RESEARCH PAPERS

POLYMORPHISM OF CORTISONE ACETATE

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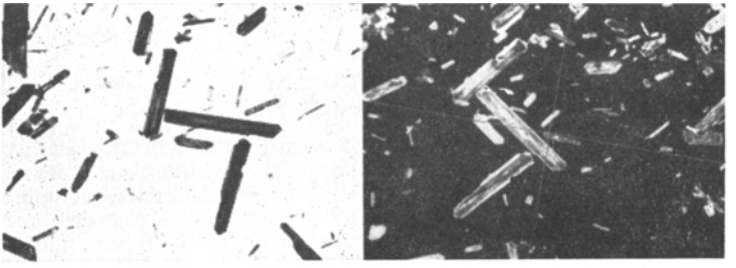
Evidence is presented that cortisone acetate occurs in at least five distinct crystalline forms. Four of these forms are unstable in presence of water and change to the stable Form I. Physical data, including spectroscopic and X-ray crystallographic constants, are recorded, and the application of these physical measurements to the recognition of the different forms, and the value of doing this with pharmaceutical preparations are discussed.

It has been reported that cortisone acetate may exist in different crystalline forms, but description of these in the scientific literature is scanty and incomplete. Accurate knowledge is important in the first place because infra-red absorption measurements on paraffin mulls or potassium halide disks prepared from crystalline cortisone acetate may be used for purposes of identification, and the curves obtained differ with the crystalline form. Garratt and Marshall (1954) deduced from their infra-red measurements that five different crystalline forms existed but their paper, which was a general one dealing with the application of infra-red spectroscopy to pharmaceutical analysis, did not give details of the characterisation of these forms. The second reason for the importance of distinguishing crystalline form finds expression in the patent literature that is concerned with processes of preparing stable, non-caking suspensions of cortisone acetate in a form suitable for intramuscular injection. The first patent application, U.S.P. (1954), B.P. (1951), claimed the discovery that three out of four, or possibly five, crystalline forms of cortisone acetate were unstable in presence of an aqueous medium and were converted into "Form 5" in the presence of water. Forms 1, 2, 3 and 5 were described and their X-ray diffraction patterns recorded. The second patent application, U.S.P. (1958), claimed the preparation of Forms A, B, and C, all, in contrast to Forms 1, 2, or 3, stable in aqueous vehicles. Forms B and C gave X-ray diffraction patterns similar to Forms 1 and 3, respectively. Form A, stable only in presence of water, differed crystallographically from any other form previously described. X-ray diffraction patterns for one form were also measured by Beher, Parsons and Baker (1955, 1958) but apparently in both these publications the figures for cortisone and for cortisone acetate were transposed. Apart from other considerations, the state of confusion of the crystallographic data appeared to us to justify a fresh examination, and it was hoped that a combination of spectroscopic and X-ray diffraction methods would provide a way of uniquely characterising and identifying these compounds.

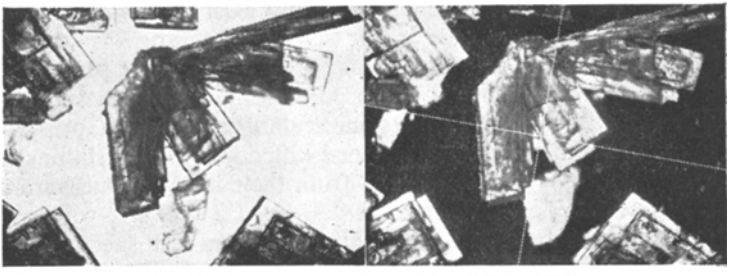
Methods

X-Ray Diffraction

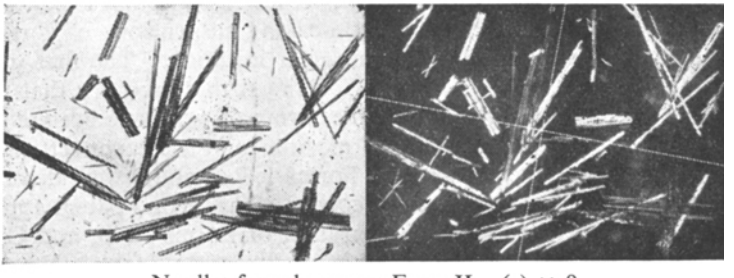
The X-ray diffraction patterns of single crystals were recorded using a Phillips X-ray tube with filtered copper radiation ($\lambda = 1{\cdot}5418$ Å) and



Roussel sample; Form I. (a) × 21



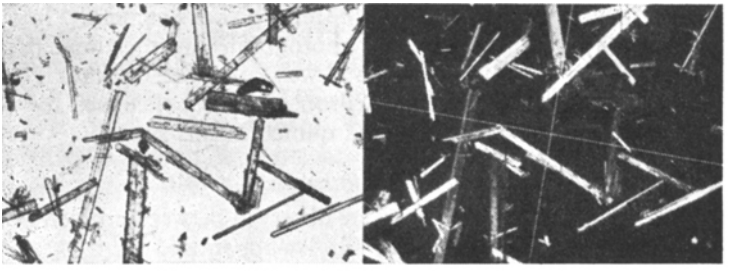
From chloroform; Form II. (b) × 5·75



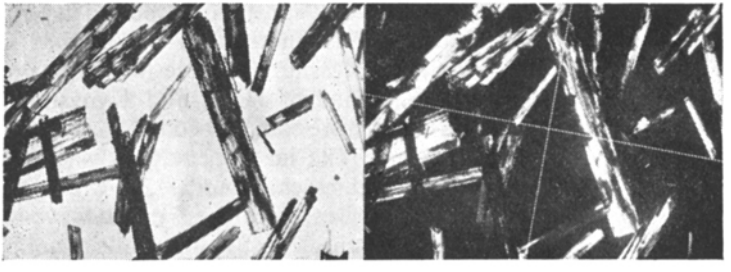
Needles from benzene; Form II. (c) × 9

Prisms from benzene; Form III. (d) × 9

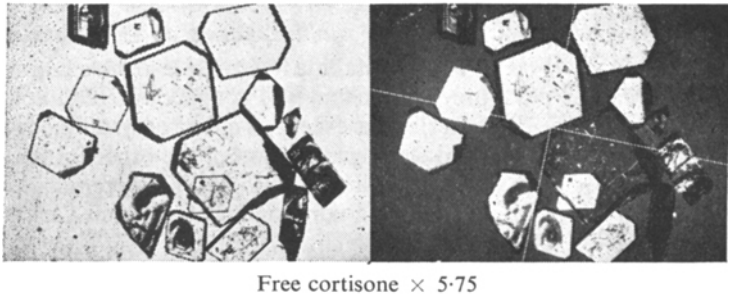
PLATE I. Photomicrographs of various forms of cortisone acetate by transmitted and polarised light. Photographs of cortisone (alcohol) crystals are included for comparison.



From CCl_4/MeOH; Form IV. (e) × 18



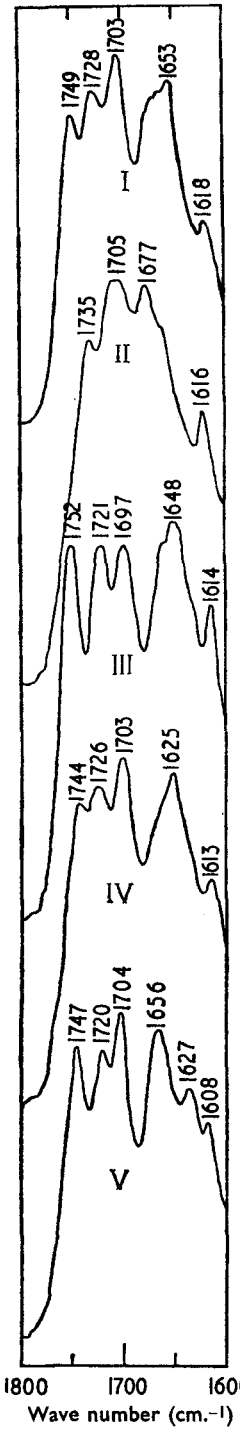
From ethanol; Form V. (f) × 9



Free cortisone × 5·75

conventional X-ray stationary and moving-film goniometers. The bulk-density of the crystals was measured in continuous gradient density columns (Low and Richards, 1952, and Linderstrom-Lang, 1937, 1938). The column containing the crystals was centrifuged and the density of the crystal layer subsequently measured by calibration with non-miscible liquids of known density. Using this method densities accurate to 0·002 g./ml. could be estimated. A combination of the X-ray and density measurements enabled the calculation of molecular weights to an accuracy of about 5 per cent.

The diffraction patterns of powdered samples were initially recorded on a 3 cm. X-ray camera. Subsequently the patterns were remeasured



more accurately from diffraction photographs taken with filtered chromium radiation on a Phillips 114·6 mm. powder-camera. Intensities from these photographs were estimated photometrically and we would like to thank Professor A. J. C. Wilson and his colleagues for all the powder-data quoted in this paper.

Melting Points

These were observed between crossed Polaroid plates in a Kofler apparatus.

Infra-red Absorption

These measurements were carried out on compressed disks of substance dispersed in potassium chloride. The material (1–2 mg.) was ground with dry potassium chloride (300 mg.) prepared by the method of Hales and Kynaston (1954) and the mixture compressed to a disk of 12·5 mm. diameter. The instrument used was a Perkin-Elmer double-beam Model 21, with a sodium chloride prism, recording on a wave-number scale. A check of wave-number calibration points, including the water-vapour peak at 1,700 cm.$^{-1}$, was made with each measurement.

Results

I. The Crystalline Forms of Cortisone Acetate

One crystalline form, I, was from a commercial preparation and was not encountered in our own experiments except as a microcrystalline material produced by alteration of the other forms. Four other distinct forms, II-V, were isolated by crystallising cortisone acetate from various solvents or solvent mixtures.

Form I

Source. The single crystals, m.p. 241–245°, used in the investigation were part of a sample given by Laboratoires Francais de Chimiotherapie (Roüssel) to the Medical Research Council Steroid Reference Collection. This form is stable in the presence of water.

Optics. The crystals were in the form of spear-shaped opaque needles, elongated parallel to the [b] crystallographic axis. They extinguished

FIG. 1. Infra-red absorption curves of crystalline modifications (Forms I-V) of cortisone acetate in potassium chloride disks.

about 3° from this axis. A typical field of these crystals in both transmitted and polarised light is illustrated in Plate I, (a).

Crystallographic data. The crystals were monoclinic with a = 15·68 Å, b = 7·52 Å, c = 26·58 Å, $\beta = 97°$. The observed density was 1·25 g./ml. for the single crystals, which gave a calculated molecular weight of 387·98. Since the molecular weight of cortisone acetate, $C_{23}H_{30}O_6$, is 402·47, Form I is not a hydrated one. The only observed absences were *o*k*o* when *k* is odd leading to a space group of $P2_1$. There are six molecules in the unit cell distributed in three crystallographically independent groups.

Infra-red absorption. The five peaks and the shoulder in the 1,800–1,600 cm.$^{-1}$ region are characteristic (see Fig. 1, Curve I). The hydroxyl band is at a high wave number (3430 cm.$^{-1}$) and there is no peak at about 850 cm.$^{-1}$ although in other respects the "finger-print" region below 1,500 cm.$^{-1}$ is practically indistinguishable from that of the other forms except as mentioned below in Form II.

Form II

Source. Cortisone acetate that had been crystallised from aqueous ethanol was dissolved in the minimum amount of boiling benzene (8 g. in 1 l.) and a little solvent was distilled to remove water. The solution, cooled overnight, deposited needles (Form II), m.p. 235–238°, and a few prisms (Form III), m.p. 251–253°. Evaporation of a chloroform solution of cortisone acetate to a concentration of about 20 to 25 per cent yielded large crystals m.p. 241–246°, which gave X-ray and infra-red absorption measurements identical with those of form II. The crystals were chunky, transparent prisms Plate I, (b) elongated parallel to the [a] axis. Extinction was inclined 7° to the edge.

Optics. The appearance of this form is illustrated in Plate I (b), (c). Though the square-ended needles that crystallised from benzene and the large flat plates from chloroform appeared superficially different, they gave identical infra-red and X-ray spectra. Both types of crystal were elongated parallel to the [b] axis and extinguished about 6° from the edge.

Crystallographic data. The crystals were orthorhombic a = 11·21 Å, b = 27·14 Å, c = 7·11 Å. The measured density of the single crystals was 1·21 g./ml. corresponding to four molecules in the unit cell. The density of the powdered samples extracted from various tablets was less homogeneous and extended between 1·20–1·21 g./ml. The calculated molecular weight was 393·01 and the space group $P2_12_12_1$ from absences.

Infra-red absorption. The curve in the 1,800–1,600 cm.$^{-1}$ region had a characteristic shape with four peaks (see Fig. 1, Curve II). In the "finger-print" region there is a prominent peak at 1,275 cm.$^{-1}$ slightly higher than the peak at 1,230 cm.$^{-1}$, much less marked in the absorption spectra of other forms. The hydroxyl peak is at 3,380 cm.$^{-1}$. As this form is the one constantly obtained by evaporation of a chloroform solution of cortisone acetate to dryness and is therefore the form into which any other can be transformed readily, it is considered worth while to reproduce the

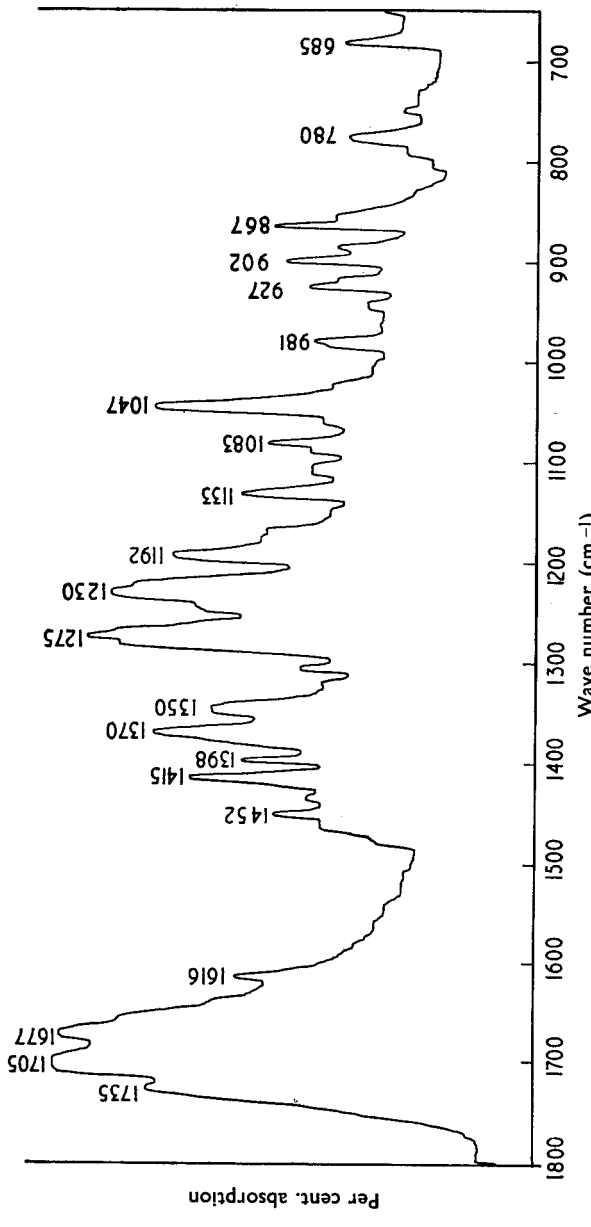


FIG. 2. Infra-red absorption of cortisone acetate (Form II) in a potassium chloride disk.

complete curve from 1,800 cm.$^{-1}$ to 650 cm.$^{-1}$, which has not been published before (Fig. 2). The curve in chloroform solution has been reproduced by Dobriner, Katzenellenbogen and Jones (1953) and by Roberts, Gallagher and Jones (1958).

Form III

Source. This form, m.p. 251–253°, crystallised from benzene at the same time as Form II and was separated by hand. Conditions for the exclusive separation of this form from benzene could not be found.

Crystallographic data. The crystals were orthorhombic a = 12·50 Å, b = 20·95 Å, c = 7·96 Å. The observed density of the single crystals was 1·24 g./ml. but that of the powdered sample was only 1·200 g./ml.—probably because of contamination. These crystals were also free of solvent of crystallisation since the calculated molecular weight was 383·00. There are four molecules in the unit cell and the space group is $P2_12_12_1$ from absences.

Infra-red absorption. The five peaks and shoulder in the 1,800–1,600 cm.$^{-1}$ region are characteristic (see Fig. 1, Curve III) differing from peaks of Form I in wave number and, especially, relative intensity. The hydroxyl band is a broad one at 3,350 cm.$^{-1}$.

Form IV

Source. A solution of 10 g. of cortisone acetate in about 300 ml. of boiling, moist alcohol deposited platy crystals on cooling. These melted at 245–247° after becoming opaque at 111–116°. Analysis gave C, 63·3; H, 7·1 per cent. Loss at 140°/15 mm. 10·25 per cent. Calc. for $C_{23}H_{30}O_6$ 2·5 H_2O: C, 62·8; H, 8·0 per cent. Loss, 10·1 per cent. Calc. for $C_{23}H_{30}O_6$, $2H_2O$. C, 63·1, H, 7·8, Loss 8·2 per cent. The material after removal of solvent gave the infra-red absorption of Form III.

Optics. Columnar crystals with spear-shaped ends. Elongated parallel to the [c] axis, which is also a cleavage direction Plate I, (e). The crystals often appeared powdery because of efflorescence. Extinction position was about 6° from the needle edge.

Crystallographic data. The crystals were orthorhombic with cell-dimensions a = 9·76 Å, b = 30·59 Å, c = 7·58 Å. The observed density was 1·26 g./ml. Density calculated for four molecules of cortisone acetate only in the unit cell was 1·18 g./ml. The difference between the observed and calculated values of the density could be accounted for by assuming the presence of two molecules of water of crystallisation per molecule of cortisone acetate.

Infra-red absorption. There is an ill-defined triple hump (3,420, 3,320 and 3,230 cm.$^{-1}$) in the hydroxyl region, and the shape of the curve in the 1,800–1,600 cm.$^{-1}$ region is characteristic (see Fig. 1, Curve IV).

Form V

Source. Cortisone acetate (1 g.) was recrystallised from a boiling mixture of carbon tetrachloride (15 ml.) and methanol (5 ml.). Needles separated on cooling, having m.p. 238–242° after becoming opaque at

105–110°. This form rapidly loses solvent of crystallisation in air and changes to Form II. X-ray diffraction patterns could only be obtained from freshly prepared material.

Optics. Large striated needles, elongated parallel to the [b] axis. Only the transparent needles were of this form, an opaque deposit indicating change to Form II. Extinction angle was about 4° from the edge.

Crystallographic data. The crystals were monoclinic with a = 9·65 Å, b = 7·46 Å, c = 16·4 Å, $\beta = 98°$. Space group was $P2_1$ from absences. The observed density of single crystals was 1·25 g./ml. which together with the unit cell measurements gave a calculated molecular weight of 438·2, indicating the presence of two molecules of water of crystallisation per molecule of cortisone acetate. Density calculated for this degree of hydration is 1·251 g./ml.

Infra-red absorption. There are three definite peaks 3,530, 3,400 and 3,280 $cm.^{-1}$ in the hydroxyl region, and in the range 1,800–1,600 $cm.^{-1}$ the curve is characteristic, with six peaks (see Fig. 1, Curve V).

II. Interconversions

Forms II, III, IV and V, as intact crystals, are stable for some time in presence of water, but when shaken or ground transformation to Form I takes place rapidly. In typical experiments it was observed that crystals of Form II were apparently unchanged in water after 126 hr., whereas in a shaking machine a change was seen after 20 hr. and was complete after 60 hr. The transformation was followed by withdrawing samples at intervals and taking an X-ray photograph of the powder directly. The time to first appreciable transformation could be decreased to 4 hr. by grinding the crystals to a fine powder before contact with water, but, probably because of caking of the suspensions, complete transformation still took about 56 hr. Continuous grinding under water brought about complete transformation within 45 min.

III. Commercial Preparations

Two specimens of cortisone acetate injection available to us gave, after separation of the powder from the suspension, the infra-red absorption and X-ray measurements characteristic of Form I—as might be expected from the systematic investigations reported above.

Five specimens of tablets of cortisone acetate from different manufacturers were available to us. All showed a moderately intense absorption band in the infra-red at 1,650 $cm.^{-1}$, which was due to the excipient, and prevented any certain conclusion about the crystalline form of the cortisone acetate.

To ascertain this the tablets were shaken for a short time with water and the insoluble material examined without delay. Of the four tablets from different manufacturers examined by infra-red absorption and X-ray diffraction after this treatment, two were found to contain Form I and two to contain Form II. After longer shaking (several hr. or overnight) all yielded Form I.

Discussion

Cortisone acetate crystallises in a variety of forms both hydrated and anhydrous which can be characterised by a combination of X-ray and infra-red spectroscopic methods. The data obtained by these methods

TABLE I

CRYSTALLOGRAPHIC CONSTANTS FOR SINGLE CRYSTALS OF CORTISONE ACETATE

Cortisone acetate $C_{23}H_{30}O_6$. M.Wt. = 402·47

	Form I	Form II	Form III	Form IV	Form V
a	15·68	11·21	12·50	9·76	9·65
b	7·52	27·14	20·95	30·59	7·46
c	26·58	7·11	7·96	7·58	16·40
β	97·0				98·0
V	3112·04	2165·58	2084·52	2263·07	1163·76
Z	6	4	4	4	2
Sp	$P2_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_1$
D_{obs}	1·250	1·210	1·250	1·260	1·250
anhyd. $D_{cal.}$	1·288	1·234	1·282	1·181	1·148
hydr. $D_{cal.}$				1·280	1·251

a, b, c = cell constants in Å. β = cell angle in degrees. V = cell volume in Å³. Z = number of molecules in unit cell. Sp = space-group. D_{obs} = density observed g./ml. anhydr. $D_{cal.}$ = density calculated without hydration. hydr. $D_{cal.}$ = density calculated assuming two molecules of water per molecule of cortisone acetate.

TABLE II

INDEXED X-RAY DIFFRACTION POWDER DATA FOR VARIOUS FORMS OF CORTISONE ACETATE

d = spacing in Ångstrom units, I/I_0 relative intensities (photometrical). hkl = Miller index of reflecting planes

FORM I

d(Å)	I/I_0	hkl	d(Å)	I/I_0	hkl
13·19	10	002	5·30 (2)	70	005, 204
10·73	30	102	4·93	30	014
8·79	30	003	4·69 (3)	60	303
7·78	20	200	4·49	10	213 (?)
7·23	10	011 (?)	4·33	60	304
6·596	10b	004	3·74	10	020
6·14 (1)	100	112	3·629	10	121
5·78	50b	104, 013	2·920	20	009
5·48	30	203	2·850	10	026

FORM II

d(Å)	I/I_0	hkl	d(Å)	I/I_0	hkl
13·57	60	020	2·759	30	420, 091
8·64	5	120	2·674	10	430
6·79	40	040	2·612	40	072
5·835 (1)	100	111	2·561	20	312, 302
5·491 (2)	90	210	2·525	20	322
5·09	50	220, 131	2·463	30	332
4·89	40	041	2·395	5	182, 342
4·77	50	230	2·360	20	272
4·484	60	141	2·322	40	352
4·322 (3)	70	240	2·280	10	1.11.1, 033
4·177	30	221	2·247	30	192
3·900	40	250	2·201	20	3.10.0
3·665	10	170	2·145	20	223
3·530	5	002	2·112	10	521, 1.10.2
3·410	50	251	2·084	5	531, 063
3·247	40	180	2·047	10	382
3·142	40	261, 042	2·002	20	
3·063	40	081	1·965	20	
2·961	5	052	1·909	20	
2·919	5	222	1·869	10b	
2·860	40	152	1·833	20	

FORM III

d(Å)	I/I_0	hkl	d(Å)	I/I_0	hkl
10·47 (5)	60	020	3·036	5	232
8·03	50	120	2·891	20	052 (spotty line)
7·44 (2)	80	011	2·805	20b	071, 421
6·71	20	101	2·688	5	440, 431
6·34	30	021	2·587	20	113
5·99	50	210	2·533	20	342
5·65	40	121	2·494	10	081
5·24 (1)	100	040, 031	2·445	30	412, 181, 203
4·83 (3)	70	140	2·384	30	223
4·45	30	221	2·328	10	521, 143
4·13	30	141	2·240	20	303
3·98	60	002	2·126	5	452
3·73	40	112	2·094	20	0.10.0
3·570	50	122	2·065	5	282
3·486	20	321	2·010	10	462
3·340	20	132	1·980	5	014, 621
3·192	30	251	1·943	10	433
3·104	20	161			

FORM IV

d(Å)	I/I_0	hkl	d(Å)	I/I_0	hkl
15·29 (3)	70	020	3·517	30	112
9·30	trace	110	3·420	30	081
8·23	70	120	3·271	40	270 (?)
7·65	30	040	3·208	60	142
6·79	10	021	2·997	50	340
6·04	30	031, 140	2·960	10	101
5·875 (1)	100	111	2·900	50	162
5·575	40	121	2·808	40	281
5·384	10	041	2·741	40	360
5·184	40	150	2·698	10	082
4·88	70	200	2·630	10	291
4·714 (2)	80	141 (?)	2·467	20	371
4·279	40	151	2·431	30	410
4·23	40	061	2·358	50	282
4·10	50	201	2·253	40	0.13.1
3·995	40	170	2·194	40	233
3·89	40	161	2·137	40	173
3·83	40	080	1·999	30	0.13.2
3·77	trace	012	1·934	20	283
3·654	50	?			

FORM V

d(Å)	I/I_0	hkl	d(Å)	I/I_0	hkl
16·24 (3)	70	001	3·195	40	300
8·79	50	101	2·984	60	015
8·12	60	002	2·918	50	204
6·66	30	102	2·766	30	024
5·88 (1)	100	110	2·693	10	
5·69	50	111	2·629	30	
5·49	70	012	2·438	10	
4·97	40	112	2·329	40	
4·77 (2)	80	201, 200	2·280	30	
4·42	40	201	2·235	20	
4·11	60b	113, 004	2·203	10	
3·939	trace	104	2·162	10	
3·859	trace	203	2·140	10	
3·75	50	020	1·991	10	
3·664	30	021	1·964	20	
3·587	trace	014	1·932	30	
3·516	20	114	1·895	10	
3·428	10	213	1·870	10	
3·253	30	122			

are summarised in Tables I–II. Plate I illustrates the appearance of the various forms for microscopic identification.

In general the most reliable X-ray diffraction data for the identification of complex organic substances are obtained from single crystals. Powder diffraction patterns are, however, technically quicker and simpler to obtain and their value in the pharmaceutical field has been demonstrated

by recent work on some barbiturates by Huang (1953) and by Williams (1959) and on antibiotics by Kennard, Cornforth, Humphrey and Lightbown (1955). The powder patterns of various forms of cortisone acetate (Table II) are sufficiently distinctive for diagnostic use.

All forms of cortisone acetate examined by us changed to the stable Form I on prolonged contact with water. The mechanism of this transformation is obscure since the stable form is not a hydrated one and represents the most complex crystallographic arrangement with three pairs of independent molecules in each cell. The transformation is usually accompanied by appreciable caking of the suspension containing the crystals and for this reason commercial preparations of cortisone-acetate are converted to Form I before the preparation of aqueous suspensions for parenteral administration. A similar procedure would appear to be possibly advantageous in the preparation of tablets of cortisone acetate for oral administration.

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