

The polymorphism of phenobarbitone

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The polymorphism of phenobarbitone has been investigated using infrared spectroscopy, X-ray diffraction and differential scanning calorimetry. Eight crystalline modifications have been isolated and shown to have distinguishable infrared absorption spectra and X-ray powder diffraction patterns. Other forms were found to be present in mixed crystals and molecular compounds obtained from mixtures of phenobarbitone with other barbiturates. Differential thermograms of three forms of thialbarbitone are also given.

THE polymorphic modifications of phenobarbitone have been investigated by several authors using various techniques and it is not easy to decide exactly how many forms have already been reported. Huang (1951a), using microsublimation, prepared four forms which he designated I-IV, and which were characterized by hot-stage microscopy and by X-ray diffraction patterns. He also reported (Huang, 1951b) a form IVa, obtained by crystallization from a melt, and subsequently (Huang, 1951c) a form IVb, though the latter was encountered only in the presence of other barbiturates.

TABLE 1. KNOWN POLYMORPHIC FORMS OF PHENOBARBITONE

Form	Melting point		Remarks
	° C (a)	° Abs (b)	
I	176	448	
II	174	446	
III	167	440	
IV	163	435	Huang's IVa probably mixture of this with XII
V	160		Probably Huang's IVb
VI	157		Huang's form IV
VII	153		Not encountered in this work
VIII	141		Not encountered in this work
IX	133		Encountered only in mixed barbiturates
X	126		Encountered only in mixed barbiturates
XI	112		Not encountered in this work
XII	not known		New form not previously reported
XIII	not known		Form V of Cleverley & Williams

(a) Taken from Brandstätter-Kuhnert & Aepkers (1962).

(b) Onset temperature of melting transitions observed by differential scanning calorimetry.

Cleverley & Williams (1959), working mainly on materials recovered from solutions, obtained Huang's forms I, II and III and a new form which they designated V. In addition to X-ray diffraction patterns they recorded infra-red spectra of these forms, though they reported that forms I and II gave identical spectra. They also noted that form V differed from the other forms in showing absorption bands attributable to OH groups, and they suggested that it existed in the enol rather than the normal keto-form.

Brandstätter-Kuhnert & Aepkers (1961, 1962), using only hot-stage microscopy, reported a total of eleven forms, though six of these were stable only in the presence of other barbiturates. Of the stable forms, I, II and III correspond to those of Huang, whilst from their melting points it is apparent that Brandstätter-Kuhnert's form IV is Huang's form IVa, and her form VI is Huang's form IV.

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In the course of the present work eight relatively stable forms of phenobarbitone have been prepared, and in trying to correlate these with the unstable forms of Brandstätter-Kuhnert, we have produced two more forms in the presence of other barbiturates, one of which is probably stable on its own. All of these ten forms were found to be distinguishable by means of their infrared spectra and of their X-ray diffraction patterns. In the account which follows, the numbering of Brandstätter-Kuhnert is used where possible, and the additional forms have been given new numbers. A list of all known forms is given in Table 1.

Experimental and results

MATERIALS

Commercial phenobarbitone was recrystallized from aqueous ethanol. It was thus obtained in form II and its melting point, as determined by normal capillary tube technique, was 174–177° C.

INFRARED SPECTROSCOPY

Infrared absorption spectra of all forms obtained were recorded as mulls in liquid paraffin using a Grubb Parsons GS2 grating spectrometer, which has a linear wavelength scale (see Figs 1–4). They were also recorded on a Perkin-Elmer 237 grating spectrometer with a linear wave-number scale. With each instrument spectra of all forms were clearly distinguishable from each other.

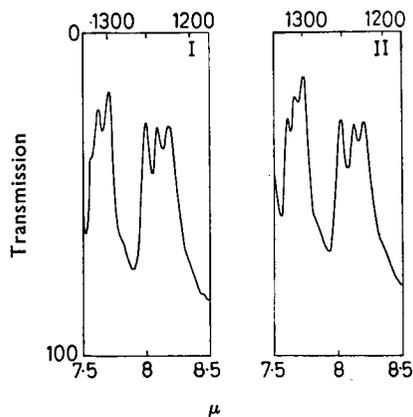


FIG. 1. Comparison of infrared spectra of forms I and II of phenobarbitone.

X-RAY DIFFRACTION

X-ray powder diffraction patterns were recorded photographically using a Unicam 9 cm camera and vanadium-filtered chromium $K\alpha$ radiation. Measurements of I/I_0 were made with a Joyce recording microdensitometer. Patterns of forms I, II, III and VI agreed with those given by Huang (1951a) as forms I–IV. Patterns of forms IV, V, XII and XIII are given in Table 2 (that for form XIII agrees with the

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pattern published by Cleverley & Williams (1959) as form V, but is included here for comparison with form XII, with which it is similar).

DIFFERENTIAL SCANNING CALORIMETRY

For the thermal analysis a Perkin-Elmer DSC-1B apparatus was used. The carrier gas was dry nitrogen at 20 ml/min, and the rate of heating employed throughout was 8°/min. Samples were hermetically sealed in aluminium pans to reduce the loss of material by sublimation, and sample weights varied from 1-10 mg. The temperatures quoted (° Abs.) represent the onset temperatures of transitions and are accurate to ±1°, indium being used for calibration. Melting points so obtained are on average one degree lower than those quoted by Brandstätter-Kuhnert & Aepkers (1962). Thermal analysis was carried out in two ways:

TABLE 2. X-RAY DIFFRACTION PATTERNS OF PHENOBARBITONE FORMS

Form IV		Form V		Form XII		Form XIII	
d(A)	I/Io	d(A)	I/Io	d(A)	I/Io	d(A)	I/Io
15.6	90	16.3	14	15.1	67	15.6	100
11.24	33	13.57	21	11.13	4	7.88	30
9.15	41	12.80	96	8.92	8	6.94	3
7.81	5	11.24	17	7.76	16	6.49	7
6.37	90	6.77	60	7.13	7	5.95	80
5.97	10	6.34	100	6.88	15	5.50	31
5.71	100	6.00	55	6.44	18	5.37	33
5.48	20	5.70	40	5.94	29	5.25	11
5.10	25	5.20	30	5.79	30	4.79	6
4.61	32	4.73	32	5.67	32	4.53	3
4.51	19	4.57	21	5.32	90	4.34	3
4.28	12	4.36	21	5.15	14	4.19	4
4.14	7	4.19	8	4.81	4	3.94	100
3.91	5	4.04	14	4.64	6	3.77	5
3.73	62	3.87	8	4.20	25	3.59	11
3.54	9	3.71	50	3.93	100	3.41	7
3.38	8	3.49	8	3.73	8	3.38	5
3.24	40	3.38	10	3.45	9	3.24	5
3.01	6	3.28	14	3.35	10	3.12	9
2.91	12	3.16	10	3.22	14	3.01	14
2.84	17	2.84	55	3.14	16	2.89	10
		2.70	14	3.07	10	2.81	31
		2.20	17	2.97	8	2.71	4
		2.01	32	2.95	11	2.63	10
				2.79	21	2.60	5
				2.71	4	2.50	4
				2.66	4	2.36	4
				2.63	6	2.24	5
				2.57	6	2.18	3
				2.35	6	1.94	8

Procedure A: a freshly prepared powdered sample was heated at 8°/min from ambient temperature to 10° above the melting point of the most stable form, i.e., 460° Abs.

Procedure B: following procedure A, the sample was held at 460° Abs for 2-3 min, then cooled quickly by means of the manual control on the instrument to 310-350° Abs, maintained at this temperature for 10-15 min and then re-heated as before.

PRODUCTION AND IDENTIFICATION OF DIFFERENT FORMS

Form I was obtained by heating all other forms at temperatures above 150°. A sample which was stored for two months at room temperature reverted to form II.

Form II is the form in which commercial samples are normally obtained, and was produced by heating all forms except I at 130–140° C. It is apparently the most stable form at room temperature. The infrared spectra of forms I and II are very similar, but may be distinguished by their absorptions near 1300 cm^{-1} (Fig. 1).

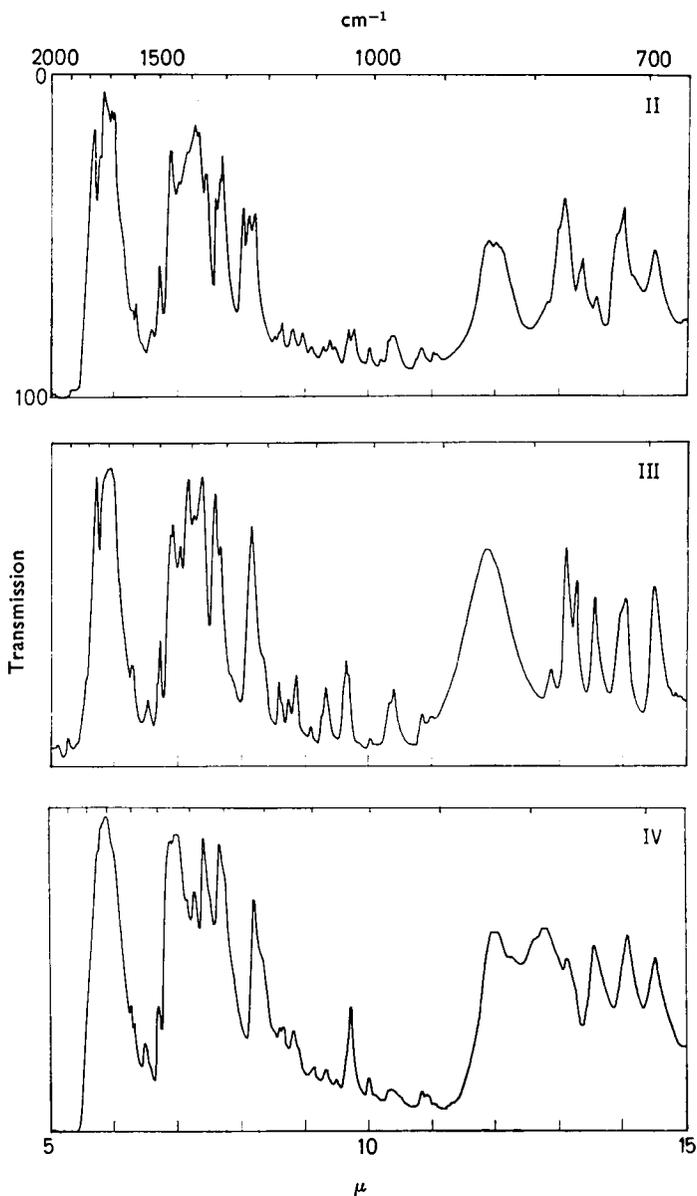


FIG. 2. Infrared spectra of phenobarbitone forms II, III and IV.

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Form III was obtained by heating most other forms at 100–120° C. On heating above 120° C, or on long standing at room temperature it was converted to form II.

Brandstätter-Kuhnert & Aepkers (1961, 1962) reported that at temperatures between 100° and 140° C crystallization from a supercooled melt could produce forms II, IV and VI. The melting point quoted for form IV, 163° C, agrees with that given by Huang (1951b) for his form IVa which he prepared by crystallization between two cover slips. Using this technique we have on several occasions obtained a homogeneous

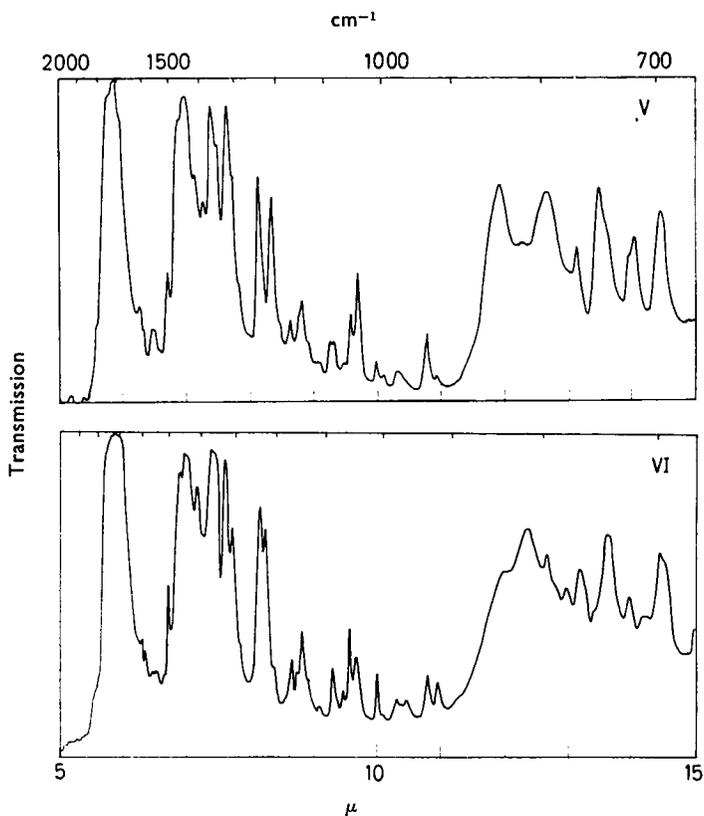


FIG. 3. Infrared spectra of phenobarbitone forms V and VI.

product giving an X-ray diffraction pattern which differs in several respects from that of Huang's form IVa. However, in his work on mixtures of barbiturates, Huang (1951c) includes patterns described as "similar to IVa" which closely resemble our pattern. If the original form IVa pattern is now compared with these, it is seen to include additional lines at 5.87, 5.44, 3.90 and 2.78 Å. These are in fact four of the strongest lines in the pattern given by Cleverley & Williams (1959) for their form V (here designated form XIII): corresponding lines also

occur in the form XII pattern. It therefore seems possible that Huang's form IVa was not a pure single form, and we believe that our product may be identified as Brandstätter-Kuhnert's form IV.

Form V was not obtained in a pure state by Brandstätter-Kuhnert & Aepkers, but was said to be stable in the presence of as little as 2½% of Rutonal. These authors also stated that it was structurally similar to form IV and showed reciprocal seeding properties with this. In the present work a form was frequently obtained which has an infrared spectrum similar to that of form IV, and which was often contaminated

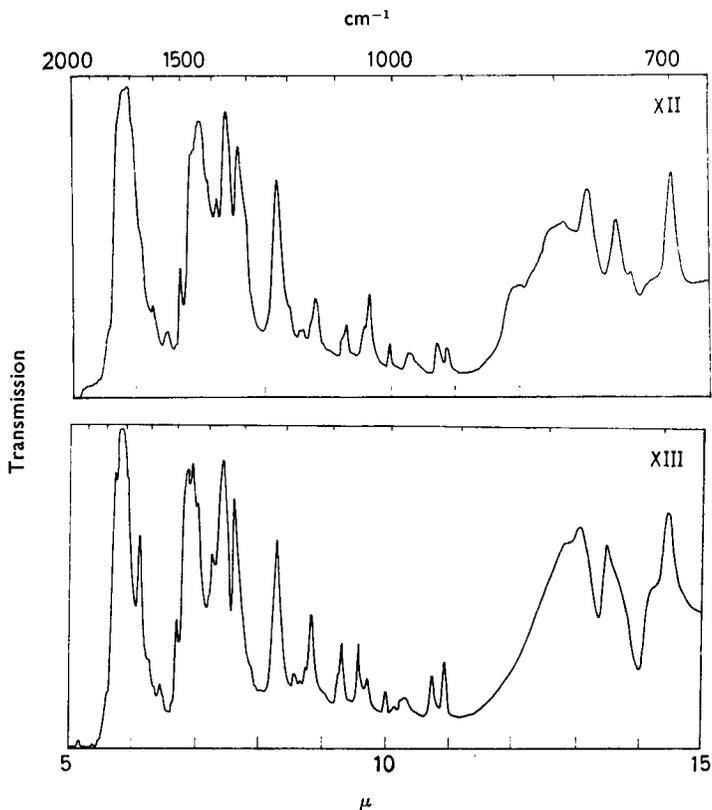


FIG. 4. Infrared spectra of phenobarbitone forms XII and XIII.

with form IV. Ether solutions of either of these forms when evaporated to dryness usually gave a mixture of the two forms. This new form, although obtained several times by crystallization from a melt of pure phenobarbitone, was most often encountered in melts containing other barbiturates, particularly pentobarbitone and amylobarbitone, and there seems little doubt that it is Brandstätter-Kuhnert's form V. The X-ray diffraction pattern shows a general resemblance to that of Huang's form IVb, obtained in the presence of 10% hexobarbitone, and that of a similar form obtained in the presence of 10% barbitone (Huang, 1951c).

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Brandstätter-Kuhnert's form VI has the same melting point (157°) as Huang's form IV, and they are therefore presumably the same. A product was sometimes obtained by crystallization from a melt maintained at about 90° C which gave an X-ray diffraction pattern corresponding to that of Huang's form IV, so this is presumably form VI.

It should be mentioned that no consistent method was found for preparing forms IV, V and VI. Crystallization from melts maintained at temperatures near 100° C, either between two glass cover slips or on a single glass plate, gave variously forms II, III, IV, V and VI and frequently two different forms crystallized simultaneously. Seeding with material previously prepared usually gave a uniform product.

Two other forms of phenobarbitone were not obtained by crystallization from melts, and it seems likely that they are not included in the eleven forms listed by Brandstätter-Kuhnert & Aepkers. The less stable form, corresponding to form V of Cleverley & Williams (1959) is here designated form XIII and was obtained as a precipitate by addition of hydrochloric acid to an alkaline solution of phenobarbitone. It could also be prepared by grinding form II in water, and this suggests that it may be hydrated; this possibility is considered below. It is difficult to remove surface moisture from this form without causing a change to another state. Cleverley & Williams found that the transition to form III began to occur at 40° C, and even at room temperature some change occurs within 24 hr.

Attempts to remove surface moisture from form XIII by storage for a week in a vacuum desiccator over concentrated sulphuric acid or phosphorus pentoxide caused complete conversion to form XII, which also appears to be an enol form, and which has a very similar X-ray diffraction pattern to form XIII. Form XII is also very unstable on heating and at as low a temperature as 60° C was completely converted to either form III or form II in 1 hr. The former is probably the normal behaviour, as the material which gave form II may have contained a trace of this modification which acted as a seed for the remainder.

Some work has been done on mixtures of phenobarbitone with other barbiturates, as a result of which two more forms were identified, apparently corresponding to Brandstätter-Kuhnert's forms IX and X. Form IX was stated (Brandstätter-Kuhnert & Aepkers, 1963) to be isomorphous with several other barbiturates, including amylobarbitone II and the stable form I of cyclobarbitone. In the present work, crystals obtained from a mixed melt containing approximately equal proportions of phenobarbitone and amylobarbitone gave a spectrum similar in certain respects to that of material obtained by seeding a phenobarbitone melt with cyclobarbitone, and both of these were attributed to the presence of form IX. In the latter instance other forms of phenobarbitone were also observed, but the proportion of form IX was quite high even in parts of the crystal mass well separated from the cyclobarbitone seed, and it seems likely that form IX is capable of independent existence in pure phenobarbitone.

Form X is also isomorphous with several other barbiturates, including amylobarbitone I and pentobarbitone I. Mixed melts containing two

parts of phenobarbitone and one part of either amylobarbitone or pentobarbitone were seeded with a crystal of the appropriate barbiturate, giving products with almost identical infrared spectra. These and the X-ray diffraction patterns showed a marked resemblance to those of amylobarbitone I and pentobarbitone I and were presumably mixed crystals of phenobarbitone X with the other barbiturates.

In the course of the work with mixtures of phenobarbitone and cyclobarbitone a product was obtained on two occasions from mixed melts which had an infrared spectrum similar to phenobarbitone IX, yet clearly distinguishable from this. The X-ray diffraction pattern of this mixture was generally similar to those of forms IX and X, and also to the two patterns given by Huang (1951c) for phenobarbitone-cyclobarbitone mixtures. Huang attributed these to a molecular compound which he

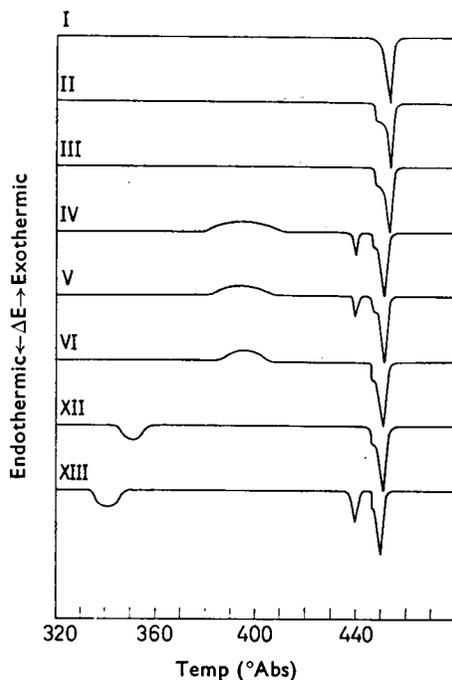


FIG. 5. Differential thermograms of eight forms of phenobarbitone (Procedure A).

thought might exist in two polymorphic modifications. Brandstätter-Kuhnert & Aepkers (1962) subsequently showed that an equimolecular mixture of these two substances could exist as either (a) a molecular compound between phenobarbitone IX and cyclobarbitone I—this molecular compound was isomorphous with phenobarbitone V and could be obtained by seeding a mixed melt with phenobarbitone IV; or (b) mixed crystals containing phenobarbitone X and the unstable cyclobarbitone II. The product obtained in the present work was indeed produced on one occasion by seeding with phenobarbitone IV, and its

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infrared spectrum showed clear differences from phenobarbitone X, so it may be assumed that this was in fact the molecular compound.

DIFFERENTIAL SCANNING CALORIMETRY

Procedure A. The thermograms of eight forms of phenobarbitone are shown in Fig. 5. Thermal analysis shows that all forms absorb approximately the same amount of energy on melting, the final transition corresponding to the melting of form I at 448° Abs.

Forms I and II show sharp melting (endothermic) transitions at 448° Abs and no other transitions are discernible. The other forms investigated all show one or two small, additional endothermic transitions at 440° and 446° Abs, corresponding to the melting of forms III and II respectively. In addition, the two enol forms, XII and XIII, show a large, broad endothermic transition in the region 330–350° Abs, the significance of which is discussed below. Forms IV, V and VI, all of which were prepared by crystallization from melts, show a small, broad (exothermic) transition in the region 390–400° Abs. These transitions are possibly due to crystallization of residual super-cooled liquid occluded in the crystals.

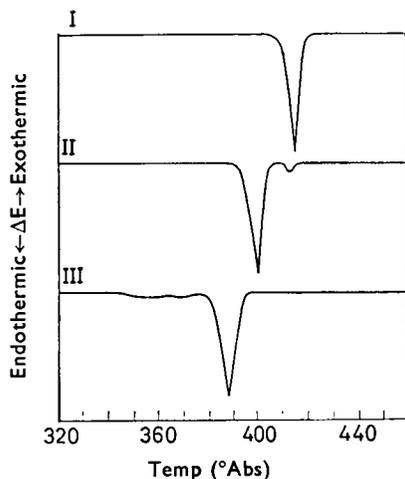


Fig. 6. Differential thermograms of three forms of thialbarbitone (Procedure A).

From Fig. 5 it is apparent that samples of, for example, forms IV, V and XIII, the homogeneity of which has been demonstrated by infrared spectroscopy, can produce transitions corresponding to forms III, II and I, and this sequence of polymorphic changes is thus confirmed. No information is given, however, regarding the possible transitions between the less stable modifications.

Thermograms are shown in Fig. 6 for the three forms of thialbarbitone. When heated in sealed pans, these give rise to distinctive thermograms, the melting transitions of the three forms being readily discernible at

approximately 378°, 393°, and 407° Abs (a commercial sample of thialbarbitone form II, when heated in a capillary tube, showed partial melting at 124° C and melted completely at 138° C). In addition a very broad endothermic transition is observed in form III commencing at about 345° Abs. As this form apparently has the enol structure (Cleverley & Williams, 1959) there is thus an analogy with the transitions in this region in the two enol forms of phenobarbitone. When thialbarbitone form III was heated in a pan which was not completely sealed, the transition at 378° Abs was not observed and the resulting thermogram was barely distinguishable from that of form II.

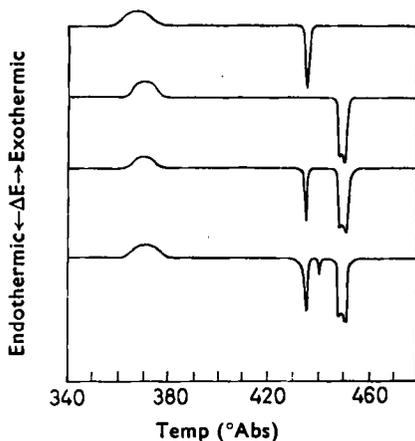


Fig. 7. Typical differential thermograms obtained from supercooled melts of phenobarbitone (Procedure B).

Procedure B. By this procedure, melted samples were cooled rapidly to 310–350° Abs (samples removed at this stage were found to be supercooled melts; when scratched they all gave form III) and then re-heated at 8°/min. The resulting thermograms showed considerable variation, typical results being shown in Fig. 7. These differ from all thermograms obtained by procedure A in two respects: firstly there is a broad exothermic transition of variable geometry in the region 360–390° Abs, which represents crystallization of the super-cooled liquid; and secondly there is a strong endothermic transition at 435° Abs in most of the thermograms. This temperature corresponds to the melting of form IV, and the presence of this was confirmed by removing some samples at about 400° Abs, after the appearance of the crystallization peak, and determining their infrared spectra. Three such samples were found to be form IV, whilst on a fourth occasion form VI was obtained.

It is significant that when powdered form IV was examined by procedure A, the transition at 435° Abs was not observed. The transition from form IV to form III, which takes place rapidly in the finely divided powder, is obviously retarded in the compact mass obtained by crystallization from the melt to such an extent that some of the form IV remains

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unchanged up to its melting point. The thermograms of the various forms of phenobarbitone as determined by procedure A, although showing certain fairly consistent features, are thus not independent of particle size, so that any attempt to identify the different forms by this means alone should be treated with caution.

Discussion

Of the many crystalline forms of phenobarbitone, two stand out from the remainder in their infrared spectra and differential thermograms, and also in their method of preparation, namely forms XII and XIII. The infrared spectra are characterized by the presence of absorptions near 3500 cm^{-1} , apparently due to OH groups, and the absence of a strong band near 840 cm^{-1} , assigned by Cleverley & Williams (1959) to the N-H out-of-plane bending vibration. These characteristics are also shown by thialbarbitone form III, and there is little doubt that they are due to enolization. Form XII of phenobarbitone differs from form XIII and from thialbarbitone form III in the absence of the peak near 1620 cm^{-1} , which could reasonably be assigned to a conjugated C=N group in the enolized structure. Also in this form the O-H stretching absorptions are relatively weaker than the N-H stretching bands, suggesting that it has less enolic character than form XIII. It seems unlikely that the two forms can be represented simply as mono- and di-enols, since the C=O stretching absorption in form XIII still appears to represent more than one carbonyl group. However, the presence of small, non-stoichiometric amounts of keto-groups in a predominantly enol structure, and vice versa, has already been suggested for certain crystalline forms of oestradiol (Smakula, Gori & Wotiz, 1957), ethinyloestradiol and spironolactone (Mesley, 1966).

An alternative explanation of the 1620 cm^{-1} band and of the stronger OH absorptions in form XIII could be the presence of water of crystallization. This would be quite possible, since form XIII is only obtained in the presence of water, whilst the transition to form XII takes place under conditions conducive to dehydration. On the other hand, the 1620 cm^{-1} band in thialbarbitone form III is not due to water, since this form has been obtained from a melt, and the thermogram for phenobarbitone form XIII is not apparently consistent with loss of water. The strong endothermic transition at about 340°C shows a superficial resemblance to those obtained by Carless, Moustafa & Rapson (1966) for the hydrated forms of cortisone acetate. However, in the latter, the transitions were observed at temperatures above the boiling point of water, whereas in the case of phenobarbitone the transition is completed well below 100°C , with no further change occurring before the melting of form III. There is a further analogy with the work of Carless, Moustafa & Rapson in that they reported that the anhydrous form III of cortisone acetate could only be prepared in the presence of water.

It must therefore be concluded that the low-temperature transitions in phenobarbitone forms XII and XIII and thialbarbitone form III are

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primarily associated with the enol-keto tautomeric change, though it is possible that a trace of water is necessary for the existence of form XIII. The greater energy absorption in form XIII relative to form XII would then be in accordance with the previous suggestion that it has a greater degree of enol character than form XII. The only other transitions observed in the thermal analysis of the various forms are those corresponding to melting, so it would appear that transitions involving only lattice re-arrangements are not detectable by this technique.

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