corresponding cortisone-21-esters.

A control experiment was performed by placing the powder in a 13 cm long by 2 cm id quartz test tube like cell which was evacuated and flushed with nitrogen ten times and then filled with nitrogen. In a second experiment a mixture (1:1 molar ratio) of 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione-I and butylated hydroxytoluene was ground into a fine powder and exposed to 15 days of 254 nm UV light. In a third experiment, large crystals of 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione-I were used instead of a powder. In a fourth experiment, a small amount of 21-(3-cyclopentyl-1-oxopropoxy)-11β,17α-dihydroxypregn-4-ene-3,20-dione-I was ground into a fine powder. This powder was placed in a 13 cm long by 2 cm id test tube like quartz cell. The cell was fitted with a two way addition stopcock and attached to a vacuum pump. The cell was evacuated and flushed with nitrogen ten times. The cell was again evacuated and this time $^{18}O_2$ gas was allowed to enter the manifold. The quartz cell was submerged in a dewar of liquid nitrogen for 15 minutes. After this time, the quartz cell was sealed using the stopcock. The quartz cell was removed from the dewar of liquid nitrogen, allowed to warm to room temperature, and then removed from the vacuum pump. The quartz cell was exposed to 254 nm UV light for 15 days.

Desolvation of 21-Oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione-I Using a Vacuum Oven. A small amount of 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione-I was ground into a fine powder. This powder was spread as a fine layer in a petri dish. The petri dish was placed in a vacuum oven (National Appliance Model 5831), a vacuum was applied and heated to 60° C for 24 hours. At the end of this time, the sample was analyzed using X-ray powder diffraction and thermal gravimetric analysis.

Desolvation of 21-Oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione-I Using Thermal Gravimetric Analysis. A 3-5 mg sample of 21-oxobutoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione-I was placed in the pan of the TGA (see analytical methods). The sample was heated to 130°C until no further weight loss could be detected by the recorder. The sample was cooled and removed from the pan. The sample was analyzed a second time using the TGA to assure no solvent was present. The sample was also analyzed using X-ray powder diffraction and differential scanning calorimetry. A small amount of the sample desolvated in the TGA was exposed to 15 days of 254 nm UV light using the same procedure as previously described. Immediately after the 15 days of exposure, a ^1H NMR spectrum was obtained. The exposed sample was also analyzed using high performance liquid chromatography.

Desolvation of Other Hexagonal Crystals Using Thermal Gravimetric Analysis. A 3-5 mg sample of each of the other hexagonal crystals was placed in the pan of the TGA. The 21-oxopentoxy and 21-(3-cyclopentyl-1-oxopropoxy) esters were heated to 130° until no further weight loss could be detected on the recorder. The 21-oxocaproxy ester was heated to 105°C. Each sample was cooled, removed from the pan, and analyzed using X-ray powder diffraction and differential scanning calorimetry. The 21-oxopentoxy and 21-(3-cyclopentyl-1-oxopropoxy) TGA treated esters were also exposed to 15 days of 254 nm UV light using the same procedure as previously described. Immediately after the 15 days of exposure, a ^1H NMR spectrum was obtained on each sample. Also the exposed samples were analyzed using high performance liquid chromatography.

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CRYSTAL DATA

Tables of the refined coordinates and bond distances have been deposited with the Cambridge Crystallographic Data Center, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW.