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Infrared identification of barbiturates with particular reference to the occurrence of polymorphism

R. J. MESLEY AND R. L. CLEMENTS

Infrared spectra of twelve substituted barbituric acids, in a total of 34 polymorphic forms, have been compared. Comparison of a sample spectrum with that of an authentic specimen provides a reliable means of identification, provided that both are in the same crystalline form. To ensure consistent production of a single form a specific treatment is recommended for each substance exhibiting polymorphism.

IN a number of monographs in the British Pharmacopoeia and the British Pharmaceutical Codex an identification test is included in which the infrared absorption spectrum of the sample under examination is compared with that of an Authentic Specimen, supplied for this purpose. It is a necessary condition for this comparison that the two specimens should be in the same physical state. Where it is possible to record the spectra of the substances in solution this presents no difficulty, but if solid-state spectra are used polymorphism may be encountered, and it may then be necessary to specify treatments for individual substances to ensure the production of consistent spectra, as in such compounds as steroids (Mesley & Johnson, 1965) and sulphonamides (Mesley & Houghton, 1967).

The substituted barbituric acids are a class of compounds to which an infrared identification procedure is particularly suited, but they are also notorious for their polymorphism.

Infrared spectra of barbiturates, examined in the form of potassium bromide discs, were recommended for identification purposes by Manning & O'Brien (1958), no allowance being made for polymorphism; however, several of their spectra refer to salts, although described as the corresponding barbituric acids. Infrared spectra of derivatives have also been used, including copper-pyridine complexes (Levi & Hubley, 1956), *p*-nitrobenzoyl derivatives (Chatten & Levi, 1957), and dixanthyl derivatives (Flann & Cloutier, 1967). The effects of polymorphism on infrared spectra of barbiturates were noted by Cleverley & Williams (1959b) and by Paulig, Gansau & others (1963). Goenechea (1966) tabulated frequencies for N–H, C–H and C=O stretching absorptions of 21 barbiturates and suggested that these were suitable for the identification of the individual substances; he also noted that the greatest differences between the spectra of polymorphic modifications were found in the N–H and C–H stretching absorptions, and commented that for analytical purposes consideration must be given to the method of purification. The C=O stretching absorptions were used by Bouché, Coclers & Delahaut (1966) for the quantitative estimation of individual barbiturates, examined as potassium bromide discs; for this purpose the sample preparation procedure was rigorously controlled to ensure that the same crystalline form was reproducibly obtained.

From the Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1, England.

Spectra recorded in chloroform solution have been used (Umberger & Adams, 1952) but, owing to the limited solubility of many of the barbituric acids, the spectra obtained were of low intensity and not always clearly distinguishable from each other. In an attempt to overcome the effects of polymorphism on solid phase spectra, Cleverley (1960) used a technique in which the substance, incorporated into a potassium bromide disc, was heated in an oven at about 10° above its melting point, then cooled and the spectrum recorded. This method suffers from the disadvantages that each compound has to be heated to a different temperature (melting points quoted by Cleverley range between 86° and 229°), and that many of the spectra obtained correspond to the amorphous forms of the substances, which again are not always distinguishable from each other (e.g. amylobarbitone and butobarbitone).

In order to make use of the more characteristic spectra given by the crystalline solids, as many forms as possible were prepared from each substance and methods devised for converting them to a common form with a reproducible spectrum. For this purpose only the free acids were considered; if samples are obtained in the salt form they may be examined as such, but if spectral differences between the sample and the authentic specimen are observed then both should be converted to the corresponding acid and re-examined.

Experimental

MATERIALS

Most of the samples used were B.P. Authentic Specimens. In some instances commercial products were also used, after checking that their infrared spectra were identical with those of the appropriate Authentic Specimens. Samples of methohexitone, methylphenobarbitone and thialbarbitone were supplied by Mr. C. A. Johnson of the British Pharmacopoeia Commission. Solvents used were of B.P. or A.R. quality.

PRODUCTION OF DIFFERENT POLYMORPHS

The production of polymorphic forms by solvent treatments has previously been investigated for most of the substances concerned by Cleverley & Williams (1959b). Polymorphs obtained by microsublimation (Huang, 1951a,b,c) and by crystallization from melts (Brandstätter-Kuhnert & Aepkers, 1961, 1962) have also been described. The present work was therefore restricted to the preparation and interconversion of the various forms already reported.

INFRARED ABSORPTION SPECTRA

Samples were prepared for infrared examination both as mulls in liquid paraffin (Nujol) and as pressed potassium bromide discs using the technique previously described (Mesley & Johnson, 1965). Spectra were recorded using Grubb Parsons GS 2 and Perkin-Elmer 237 grating spectrometers.

Results

The barbiturates examined were those which are currently included in the British Pharmacopoeia and British Pharmaceutical Codex. These are listed in Table 1, which shows the number of crystalline forms encountered. The individual substances are considered below.

TABLE 1. INCIDENCE OF POLYMORPHISM IN THE BARBITURIC ACIDS EXAMINED

Substance	Substituents	No. of crystalline forms encountered
Amylobarbitone	5-ethyl-5-isoamyl	2
Barbitone	5,5-diethyl	4*
Butobarbitone	5-butyl-5-ethyl	3*
Cyclobarbitone	5-cyclohex-1′-enyl-5-ethyl	1*
Methohexitone	5-allyl-1-methyl-5-(1-methylpent-2-ynyl)	1
Methylphenobarbitone	5-ethyl-1-methyl-5-phenyl	1
Nealbarbitone	5-allyl-5-neopentyl	2
Pentobarbitone	5-ethyl-5-(1-methylbutyl)	4*
Phenobarbitone	5-ethyl-5-phenyl	10*
Quinalbarbitone	5-allyl-5-(1-methylbutyl)	1
Thialbarbitone	5-allyl-5-cyclohex-1′-enylthiobarbituric acid	3
Thiopentone	5-ethyl-5-(1-methylbutyl)thiobarbituric acid	2

* Additional forms have been reported elsewhere.

Amylobarbitone. Two forms were distinguished by Cleverley & Williams (1959b) and by Brandstätter-Kuhnert & Aepkers (1962). The recrystallization procedure prescribed in the British Pharmacopoeia consistently gives form II.

Barbitone. Huang (1951b) reported X-ray diffraction patterns of four distinct forms. Cleverley & Williams (1959b) published infrared spectra of forms I and II, and stated that the spectrum of form IV was identical to that of form II. In the present work all four of Huang's forms were obtained, and all were distinguishable by means of their infrared spectra; in particular, the spectrum of form IV was found to be quite different from that of form II. In addition to these four forms, Huang (1951c) has reported a fifth form present in mixed crystals with cyclobarbitone, and Brandstätter-Kuhnert & Aepkers (1962) have detected two such additional forms in barbiturate mixtures.

The procedure recommended below yields form II, which was found to give a consistent spectrum from either a Nujol mull or a potassium bromide disc, though Cleverley & Williams (1959a) have reported that this form is unstable on grinding.

Butobarbitone. Cleverley & Williams (1959b) described three forms and on occasions found infrared and X-ray evidence of a fourth form. In the present work only forms I–III were encountered. The recommended procedure gives form I, which should be examined as a Nujol mull, as this form may give a different spectrum after grinding with potassium bromide (Cleverley & Williams, 1959a).

Cyclobarbitone. It appears that only one crystalline form can be obtained from pure cyclobarbitone, though Brandstätter-Kuhnert & Aepkers (1962) have reported a second form in mixed crystals with phenobarbitone. Nevertheless, published infrared spectra and X-ray diffraction patterns show some variation. The spectrum published by

Manning & O'Brien (1958) is in fact that of the calcium salt, and other spectra, particularly when recorded from potassium bromide discs, probably show some evidence of amorphous material. Two portions of material recovered from different solvents showed slight spectral differences, but they both gave the same X-ray pattern, which corresponded to that of Huang (1951a); rather different patterns have been published by Penprase & Biles (1956) and by Williams (1959).

Methohexitone. No evidence of polymorphism was detected.

Methylphenobarbitone. No evidence of polymorphism was detected.

Nealbarbitone. This substance has not been investigated by previous workers. Two forms were encountered, one of which tended to contain residual solvent when recovered from chloroform solution. The recommended procedure gives a form with a consistent spectrum, whether recorded as a Nujol mull or as a potassium bromide disc.

Pentobarbitone. At least five forms of pentobarbitone have been reported. Cleverley & Williams (1959b) described four forms, of which I–III were said to have identical infrared spectra and closely similar X-ray diffraction patterns. Of these the pattern of form II was found to correspond to that already published by Huang & Jerslev (1951). Subsequently Jerslev & Ravn-Jonsen (1960) asserted that this latter pattern was not characteristic of pure pentobarbitone, as it corresponded to mixed crystals obtained from a melt containing about 15% of the isomeric 5-ethyl-5-(1-ethylpropyl)barbituric acid, whereas pure pentobarbitone crystallized as form I. This assertion may not be wholly justified since (*a*) the material of Huang & Jerslev was obtained by precipitation and not by crystallization from a melt, and (*b*) the published pattern in fact corresponds to a polymorphic form prepared from pure pentobarbitone by Cleverley & Williams. Jerslev & Ravn-Jonsen also gave X-ray data for forms I and II (corresponding to forms I and IV of Cleverley & Williams) and for a form IIb, which was obtained as a commercial sample and does not correspond to any of the four forms of Cleverley & Williams. Brandstätter-Kuhnert & Aepkers (1962) have reported the existence of three forms, but from the melting points alone it is not possible to correlate these with the forms reported elsewhere.

In the present work the four forms described by Cleverley & Williams were encountered and their infrared spectra recorded. Forms I–III certainly give similar spectra, and it is doubtful whether forms II and III can be distinguished, but consistent small differences were observed in the spectrum of form I. The recommended procedure normally gives form I, but occasionally some form II may be present; however the presence of a small amount of form II produces no significant change in the spectrum.

Phenobarbitone. Brandstätter-Kuhnert & Aepkers (1961, 1962) decribed eleven forms, some of which were found only in mixed crystals with other barbiturates. In the present work ten forms were obtained, including two not mentioned by Brandstätter-Kuhnert & Aepkers, and the individual forms have already been described (Mesley, Clements, Flaherty & Goodhead, 1968). The consistent conversion of all of these

to a single form presents some difficulty. No solvent treatment was found to be universally applicable, although recrystallization from aqueous ethanol usually gave form II. The only satisfactory technique was to heat the substance to such a temperature that only form I could remain, precautions being taken to prevent loss of the sample by sublimation.

Quinalbarbitone. Attempts to recover quinalbarbitone from preparations, in which it is normally present as the sodium salt, or from solutions in organic solvents, normally give a viscous oil which crystallizes only on long standing (usually 1–2 weeks). Oils from three different treatments all gave the same crystalline form, the infrared spectrum of which agreed with those published by Levi & Hubley (1956) and Chatten & Levi (1957), whilst the X-ray diffraction pattern agreed with that of Williams (1959). The spectrum published by Manning & O'Brien (1958) is quite different and refers to the sodium salt, but possible evidence of a second crystalline form is provided by a different X-ray pattern included in the Powder Diffraction File (No. 9–520) and also obtained by Penprase & Biles (1956).

Thialbarbitone. Cleverley & Williams (1959b) described three forms, and concluded from its infrared spectrum that form III was an enol form. The procedure given in the British Pharmaceutical Codex for the precipitation of thialbarbitone from a solution of its sodium salt initially yields form III, which is converted to form II on heating at 100°; the latter form, however, is much more soluble than the precipitated form III and may therefore dissolve in any water still present, so that a period of preliminary drying at 60° is necessary. A quicker procedure, given below as an alternative, yields form III.

Thiopentone. Cleverley & Williams (1959b) described two crystalline forms, but stated that their infrared spectra are identical. This has been confirmed, and no treatment is therefore recommended.

Discussion

Comparing the spectra of the 34 polymorphic modifications encountered, it is apparent that, although different forms of the same substance may not always be distinguishable from each other, there is no difficulty in distinguishing them from any of the other barbiturates examined. Because of their similarity, however, it is not always possible to detect the presence of a second barbiturate in a mixture, and this is particularly the case where the two substances may have forms which are isomorphous with each other, or where molecular compounds can exist. Spectra may then be obtained which do not correspond to the stable forms of either component of the mixture.

In general, however, when comparing a barbiturate sample with an authentic specimen of the same substance, in most instances it will be found that they give the same spectrum. If they do not, then the possibility of polymorphism must be considered. If both samples are subjected to the appropriate treatment indicated below, they should yield the

same crystalline form. These treatments were effective for all the forms encountered in this work, and although other forms may exist it is probable that the treatments would be effective for them as well. Since the publication of the earlier reports on steroids (Mesley & Johnson, 1965) and sulphonamides (Mesley & Houghton, 1967) several new polymorphic forms have been encountered, but all of these have proved amenable to the treatments previously recommended.

RECOMMENDED PROCEDURES

Amylobarbitone. Recrystallize from ethanol (25% v/v).

Barbitone. Dissolve in chloroform, evaporate the solution to dryness at room temperature in a current of air.

Butobarbitone. Dissolve in ether, evaporate solution to dryness on water bath, leave on water bath until material crystallizes.

Cyclobarbitone. No recommendation necessary.

Methohexitone. No recommendation necessary.

Methylphenobarbitone. No recommendation necessary.

Nealbarbitone. Recrystallize from ethanol (25% v/v).

Pentobarbitone. Recrystallize from ethanol (25% v/v).

Phenobarbitone. Heat in covered vessel for 1 hr at 150° or overnight at 140°.

Quinalbarbitone. If material is not crystalline, either (*a*) examine as chloroform solution, or (*b*) dissolve in the minimum of chloroform, place a drop or two on a rock salt plate, heat to 100° to remove solvent, place second heated plate on top of sample, allow to cool and record spectrum of resulting film.

Thialbarbitone. (*a*) Dissolve in minimum of dilute sodium hydroxide solution, acidify by dropwise addition of 0·1N hydrochloric acid until no further precipitation occurs, allow to stand until precipitate coagulates, filter, wash precipitate with water and dry for 2 hr at 60°, then for 2 hr at 100°, or (*b*) dissolve in ethanol, evaporate to dryness on water bath, allow to cool, add sufficient ethanol (25% v/v) to cover the glassy product, allow to crystallize, decant off the liquid and dry in a current of air.

Thiopentone. No recommendation necessary.

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