J. Pharm. Pharmac., 1966, **18**, *Suppl.*, 190S–197S Received April 27, 1966

Cortisone acetate crystal forms

J. E. CARLESS, M. A. MOUSTAFA* AND H. D. C. RAPSON

Infrared spectroscopy and differential scanning calorimetry data are given for the characterisation of three anhydrous and two hydrated crystal modifications of cortisone acetate. All forms revert to the same form on heating to 200°. The amount of hydration estimated from thermal analysis varies from half to one molecule of water per molecule of cortisone acetate. Methods for the preparation of the five forms are described emphasising the importance of solvent composition, particularly its water content. Interconversion of these forms under various conditions such as grinding, contact with water and heat treatment is discussed.

CORTISONE acetate is reported to exist in different polymorphic forms (Garratt & Marshall, 1954; Callow & Kennard, 1961; Mesley & Johnson, 1965). Published spectra from different sources show distinct differences (Neudert & Röpke, 1957; Tarpley, Yudis, Manowitz, Horrigan & Weiss, 1954; Meda, 1958; Hayden, Sammul, Selzer & Carol, 1962). The characterisation and preparation of these forms has not been adequately described in the literature. There has been much confusion about the number of these forms and the exact methods to be used for their identification. Using infrared spectroscopy and differential scanning calorimetry, the present inquiry has investigated the physical factors responsible for the formation and stability of these different forms. The identification of different crystalline modifications is essential for the study of polymorphic changes of the material in suspension or in the dry state. Methods for preparing the different crystal forms are described in the present paper.

Experimental and results

MATERIAL AND APPARATUS

Two batches of cortisone acetate (British Drug Houses Ltd.) were used and found to belong to different crystalline forms. A third sample was a micronised product from Roussel Laboratories.

Infrared spectroscopy. Spectra were determined with a Unicam SP200 double-beam spectrometer with a sodium chloride prism. A Unicam SP100 grating spectrometer was used to confirm some of the results and to identify effluents collected in a gas cell.

Differential scanning calorimetry. A Perkin-Elmer DSC-1 apparatus fitted with effluent analyser was used (see Wendlandt, 1964). Dry nitrogen at 30 ml/min was used as the carrier gas. The rate of heating generally adopted was 32°/min and sample weight varied from 1–10 mg. The heat of fusion of tin was used to calibrate the response of the calorimeter. The thermal conductivity analyser was calibrated for water vapour measurement by heating varying amounts (0·5–10 mg) of $CuSO_4.5H_2O$

From the Department of Pharmacy, Chelsea College of Science and Technology (University of London), Manresa Road, S.W.3.

* Permanent Address: Faculty of Pharmacy, University of Alexandria, Alexandria, U.A.R.

in the sample pan of the calorimeter. Peak areas recorded were determined by planimeter. For the identification of volatile products evolved from cortisone acetate during heating in the calorimeter, the effluent was passed through a specially designed trap, cooled in liquid nitrogen and the volatile material subsequently transferred to a gas cell for identification by infrared spectroscopy.

Thin-layer chromatography. The technique described by Hall (1964) was used to check the purity of starting materials and to follow the stages in purification by crystallisation or in heating at various temperatures.

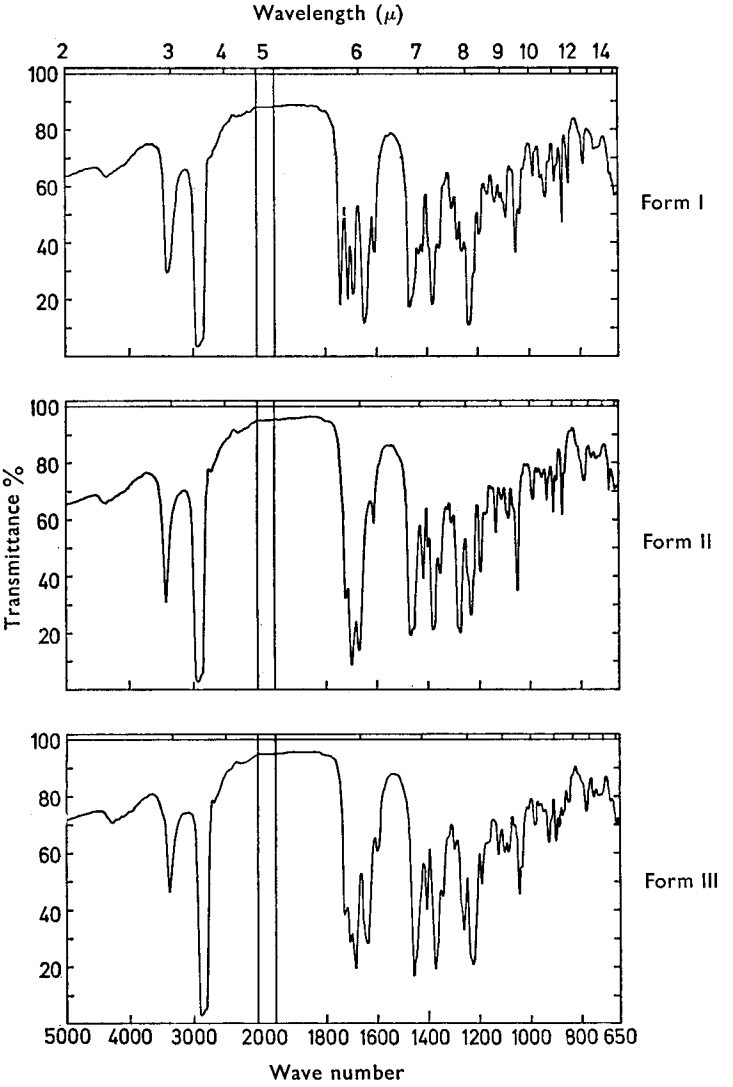

FIG. 1. Infrared spectra of cortisone acetate crystal forms in Nujol mulls.

Nomenclature. The numbering system used for nomenclature is the same as used by Callow & Kennard (1961), except that Forms I and III have inverted notation.

PREPARATION OF THE DIFFERENT FORMS

Form I was only obtained by heating any other form to a temperature of 200°. The solvents used for crystallisation of Forms II–V are as follows: Form II—chloroform, benzene; Form III—30% v/v water in acetone, water at 100°; Form IV—95% aqueous ethanol; Form V—carbon tetrachloride–anhydrous methanol (3:1 v/v). The cortisone acetate was dissolved in boiling solvent and then rapidly cooled in a refrigerator for about 1 hr and the separated crystals stored over silica gel and concentrated sulphuric acid in a desiccator. Prolonged contact of crystals with certain solvents, e.g. carbon tetrachloride or dimethylformamide, led to discolouration and to an increased number of steroid impurities.

CHARACTERISATION OF THE DIFFERENT FORMS BY INFRARED AND DIFFERENTIAL SCANNING CALORIMETRY

Infrared spectra of Forms I–V are presented in Fig. 1. Main characteristics other than those in the carbonyl and hydroxyl stretching regions include a prominent moderately strong single band at 870 cm^{-1} in Form I, a prominent strong band at 1,275 cm^{-1} of higher absorbance than the band at 1,230 cm^{-1} in Form II, and a band at 3,540 cm^{-1} probably due to an enol in Form V.

TABLE 1. QUANTITATIVE DATA FROM THERMAL ANALYSIS

Form		Energy uptake during melting kcal $mole^{-1}$	Energy uptake during loss of solvent kcal $mole^{-1}$	Quantity of water detected mol/mol
I	(raw data)	8·78, 8·56, 9·74	—	—
	(average)	9·03		
II	(raw data)	10·12, 8·83, 9·20	—	—
	(average)	9·38		
III	(raw data)	8·27, 10·21, 9·85	—	—
	(average)	9·44		
IV	(raw data)	8·52, 8·01, 10·12	10·12, 9·81, 9·69	0·58, 0·59, 0·40
	(average)	8·88	9·87	0·53
V	(raw data)	10·42, 7·93, 10·13	8·86, 7·77, 7·82	0·98, 0·98, 0·88
	(average)	9·49	8·15	0·95

Thermal analysis (Table 1, Figs 2, 3 and 4) shows that about 8–10 kcal $mole^{-1}$ are absorbed by all forms on melting, although the exact temperature of peak melting varies slightly from one form to another. In addition, Forms IV and V show a second (endothermic) transition of 8–10 kcal $mole^{-1}$ far below their melting point. This arises from loss of water together with a small amount of carbon dioxide. For freshly prepared

samples of Form IV, it corresponds to half a molecule of water, per molecule of cortisone acetate and for Form V to one molecule of water. Forms I, II and III are anhydrous.

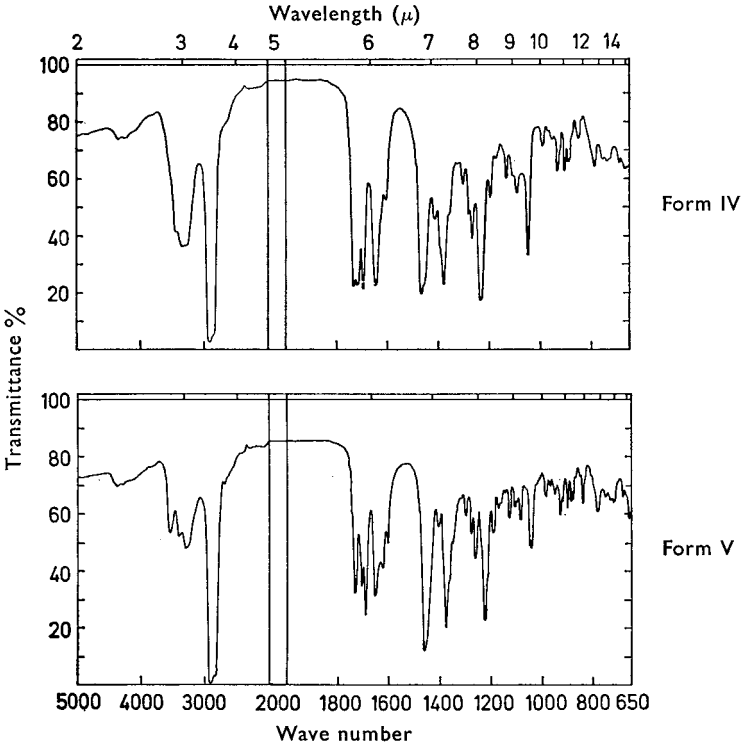

FIG. 1 (continued). Infrared spectra of cortisone acetate crystal forms in Nujol mulls.

STABILITY AND INTERCONVERSION OF THE DIFFERENT FORMS

Forms II–V can be prepared from any form of cortisone acetate by crystallising from the appropriate solvent as described under 'Preparation of different forms.' The final crystal form was independent of the original form.

The different forms were ground in an agate mortar for periods up to 45 min; grinding was also carried out in an agate ball mill (Glen Creston—Model M.270) for periods up to 15 min. Such treatment had little effect on the crystal form.

When an aqueous suspension of *any* form was stored (2 weeks) at room temperature, it underwent a change to Form IV (an extra band at 870 cm^{-1} appeared in the infrared spectrum). Only in the transformation of Form II was Form III detected as an intermediate. Continuous grinding of all forms (except Form IV) under water for 45 min in an agate mortar produced Form III. When an aqueous suspension of Form II was maintained at 100° for 30 min it showed no change in form but an increased number of steroid impurities were detected by chromatography.

If heated to 200° all forms change rapidly to Form I without an increase in the number or amount of related steroid impurities. Prolonged heating at lower temperatures favours the change from Form V to III to II and finally to I. The last stage in that sequence is particularly slow.

A summary of the course of interconversions involved under various conditions is illustrated diagrammatically in Fig. 5.

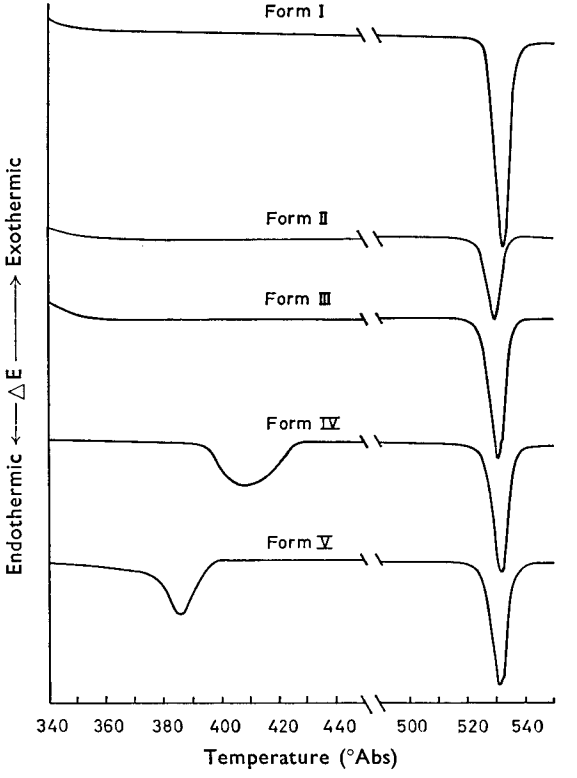

FIG. 2. Thermal analysis curves of cortisone acetate crystal forms. (△E is proportional to millicalories per sec).

Discussion and conclusions

Cortisone acetate in the solid state exists in one of five different crystalline modifications; Forms I, II and III are anhydrous; Forms IV and V are hydrated. In defining the stability of any one of these, it is important to specify the storage conditions used. The three anhydrous forms show true polymorphic behaviour, the other two represent different degrees of hydration, probably of one or other of the anhydrous forms. The way in which water molecules are linked to cortisone acetate molecules is still obscure, although the relatively high energy required for their release suggests some form of bonding to the cortisone acetate molecules.

Callow & Kennard (1961) described methods for the preparation of the different forms of cortisone acetate, but in the present investigation

it was found difficult to reproduce all their results. In some cases the solvent composition, and in particular the water content, are critical. For Form III, the acetone must contain *some* water (>10%), otherwise mixtures of various forms tend to be produced. In case of Form V, methanol must be anhydrous; ~2% water content produced Form IV instead.

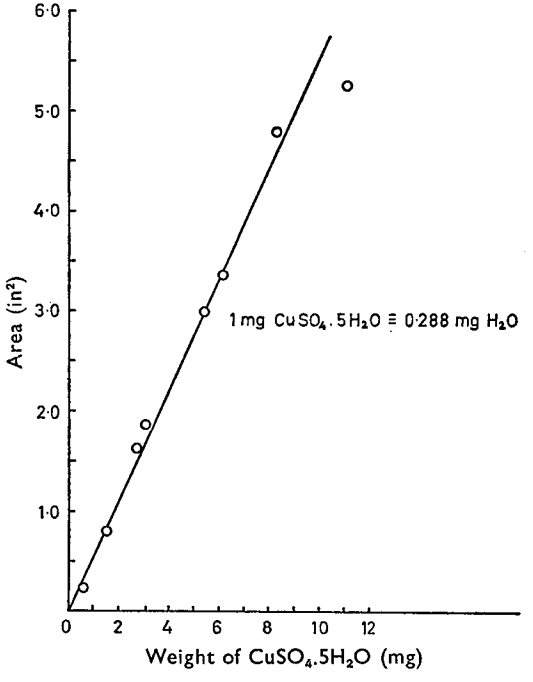

FIG. 3. Differential scanning calorimeter effluent analyser calibration curve for water vapour (scanning speed = 32° per min). ($CuSO_4 \cdot 5H_2O \rightarrow CuSO_4 \cdot H_2O + 4H_2O$).

In general, crystallisation from non-polar solvents gives rise to anhydrous forms (Form II in most cases). On the other hand, polar solvents lead to different forms depending mainly on the water content of the system. Unexpectedly, solvents containing too much water favoured the formation of anhydrous forms (Form III in most cases), whereas very *slightly* hydrated solvents (<1% water) gave rise to highly hydrated forms. Difficulties in preparing any of these forms often arise because the above factors are overlooked. It has been calculated from elemental analysis and loss of weight at 140°/15 mm (Callow & Kennard, 1961) that Form IV contains two molecules of water. Our evidence shows that only half a molecule of water is associated with each molecule of cortisone acetate.

For the preparation of stable aqueous suspensions, Form IV appears to be most suitable. This conflicts with Callow & Kennard (1961), who reported that Form III was the stable form.

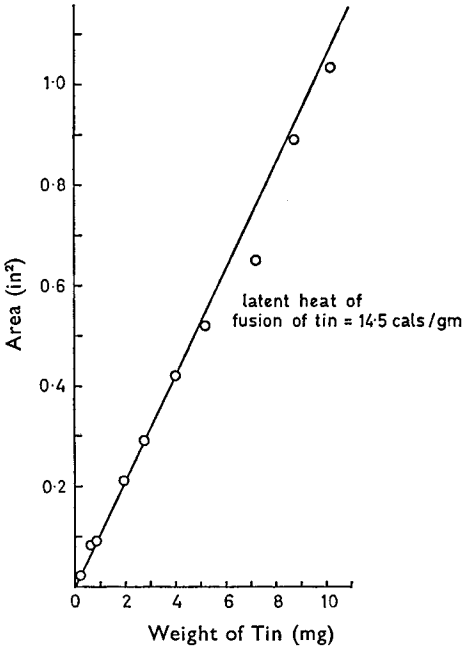

FIG. 4. Differential scanning calorimeter calibration curve for energy uptake (scanning speed = 32° per min).

High temperature treatment causes a decrease in the water content, then produces the thermally stable Form I which resists further heating up to its melting point. The constancy of the amount of energy required for melting supports the view that all forms change to Form I before melting. The transformations mentioned above still take place at room temperature, but at a much slower rate. The kinetics of these transformations are being studied and the work is still in progress.

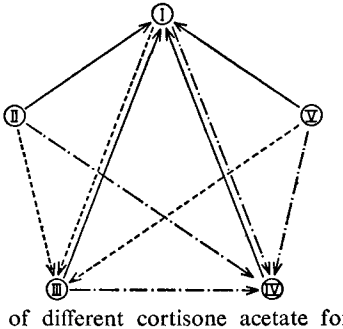

FIG. 5. Interconversion of different cortisone acetate forms. ———heating to 200°. --------grinding under water. —·—·—·— suspension in water.

Form V is very labile to heat and water treatments. The first sign of transformation being loss of its enol band (3540 cm^{-1}) from the infrared spectrum.

The infrared spectrum of Form I may be regarded as definitive for identification purposes. Nujol mulls are preferable because of the sharpness of the bands produced, in comparison to the blunt bands seen in the spectrum of a halide disc. The high pressure applied during the preparation of a disc brings about a partial change of form and this is particularly seen in the hydroxyl stretching region.

Acknowledgement. The authors wish to thank the Westminster Hospital, London, for a sample of cortisone acetate and Dr. J. E. Page of Glaxo Laboratories Ltd. for a helpful discussion.

References

Callow, R. K. & Kennard, O. (1961). *J. Pharm. Pharmac.*, **13**, 723–733.
Garratt, D. C. & Marshall, P. G. (1954). *Ibid.*, **6**, 950–959.
Hall, A. (1964). *Ibid.*, **16**, *Suppl.* 9*T*.
Hayden, A. L., Sammul, O. R., Selzer, G. B. & Carol, J. (1962). *J. Ass. Off. agric. Chem.*, **45**, 797–900.
Meda, F. (1958). *Spectrochim. Acta*, **13**, 75–79.
Mesley, R. J. & Johnson, C. A. (1965). *J. Pharm. Pharmac.*, **17**, 329–340.
Neudert, W. & Röpke, H. (1957). *Spectra Contributed to Documentation of Molecular Spectroscopy*. London: Butterworths.
Tarpley, W., Yudis, M., Manowitz, B., Horrigan, R. V. & Weiss, J. (1954). *Industr. Engng Chem.*, **46**, 1458–1464.
Wendlandt, W. W. M. (1964). *Thermal Methods of Analysis*. New York: Interscience.