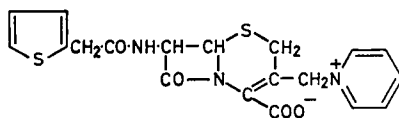


Polymorphism of cephaloridine

J. H. CHAPMAN, J. E. PAGE, A. C. PARKER, D. ROGERS*, C. J. SHARP AND SUSAN E. STANIFORTH

Infrared spectra of Nujol mulls and X-ray powder photographs have shown that cephaloridine can exist in at least six different crystalline forms. The preparation, interconversion and properties of these forms are discussed. Proton magnetic resonance spectroscopy has been used to check their chemical identity.

INFRARED measurements have shown that cephaloridine (I) (Martin & Shaw, 1965; Spencer, Siu & others, 1967), like other cephalosporin C derivatives, when recrystallized under different conditions gives rise to several crystalline forms (Green, Page & Staniforth, 1965); the most important of these we have called the α -, β -, δ -, ϵ -, ζ - and μ -forms.



I

The α - and β -forms are prepared by treating with triethylamine, solutions of cephaloridine hydronitrate in *NN*-dimethylacetamide and *NN*-dimethylformamide, respectively. The same treatment of solutions of cephaloridine hydronitrate in dimethyl sulphoxide, ethylene glycol, diethylene glycol and benzyl alcohol gives the respective solvates all having the ζ -form; while from methanolic solutions a methanolate, the μ -form, is obtained. Recrystallization of any of the forms from water leads to the δ -form, while prolonged drying of the μ -form *in vacuo* leads to the ϵ -form. The various forms may be inter-converted *via* the μ -form. Thus, treatment of the μ -form with *NN*-dimethylacetamide, *NN*-dimethylformamide, water vapour or dimethyl sulphoxide gives the α -, β -, δ - and ζ -forms, respectively; these may be reconverted to the μ -form by treatment with methanol or methanol vapour. Details for the preparation and interconversion of the six crystalline forms are given in the Experimental section.

SPECTROSCOPIC MEASUREMENTS

Dickson, Page & Rogers (1955) have shown that the various crystalline forms of a polymorphic substance give different infrared spectra when examined in the solid state as Nujol mulls or alkali halide discs, but identical spectra when examined in solution. However, since water transmits over only a narrow range in the infrared region, aqueous solutions of water-soluble compounds, such as cephaloridine, do not give solution spectra that are sufficiently reliable for checking chemical identity and, hence, confirming the chemical identity of the polymorphs.

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We have found that a combination of solid-state infrared with proton magnetic resonance (pmr) measurements on heavy-water solutions provides a better test for polymorphism in water-soluble compounds. Thus, the crystalline forms of cephaloridine may be distinguished by solid-state infrared (Fig. 1) or X-ray measurements (Table 1), and the chemical identity (but see below) of the different forms established by pmr measurements on heavy water solutions. The pmr measurements not only confirm the structure of cephaloridine (Green, Page & Staniforth, 1965), but also yield quantitative information on solvent and other impurities.

TABLE 1. X-RAY POWDER DATA FOR α -, β -, ϵ -, ζ - AND μ -FORMS OF CEPHALORIDINE (CU-K α -RADIATION)

α -Form		α -Form		β -Form		β -Form		ϵ -Form		ζ -Form		μ -Form	
d (Å)	I*	d (Å)	I*	d (Å)	I	d (Å)	I	d (Å)	I	d (Å)	I	d (Å)	I
14.5	m	3.47	wd	14.5	m	3.58	vw	10.2	wd	7.4	vw	7.3	m
7.4	m	3.39	m	7.4	w	3.52	vw	7.7	md	6.9	w	6.7	w
6.4	w	3.35	w	6.8	w	3.50	w	6.3	wd	6.2	wd	5.8	vw
5.7	s	3.29	vw	6.01	w	3.42	w	5.4	s	5.8	vw	5.45	md
5.55	s	3.12	wd	5.75	s	3.41	w	5.25	w	5.6	w	5.15	md
5.25	s	2.96	vw	5.65	w	3.35	vw	5.1	vwd	5.4	vwd	4.5	md
4.9	w	2.82	wd	5.3	wd	3.30	vw	4.8	m	5.2	w	4.4	m
4.8	vw	2.76	vw	5.29	vw	3.22	vw	4.4	vw	4.95	w	4.0	vw
4.5	m	2.72	vw	4.95	s	3.18	vw	4.25	w	4.7	vw	3.90	m
4.2	m	2.67	vw	4.85	m	3.02	w	4.15	w	4.5	w	3.65	w
4.05	m	2.64	w	4.5	w	2.86	w	4.0	w	4.4	w	3.60	vw
3.87	w	2.57	vw	4.3	s	2.82	w	3.90	w	4.05	w	3.50	w
3.83	vw	2.54	w	4.25	m	2.78	w	3.65	w	3.95	w	3.35	m
3.74	w	2.51	vwd	4.1	m	2.77	vw	3.60	w	3.80	w	3.25	m
3.70	w	2.47	vwd	3.96	m	2.68	w	3.55	vwd	3.75	w		
3.67	wd	2.43	w	3.85	m	2.58	w	3.47	vw	3.55	m		
3.55	vw			3.79	wd	2.56	w	3.40	w				
				3.72	wd	2.52	vwd	3.30	w				
								3.13	m				
								2.82	wd				

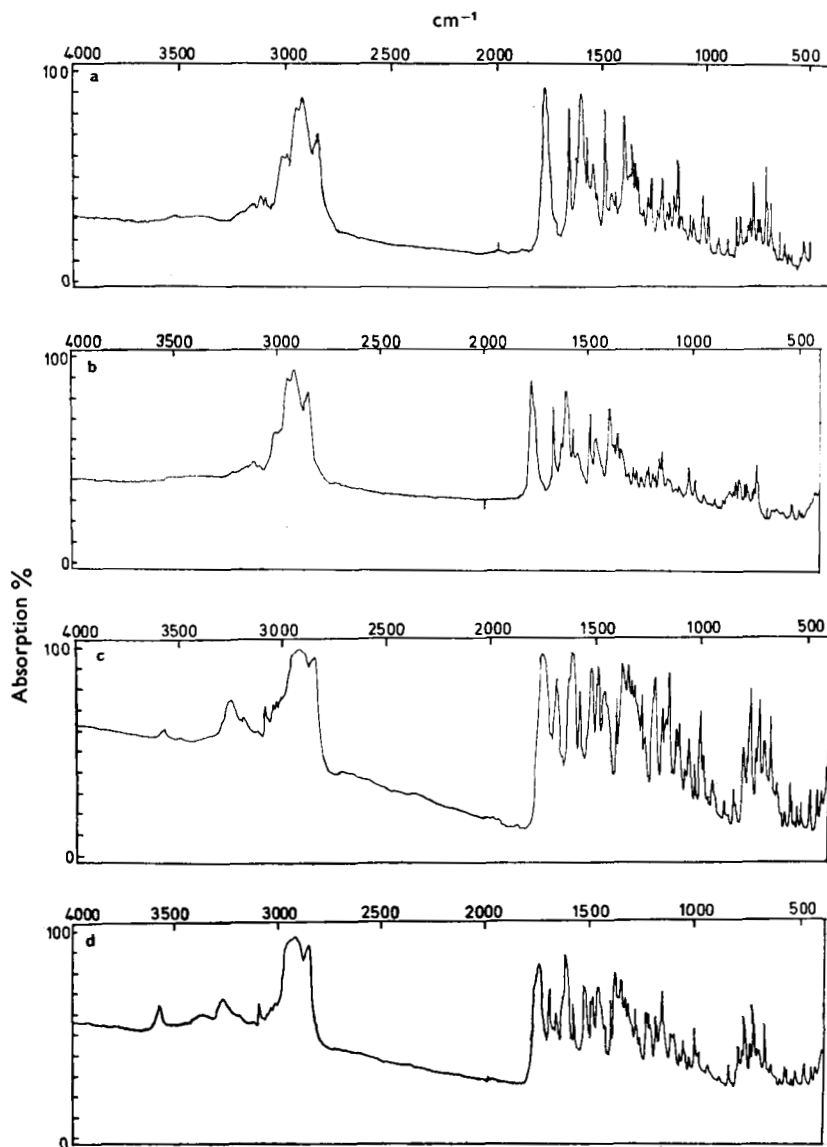
* s = strong, m = medium, w = weak, vw = very weak, d = diffuse line.

Proton magnetic resonance spectroscopy showed that although the μ -form of cephaloridine contains about one mole of methanol and the ζ -form about one mole of either dimethyl sulphoxide, ethylene glycol, or diethylene glycol, depending on the solvent used for its preparation, the α -, β -, anhydrous δ - and ϵ - forms contain less than 0.1 mole (i.e. non-stoichiometric amounts) of solvent. The α -form is characterized by containing about 0.05 mole of *N,N*-dimethylacetamide. This small amount of "impurity," which cannot be removed by prolonged drying at 10^{-5} - 10^{-6} mm, is apparently able to "lock" the cephaloridine molecule in a particular crystal lattice. However, if the cephaloridine is reprecipitated or recrystallized from some other solvent, the cephaloridine takes on the crystal form associated with the new solvent.

Mesley (1965) has reported somewhat similar non-stoichiometric chloroform-adducts of hydrocortisone and dexamethasone, which are believed on thermogravimetric evidence to be clathrates. Chapman (1965) mentions that polymorphism in lipids often depends on the presence

or absence of impurities, but unfortunately does not take the matter further.*

The infrared spectra of Nujol mulls of the six crystalline forms of cephaloridine differ considerably (see Fig. 1). The α - and β -forms show



* After this paper was submitted for publication two papers by M. Kuhnert-Brandstätter and H. Grimm (*Mikrochim. Acta*, 1968, 115-126; 127-139) appeared in which solvent-containing crystal forms, "pseudopolymorphic" forms, of various steroids are described.

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FIG. 1.—*continued.*

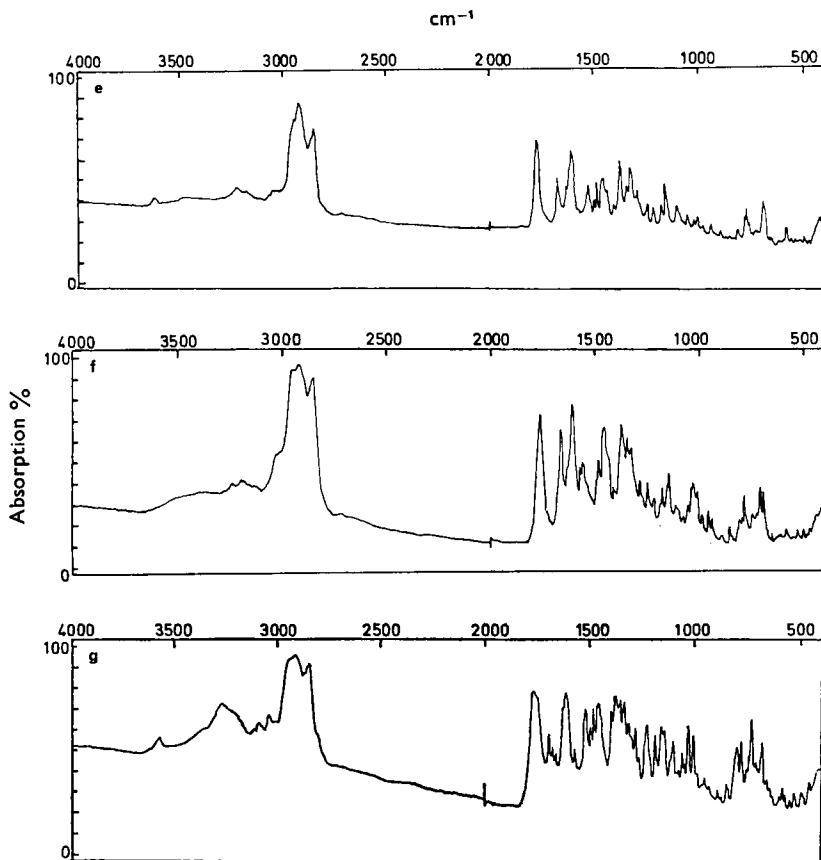


FIG. 1. Infrared spectra of Nujol mulls of (a) α -, (b) β -, (c) anhydrous δ -, (d) equilibrated δ - ($\frac{1}{2}$ mole of water), (e) ϵ -, (f) ζ - (*ex* dimethyl sulphoxide) and (g) μ -forms of cephaloridine.

a broad band for bonded N-H at about $3,120\text{ cm}^{-1}$ and no O-H absorption, confirming that the two forms are anhydrous. They give intense β -lactam ($1,774\text{--}1,776\text{ cm}^{-1}$), ionized carboxyl ($1,602\text{ cm}^{-1}$) and secondary amide ($1,666\text{--}1,670$ and $1,548\text{--}1,554\text{ cm}^{-1}$) bands and may be distinguished by strong C-H bending bands at 718 and 700 cm^{-1} , respectively.

The spectrum of the μ -form, which contains about 1 mole of methanol, is characterized by broad O-H and N-H absorption bands at $3,350$ and $3,270\text{ cm}^{-1}$, secondary amide peaks at $1,690$ and $1,518\text{ cm}^{-1}$ and C-H bending bands at 730 and 680 cm^{-1} . The O-H and C-O bands at $3,270$ and $1,028\text{ cm}^{-1}$, respectively, which disappear when the methanol is removed and the sample converted to the ϵ -form, are attributed to methanol. The ϵ -form shows a weak N-H peak at $3,225\text{ cm}^{-1}$ and secondary amide bands at $1,678$ and $1,528\text{ cm}^{-1}$; the C-H bending peak is at 690 cm^{-1} . In all forms, the carbonyl bands for the β -lactam and

ionized carboxyl groups are much stronger than the corresponding secondary amide bands.

The anhydrous δ -form (from water) and μ -form (from methanol) are markedly hygroscopic; the weak peaks at about $3,575\text{ cm}^{-2}$ in Fig. 1c and g are attributed to traces of water picked up during the mulling operation. Samples of the δ -form in equilibrium with the normal laboratory atmosphere are repeatedly found to contain about $\frac{3}{4}$ mole of water, and some effort has been made to discover whether a discrete hydrate exists; so far without decisive result.

Measurements of partial pressures of water vapour in equilibrium with solids of various water contents give no indication of the formation of stoichiometric hydrates by the α -, β - or δ -forms. Thermogravimetric analysis of the hydrated δ -form gives some support to the existence of a hydrate containing $\frac{3}{4}$ mole of water; but the principal positive evidence for such a hydrate comes from infrared spectra and from the demonstration of a distinct break at about $\frac{3}{4}$ mole in the rate of water-uptake plot, measured on δ -form in contact with a saturated atmosphere. The evidence from powder and single crystal X-ray data is, however, rather strongly against this hydrate having a definite stoichiometry.

The infrared spectrum of an anhydrous sample of the δ -form shows N-H bands at $3,260$ and $3,196\text{ cm}^{-1}$ and no other absorption between $3,600$ and $3,100\text{ cm}^{-1}$. The relatively strong $3,260\text{ cm}^{-1}$ band probably accords with the stretching vibration of an N-H group that is *trans* N-H...O=C hydrogen-bonded, but the weaker $3,196\text{ cm}^{-1}$ band represents either the stretching vibration of an N-H group that is *cis* N-H...O=C hydrogen-bonded (Darmon & Sutherland, 1949) or the Fermi resonance of the secondary amide I and II vibrations (Cannon, 1960). Absorption bands for the β -lactam and ionized carboxyl groups appear at $1,762$ and $1,620\text{ cm}^{-1}$, respectively, and for the secondary amide I and II vibrations at $1,695$ and $1,525\text{ cm}^{-1}$, respectively.

On hydration with up to about $\frac{3}{4}$ mole of water, characteristic O-H stretching and O-H bending bands for water-of-crystallization appear at $3,575$ and $3,366$, and at $1,665\text{ cm}^{-1}$, respectively; the intensity of these bands is directly related to the water-content of the sample as measured by the Karl Fischer method. The water-of-crystallization hydrogen-bonds onto the carbonyl of the β -lactam ring and causes a decrease in the intensity of the carbonyl band at $1,762\text{ cm}^{-1}$ and a corresponding increase in the intensity of a new carbonyl band at $1,745\text{ cm}^{-1}$. The relative intensity of the two carbonyl bands provides a measure of the amount of free and hydrogen-bonded β -lactam carbonyl. In the hydrate containing $\frac{3}{4}$ mole of water, the absorption band for unbonded β -lactam carbonyl appears as a shoulder on, and has about one-third of the intensity of, that for the bonded β -lactam carbonyl. The secondary amide and ionized carboxyl bands are unaffected by water uptake.

If the water-content of the δ -form is further increased up to 4 moles, the general pattern of the water bands at $3,575$ and $3,366\text{ cm}^{-1}$ and of the β -lactam bands at $1,762$ and $1,745\text{ cm}^{-1}$ does not change significantly. The additional water, which gives rise to broad absorption centred at

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3,380 and 1,665 cm^{-1} and does not hydrogen-bond to the β -lactam carbonyl, is held less strongly than the first $\frac{3}{4}$ mole and is readily lost when the sample is exposed to an atmosphere of normal humidity, the spectrum reverting to that of the equilibrium form containing about $\frac{3}{4}$ mole of water.

Similar results were obtained with a specimen of cephaloridine in which the imido hydrogen had been replaced by deuterium. The anhydrous material showed N-D bands at 2,444 and 2,400 cm^{-1} and displaced amide I and II bands at 1,690 and 1,418 cm^{-1} , respectively; the other bands above 1,500 cm^{-1} had essentially the same frequencies as those for the δ -form of undeuterated cephaloridine. The relatively strong 2,400 cm^{-1} band is probably associated with the stretching vibration of an N-D group that is *trans* N-D...O=C hydrogen bonded, but the weaker 2,444 cm^{-1} band may be associated with Fermi resonance of the deuterated amide II' and amide III' vibrations. When deuterated cephaloridine is equilibrated with ordinary water, the water again hydrogen-bonds onto, and displaces from 1,762 to 1,745 cm^{-1} the absorption band for, the β -lactam carbonyl. The relative intensities of the β -lactam bands suggest that the sample has about $\frac{3}{4}$ mole of water. The water-of-crystallization bands that appear at 3,575, 3,368 and 1,664 cm^{-1} are similar to those shown by ordinary hydrated cephaloridine.

If deuterated cephaloridine is equilibrated with heavy water, the β -lactam carbonyl band splits as for the ordinary hydrate, but the O-H bands are replaced by O-D bands at 2,645, 2,630, 2,478 and 1,490 cm^{-1} . The δ -form of ordinary cephaloridine forms a similar "hydrate" with heavy water.

These results suggest that in every group of four molecules in the δ -form of cephaloridine, the β -lactam carbonyl groups of three can form hydrogen bonds with water and thereby prevent the fourth from doing so.

X-RAY MEASUREMENTS ON THE δ -FORM

X-ray powder photographs were taken of anhydrous, equilibrated ($\frac{3}{4}$ mole of water) and "wet" (about 1.0 mole of water) specimens of the δ -form suitably encapsulated, using a Guinier camera and Fe-K α radiation (see Table 2).

Single-crystal studies were confined to the equilibrated form, but, as the crystals were small fragile flakes and always seemed to incorporate some strains, the spots on the film were not sharp. The thermal attenuation was also marked. Weissenberg photographs established that these crystals have Laue symmetry *mmm* and screw absences were noted on each axis to $h = 8$, $k = 5$, $l = 30$, so that the space group is uniquely identified as $P2_12_12_1$. The cell parameters were estimated as $a = 7.2_9$, $b = 8.1_9$ and $c = 32.1 \text{ \AA}$ to an accuracy of little better than 1%. By noting the relative strengths of the single-crystal reflexions it was possible to index the first 26 lines ($d \geq 3.24 \text{ \AA}$) in its powder pattern. Of these, 22 lines were uniquely indexed and were used to calculate by least-squares the cell parameters and standard deviations in Table 3. With this as a guide it has been possible to index the powder patterns of the "wet" and

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TABLE 2. OBSERVED AND CALCULATED POWDER DATA FOR "WET" (ABOUT 1 MOLE OF WATER), EQUILIBRATED (2/3 MOLE OF WATER) AND ANHYDROUS δ-FORMS OF CEPHALORIDINE (Fe-Kα-RADIATION)

hkl	"Wet"				Equilibrated				Anhydrous			
	d(Å)	I*	10 ⁵ × sin ² θ _o	10 ⁵ × sin ² θ _c	d(Å)	I	10 ⁵ × sin ² θ _o	10 ⁵ × sin ² θ _c	d(Å)	I	10 ⁵ × sin ² θ _o	10 ⁵ × sin ² θ _c
002	16.21	f	357	357	16.10	vvf	362	362	15.94	f	370	366
004	8.06	vf	1,443	1,429	—	—	—	1,448	7.96	vf	1,480	1,465
011	7.98	vf	1,475	1,483	7.91	f	1,501	1,499	7.84	f	1,528	1,519
012	7.34	ms	1,743	1,751	7.28	s	1,772	1,771	7.24	ms	1,789	1,794
101	—	—	—	1,852	—	—	—	1,848	—	—	—	1,823
102	6.65	ms	2,121	2,120	6.65	sv	2,121	2,120	6.69	ms	2,096	2,098
013	6.53	vf	2,204	2,198	6.49	vf	2,230	2,223	6.44	w	2,262	2,252
103	6.05	w	2,563	2,567	6.03	w	2,584	2,572	6.05	w	2,563	2,556
014	5.75	vf	2,832	2,823	5.72	f	2,868	2,857	5.68	w	2,904	2,893
110	—	—	—	3,157	—	—	—	3,166	—	—	—	3,159
104	5.423	—	3,190°	3,191	5.410	—	3,205°	3,206	5.417	—	3,197°	3,197
006	—	vs b	—	3,215	—	vs b	—	3,258	—	vs vb	—	3,298
111	5.379	—	3,243°	3,246	5.353	—	3,274°	3,274	5.340	—	3,290°	3,251
112	5.181	vvf	3,495°	3,514	5.158	w	3,527	3,528	5.128	f b	3,568°	3,526
015	5.088	f	3,624	3,628	5.059	w	3,665	3,671	5.020	f b	3,723	3,717
113	—	—	—	3,960	—	—	—	3,981	—	—	—	3,984
105	4.854	m b	3,983	3,996	4.838	ms	4,009	4,020	4.828	m	4,026	4,021
114	4.520	s b	4,593	4,586	4.506	vs	4,620	4,614	4.520	ms vb	4,593°	4,725
016	—	—	—	4,609	—	—	—	4,667	—	—	—	4,725
106	—	—	—	4,977	—	—	—	5,016	—	—	—	5,029
115	4.172	ms	5,390	5,390	4.157	s	5,430	5,429	4.168	ms b	5,400°	5,449
020	4.105	w	5,569	5,576	4.083	ms	5,629	5,635	4.134	—	5,489°	5,710
021	—	—	—	5,666	—	—	—	5,725	4.054	m	5,710	5,710
008	—	—	—	5,715	—	—	—	5,792	—	—	—	5,802
017	4.039	f	5,751	5,772	4.011	vvf	5,832	5,843	3.990	f	5,894	5,915
022	3.969	vf	5,955°	5,933	3.952	vvf	6,007	5,997	—	—	—	6,077
107	3.905	vs	6,153	6,140	3.892	s	6,195	6,191	3.888	s b	6,207°	6,220
—	—	—	—	—	—	—	—	—	3.872	—	6,258°	—
116	—	—	—	6,371	3.820	ms	6,429°	6,424	—	—	—	6,457
023	3.836	m	6,375°	6,380	—	—	—	6,449	3.795	m b	6,514°	6,535
024	—	—	—	7,005	—	—	—	7,083	—	—	—	7,176
200	—	—	—	7,050	—	—	—	7,030	—	—	—	6,927
018	—	—	—	7,109	3.610	f b	7,198	7,200	—	—	—	7,290
201	3.627	m	7,131°	7,140	3.630	f b	7,119	7,121	3.656	f	7,019	7,018
120	—	—	—	7,339	—	—	—	7,392	—	—	—	7,442
202	—	—	—	7,408	3.558	vs	7,414°	7,392	3.585	w	7,300	7,293
121	3.554	m b	7,425°	7,428	—	—	—	7,483	—	—	—	7,534
108	—	—	—	7,478	—	—	—	7,550	—	—	—	7,594
117	3.525	m	7,551	7,534	3.512	f	7,609	7,601	3.495	ms	7,679	7,648
122	—	—	—	7,696	—	—	—	7,754	—	—	—	7,809
025	—	—	—	7,809	—	—	—	7,897	—	—	—	8,000
203	—	—	—	7,854	—	—	—	7,845	—	—	—	7,751
123	3.393	vf	8,150	8,143	3.383	f	8,198	8,207	—	—	—	8,267
210	—	—	—	8,434	—	—	—	8,439	—	—	—	8,354
204	3.327	ms	8,475	8,480	3.327	s	8,475	8,478	3.347	s	8,378	8,392
211	—	—	—	8,534	—	—	—	8,530	—	—	—	8,446
019	—	—	—	8,627	—	—	—	8,739	—	—	—	8,847
124	—	—	—	8,767	3.262	ms	8,818°	8,840	—	—	—	8,908
212	3.269	ms	8,781	8,802	—	—	—	8,801	3.266	f	8,794°	8,721
026	—	—	—	8,791	—	—	—	8,893	3.228	f	9,005	9,007
00.10	3.242	f	8,930	8,934	—	—	—	9,050	—	—	—	9,160
109	—	—	—	8,996	—	—	—	9,088	—	—	—	9,151
213	—	—	—	9,248	—	—	—	9,254	—	—	—	9,179
205	3.179	ms	9,282	9,284	3.177	s	9,294	9,293	3.190	s	9,218	9,216

* s = strong, m = medium, w = weak, f = faint, b = broad line; ° = line omitted from least-squares calculation.

anhydrous forms and to get least-squares estimates of the cell dimensions of these forms (see Table 3). Table 2 shows the indexing and the agreement between sin²θ_{obs} and sin²θ_{calc} for the three forms.

Comparison of the powder photographs showed that:

(1) small progressive changes of cell parameters occur (*a* increasing, *b* and *c* decreasing) and that the volume decreases with loss of water.

(2) most lines become progressively less sharp as water is lost, indicating the onset of disorder and strain.

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(3) all three patterns appear to be orthorhombic.

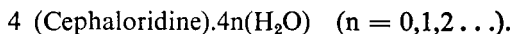
(4) a few lines change their relative intensities rather conspicuously, indicating appreciable rearrangement of the packing in at least parts of the molecule.

TABLE 3. CRYSTALLOGRAPHIC DATA FOR "WET," EQUILIBRATED AND ANHYDROUS δ -FORMS OF CEPHALORIDINE. (Cell parameters from least-squares calculations; standard deviations are quoted in brackets.) M_x is experimental value of molecular weight of asymmetric unit; M'_x is the value of M_x after scaling to make $M'_x = 433.5$ for "wet" δ -form.

	"Wet"	Equilibrated	Anhydrous
a	7.296(4)	7.306(2)	7.361(3)
b	8.204(5)	8.161(3)	8.107(5)
c	32.407(14)	32.201(12)	32.008(24)
V (\AA^3)	1940.9(1.7)	1920.1(1.1)	1910.1(2.1)
D_x (g. cm^{-3})	1.473(4)	1.470(4)	1.461(4)
M_x	430.5(1.2)	425.1(1.2)	420.3(1.2)
M'_x	433.5(1.2)	428.1(1.2)	423.3(1.2)
Expected M.	433.5(H_2O)	429.0($\frac{3}{2}$ H_2O)	415.5(no H_2O)
No. of lines used in least squares calculation	20	22	18

Other photographs taken at intermediate stages of hydration gave intermediate features on the powder patterns, and both the "wet" and anhydrous forms on exposure eventually gave the pattern of the equilibrated form. The evidence suggests that the lattice changes are smooth and reversible.

The space group $P2_12_12_1$ has four equivalent general positions and no special positions, so that if the space group symmetry is adhered to strictly, the only permissible stoichiometries for the cell contents would be



A water content of 3 molecules per cell could only occur by random partial occupancy of water sites. The level of hydration can be checked only if both the cell densities and parameters are measured accurately enough. The densities were measured by flotation in a centrifuge in mixtures of trichloroethylene and carbon tetrachloride. The cell parameters quoted are all affected equally by a small correction for film shrinkage for which allowance has not been made; this has apparently led to values for cell volume being about $\frac{3}{4}\%$ too small and to a similar error in the magnitudes of the molecular weights, M_x . Scaling up the values so that the fully hydrated form has 4 molecules of water per cell gives the values, M'_x , in Table 3. The loss in passing from "wet" to equilibrated δ -form is 0.30 ± 0.13 mole (and is not, of course, affected by rescaling), but the anhydrous δ -form has too high a value of M_x ; it corresponds to 0.43 mole of water. The density of this form was measured on a freshly prepared specimen under conditions that should prevent absorption of water. Nevertheless, it is likely that despite efforts to encapsulate and photograph the crystals without delay, water was absorbed during the exposure: a swelling of the lattice during the exposure may have contributed to the broadness of the lines for the anhydrous δ -form.

The crystallographic results in general corroborate the spectroscopic

conclusions, but indicate that loss of water must occur randomly from the water sites and that there cannot be a sharply defined, stable stoichiometry of $\frac{3}{4}$ mole of water. The deterioration of crystallinity, the progressive changes in cell size, the changes in relative intensity of some lines as water is lost and the apparently reversible nature of the changes suggest that the δ -form is a smoothly and reversibly swellable structure, in which there is some rearrangement of the packing (of some at least) of the cephaloridine molecules. Similar reversible variation in hydration, which ignores strict space group requirements, is known among zeolites (e.g., faujasite), clay minerals that swell in only one dimension (e.g., montmorillonite or vermiculite), and large organic molecules (e.g., vitamin B₁₂ and proteins), but the number of water molecules is then much larger and considerable disorder occurs. Partial occupancy, defying space group requirements, is not rare when the number of solvated water molecules is low, but we have not so far found a clear precedent for it occurring as an equilibrium condition in a swellable lattice.

The density changes are proportional to the water content, but the change from "wet" to dry δ -form is only 0.7% compared with a weight loss of 4.3%. The major consequence of this loss of water is, therefore, a reduction of 3.6% in the cell volume, i.e., the molecules collapse to fill the holes vacated by the water.

The density is high for a material of this constitution, and consideration of ways of packing the molecules into the cell suggest that the packing must be tight. Presumably long-range electrostatic and intermolecular amide dipole-dipole forces and hydrogen bonding are responsible for the collapse of the structure in the absence of water. But, since the infrared spectra show no significant changes in the secondary amide hydrogen-bond frequencies on dehydration it can be deduced that the molecule is flaccid and offers little resistance to crumpling, i.e. the lattice can contract and the holes can be filled easily without significant alteration of the hydrogen-bonding distances. Changes of lattice parameter have little effect on the electrostatic and dipolar energies, so that there is little inducement to squeeze out the three molecules of water per cell.

But for the infrared evidence, the crystallographer, in the absence of a detailed knowledge of atomic positions and, in view of the sharpness of the powder lines, would have expected the extra water in the fully hydrated form to go into vacant sites crystallographically equivalent to those occupied by the other water molecules. The conclusion seems inescapable, that if in the "wet" δ -form structure all atoms had gone into strictly equivalent positions, the water molecules would have been unable to link effectively with the β -lactam carbonyl groups. Instead, a free-energy minimum has been achieved by accepting some measure of structural disorder. Roughly three in every four water molecules make effective links, but in so doing displace and distort the cephaloridine molecule somewhat (and probably themselves move from strictly equivalent sites), thus effectively closing the "hole" adjacent to the fourth β -lactam carbonyl group. The "extra" water is then loosely held and is easily squeezed out. Removal of water from the " $\frac{3}{4}$ mole hydrate" results in further crumpling and disorder.

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The broadness of some of the lines for the anhydrous δ -form and the higher standard deviations of its cell parameters may represent an incipient departure from orthorhombic symmetry, but there is no clear evidence for this, nor is it evident how $P2_12_12_1$ might degrade.

Consideration has been given [on the lines considered by Dunitz (1964)] to the possibility that the orthorhombic symmetry might only be simulated by multiple twinning, but we find it difficult to see any explanation for the observed systematic absences.

We can only conclude that the whole process involves progressive random disorder from space group $P2_12_12_1$. The equilibrium is then thermodynamically controlled and represents a balance between the energies associated with (1) water attached to the β -lactam carbonyl group, and (2) the squeezing action of the electrostatic and dipolar forces. Presumably the equilibrium hydration level could be modified by temperature, pressure and ambient humidity changes. The crystallographic results corroborate all other findings, but indicate that the equilibrated δ -form is not a sharply defined stoichiometric entity as are most other hydrates. It has the composition 4 : 3 only by accident and within present experimental accuracy. From the poor quality of even the best single crystals examined it seems unlikely that a detailed study of its structure will be achieved.

Experimental

The infrared spectra (4,000–400 cm^{-1}) were recorded on a Perkin-Elmer model 521 spectrophotometer fitted with a diffraction grating. The compounds were examined as Nujol mulls mounted between potassium bromide plates. The mulls of hygroscopic forms of cephaloridine were prepared under anhydrous conditions in a dry-box; care was taken not to expose the mulls to a moist atmosphere. The cephaloridine samples satisfied the tests described by Martin & Shaw (1965) and were examined by pmr spectroscopy for solvent impurities, such as acetone, *NN*-dimethylacetamide, *NN*-dimethylformamide, dimethyl sulphoxide, ether, methanol and triethylamine. The pmr spectra of 10% solutions in heavy water were measured at 38° on a Varian Associates A60 Spectrometer at a sweep rate of 1 cycle/sec/sec and calibrated against sodium 3-(trimethylsilyl)-propane-1-sulphonate used as an internal standard. The X-ray powder photographs were taken on a Nonius Guinier (Mark 2) camera with either Cu- or Fe-K α -radiation.

Water-content/vapour-pressure measurements were conducted at 17.5° on samples of cephaloridine contained in a closed, evacuated system in which the pressure of the water vapour could be measured directly on a simple manometer. Measurements were made by filling the apparatus (of known volume) with water vapour at its saturation pressure, allowing the cephaloridine crystals to absorb some of, and reach equilibrium with, the water vapour, and then recording the pressure. An adsorption isotherm was thus plotted. By a similar procedure step-wise desorption isotherms were also obtained.

The rate of intake of water vapour was measured by placing samples (about 4 g) of dried δ -form on a top-loading balance (Mettler P120) alongside suitable wicks arranged to give a substantially saturated atmosphere under a bell-jar that covered the pan area. A curve of weight-gain against time was plotted and showed a break at a point corresponding to approximately $\frac{3}{4}$ mole of water.

α -Form of cephaloridine. Triethylamine (2.04 ml) was added dropwise with stirring to a filtered solution of cephaloridine hydronitrate (6.89 g) (Eardley, Stocker & Long, 1966) in *NN*-dimethylacetamide (35 ml). After being kept at 4° for 4 hr, crystals of the α -form were filtered off, washed with *NN*-dimethylacetamide (10 ml) and acetone (3 \times 25 ml), and dried at room temperature under reduced pressure. Prolonged drying at pressures down to 10⁻⁵–10⁻⁶ mm did not reduce the *NN*-dimethylacetamide content of the sample below 0.05 mole, as indicated by the intensity of pmr peaks at τ 6.92, 7.07 and 7.90.

β -Form of cephaloridine. The β -form of cephaloridine was prepared by a similar method to that used for the α -form except that *NN*-dimethylacetamide was replaced by *NN*-dimethylformamide; the intensity of the pmr peaks at τ 6.97 and 7.13 indicated that the product contained about 0.1 mole of *NN*-dimethylformamide.

δ -Form of cephaloridine. The α -form of cephaloridine (14.5 g) was dissolved in water (40 ml) by warming and the filtered solution kept at room temperature until crystallization started. The solution was then stored for 16 hr at, and filtered at, 4°. The δ -form crystals were washed, in turn, with ice-water (4 \times 10 ml) and acetone, and dried at 0.1 mm and room temperature over P₂O₅ overnight; this gave a product containing about $\frac{3}{4}$ mole of water. Anhydrous δ -form was obtained by drying the hydrated form at 40° at 0.01 mm over P₂O₅ for 6 days. On exposure to a normal laboratory atmosphere the anhydrous material picked up moisture and reverted to the equilibrium hydrate containing $\frac{3}{4}$ mole of water. Crystals containing about $\frac{3}{4}$ mole of heavy water were produced by exposing the anhydrous material to heavy water vapour in an otherwise evacuated system (0.1 mm) for 12 hr.

δ -Form of deuterated cephaloridine. The δ -form of cephaloridine was recrystallized from heavy water, washed with acetone and dried under reduced pressure to remove acetone. The deuterated product obtained in this way contained about $\frac{3}{4}$ mole of heavy water. Prolonged drying below 0.01 mm gave the anhydrous δ -form of deuterated cephaloridine, which on exposure to either water or heavy water vapour for 12 hr in an otherwise evacuated system (0.1 mm) gave crystals containing about $\frac{3}{4}$ mole of water or heavy water, respectively.

ϵ -Form of cephaloridine. The μ -form (see below) was dried *in vacuo* for 60 hr at room temperature to give the ϵ -form. The intensity of the

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pmr peak at τ 6.60 indicated that the product contained about 0.01 mole of methanol.

ζ -Form of cephaloridine. Triethylamine (0.8 ml) and a few drops of acetone were stirred into a filtered solution of cephaloridine hydronitrate (2 g) in dimethyl sulphoxide (15 ml). Acetone (90 ml) was added and, after 10 min, the ζ -form crystals were filtered, washed with acetone and dried for 2 hr at room temperature under reduced pressure. The pmr spectrum of the material was identical with that for the other forms except for a peak at τ 7.25, the intensity of which indicated the presence of about 1 mole of dimethyl sulphoxide. Similar solvates were formed with ethylene glycol, diethylene glycol and benzyl alcohol.

μ -Form of cephaloridine. The preparation of the μ -form must be carried out in a dry-box. Triethylamine (1.0 ml) was added dropwise, with stirring, to a mixture of absolute methanol (25 ml) and a filtered solution of cephaloridine hydronitrate (2.5 g) in *NN*-dimethylacetamide (10 ml). A further 25 ml of methanol were added, and the white crystals of μ -form filtered, washed with methanol (20 ml) and acetone (2×20 ml), and dried. The intensity of the peak at τ 6.60 in the pmr spectrum showed the presence of about one mole of methanol.

When the μ -form was exposed to a normal laboratory atmosphere, it reverted to δ -form containing about $\frac{3}{4}$ mole of water; this process was reversed by exposing the hydrate to dry methanol vapour. Shaking the μ -form with liquid *NN*-dimethylacetamide, *NN*-dimethylformamide or dimethyl sulphoxide at 4° for 16 hr gave the α -, β - or ζ -forms, respectively. Shaking these forms with methanol under the same conditions gave the μ -form.

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