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Thermal stability of bropirimine

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Summary

A differential scanning calorimetric study of bropirimine reveals that the thermal stability is decreased upon admixture with polyethylene glycol and other excipients added to enhance solubility profiles. The degradation involves the substitution of the bromine atom in the molecule to generate 2-amino-6-phenylpyrimidin-4(3H)-one. The reaction occurs with other 5-bromo and 5-iodo pyrimidinones but not with 5-fluorouracil or its chloro analogue. Evidence is presented to suggest that the instability is due to an increasing proportion of the halogeno compound in the liquid state rather than a direct chemical involvement of the additive in the reaction.

Introduction

The derivative 5-bromo-2,3-dihydro-2-imino-6-phenyl-4(1H)-pyrimidinone (ABPP, bropirimine, 2-amino-5-bromo-6-phenylpyrimidin-4(3H)-one; I) possesses anti-viral and anti-tumour activity together with a novel immunomodulatory action causing enhancement of serum interferon levels. The 6-phenyl series was introduced to overcome the toxicity due to crystal deposition in the renal papillae of rats during chronic dosage observed during the testing of the 6-methyl analogues (Nelson and Grassi, 1980; Skulnik et al., 1985). The compound is poorly soluble in many solvents and formulation for intravenous delivery requires a combination of pH control and cosolvent optimisation (Alpar et al., 1986). Significant improvements in solubility and bio-availability have also been reported for many poorly-soluble drugs when converted into solid dispersions (Ford, 1986). To further investigate the optimum formulation of bropirimine and analogues the role of additives has been examined in an attempt to form mixtures or solid dispersions with enhanced solubility profiles. Thermal analysis has revealed that the presence of additives in such mixtures destabilises the bropirimine, although the decomposition temperatures are still above 200 °C and the interactions should not cause any formulation or manufacture problems.

Experimental

Apparatus

Differential scanning calorimetry was undertaken with a Perkin-Elmer DSC-4 instrument using

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the Thermal Analysis Data Station (TADS) for data collection, handling and presentation. ^{1}H-NMR spectra were recorded in dimethyl sulphoxide-d_6 solution, with tetramethylsilane as internal standard at 360 MHz by means of a Bruker Spectrospin spectrometer. Mass spectra were obtained with a VG Micromass MM12 mass spectrometer using a direct insertion probe with an inlet temperature of 250 °C, an ionisation energy of 70 eV, an accelerating voltage of 3 kV and a trap current of 100 μA.

HPLC analyses were undertaken using a system constructed from an Altex 100A dual-piston reciprocating solvent-metering pump and a reversed-phase stainless steel Shandon-type column (10 cm × 4.6 mm i.d.) packed with Hypersil-ODS (5 μm) stationary phase. Samples were introduced by means of a Rheodyne 7125 injection valve, fitted with a 20 μl loop, and detection was accomplished with a Pye LC3 variable wavelength UV detector, fitted with an 8 μl flow cell, and operated at a wavelength of 305 nm with a sensitivity of 0.08–0.16 AUFS. The mobile phases consisted of: (i) aqueous acetonitrile (20%), adjusted to pH = 2.0 with phosphoric acid, containing diethylamine (0.1%) as moderator; or (ii) 20% aqueous methanol, containing diethylamine (0.1%) and adjusted to pH = 2.5 with phosphoric acid and were delivered at a flow rate of 1 ml · min^{-1}. Respective retention times for bropirimine and 2-amino-6-phenylpyrimidin-4(3H)-one, the debrominated analogue, in these phases were: (i) 5.8 and 2.7 min; and (ii) 20.0 and 6.5 min.

Methods

Physical mixtures of drug and additive were prepared by trituration on a glass tile or by ball milling, by evaporation from methanolic solution or by melting at 90 °C. Samples for dissolution were accurately weighed (200 mg) and compressed under vacuum at a pressure of 8000 kg for 2 min. The compressed discs were stirred at 150 rpm while suspended in one litre of distilled water at 37 °C. The dissolution medium was continuously monitored by UV spectroscopy at 305 nm with a sensitivity of 0.2 AUFS. Samples for thermal analysis were accurately weighed (1–4 mg) into an aluminium pan, covered with an aluminium lid and crimped into position. The pan was placed in the DSC oven together with a blank, prepared in exactly the same way but without the sample. The sample and blank were continuously purged with nitrogen gas at a flow rate of 25 cm^3 · min^{-1} (1.4 kg · cm^{-2}) and thermograms were recorded over a temperature range of 40–350 °C with a programmed heating rate of 10 °C · min^{-1}. Temperature calibration was made with an indium standard (onset temperature 156.6 °C).

Results and Discussion

Mixtures of bropirimine with polyethylene glycol (PEG, Macrogol, Carbowax; 20M) were prepared either by dry mixing, by evaporation or by melting. Typical dissolution profiles are displayed in Fig. 1 which shows the enhanced rates of dissolution obtained from the mixed systems. Thermal analysis (Hardy, 1982; Pope and Judd, 1977) of the mixtures, using differential scanning calorimetry (DSC), showed that the resulting thermograms differed little between samples prepared by any of these methods but differed significantly from that of the pure bropirimine. Fig. 2 records the thermogram of bropirimine and reveals an endotherm with an onset temperature near 289 °C. This develops into a peak showing irregular variations which are characteristic of a thermal degradation reaction. The exact temperature range

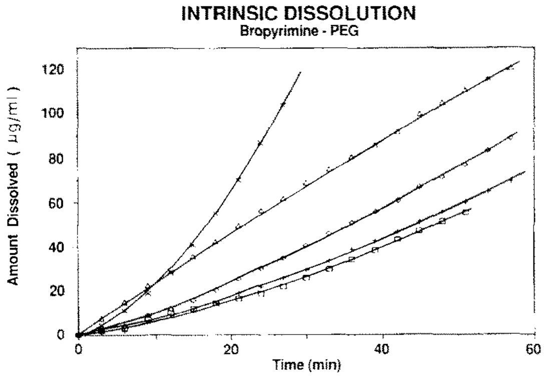


Fig. 1. Dissolution profiles of compressed discs of bropirimine–PEG 20M mixtures into distilled water at 37 °C. □, 10% PEG; +, 20% PEG; ◇, 30% PEG; △, 40% PEG; ×, 60% PEG.

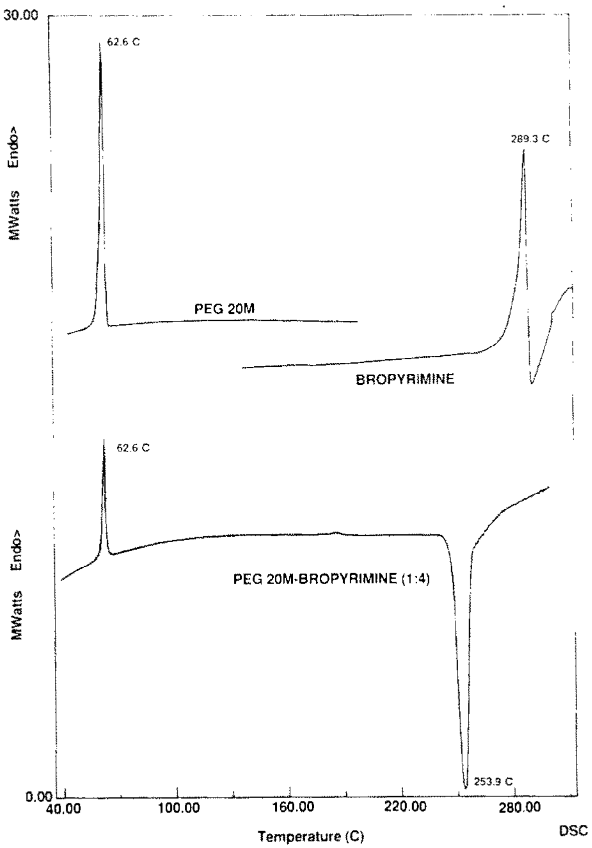


Fig. 2. Thermograms from differential scanning calorimetry of PEG 20M, bropirimine and a 4 : 1 physical mixture.

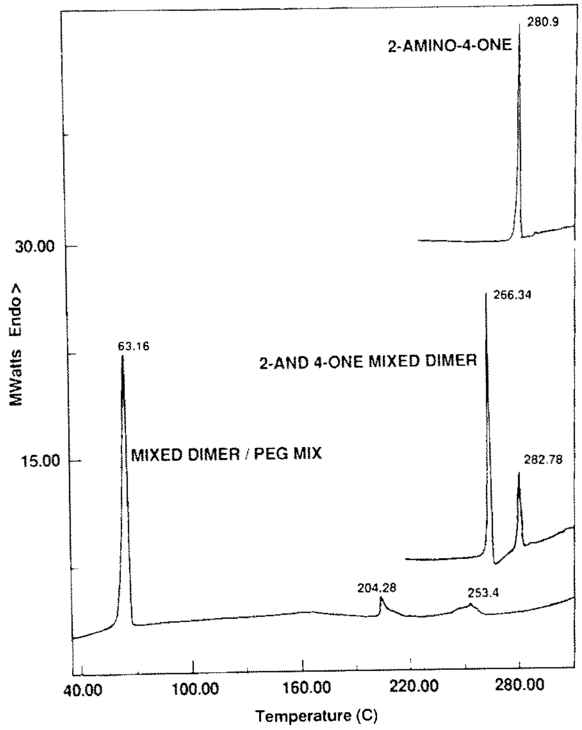


Fig. 3. DSC trace of the co-crystallised mixture of 2-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-4(3H)-one alone, co-crystallised with the corresponding 4-aminopyrimidin-2(1H)-one and this dimer in admixture with PEG 20M.

over which the transformation occurs varies somewhat with the source of the sample, the sample size and the heating rate employed but all show similar profiles. Fig. 2 also shows the DSC trace for PEG 20M, with the onset temperature of the single endotherm representing melting showing near to 63° C, and a thermogram from a mixture of bropirimine and PEG 20M. It can be seen that the PEG endotherm is still present in the mixture but that corresponding to the melting of the bropirimine has been lost and in its place a new, exothermic transition is now observed. This exothermic event, occurring with an onset temperature in the 230–250° C range, is largely independent of the ratio of PEG and bropirimine and could perhaps be due to dissolution of the pyrimidinone in the PEG melt, to a crystallisation phenomenon or to degradation. To test these possibilities the DSC curves of some related compounds, both alone and in admixture, were determined.

The acid hydrolysis of pyrimethamine under controlled conditions gives rise to a mixture of 2-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-4(3H)-one and the corresponding 4-aminopyrimidin-2(1H)-one. These compounds co-crystallise as a paired dimer showing strong association of the Watson-Crick type (Griffin et al., 1985). This interaction is analogous to that observed in bropirimine which also forms strong associations in the crystal (Schwalbe and Bryant, unpublished data) as a result of strong intermolecular hydrogen bonds. To assess the role of this association on the appearance of the thermogram, the DSC curve of the co-crystallised mixture of these two components was determined. This is displayed in Fig. 3 together with the effect of added PEG 20M. Although a rather complex thermal profile is ob-

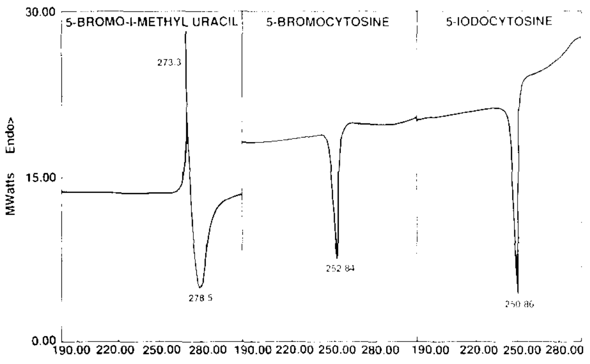


Fig. 4. DSC thermograms of: 5-bromo-1-methyluracil; 5-bromocytosine; and 5-iodocytosine.

tained there is no indication that any exothermic events, characteristic of the bropirimine–PEG interaction, are initiated upon admixture. Indeed, the major effect appears to be a large reduction in the intensity of the endotherms, perhaps due to progressive dissolution of the pyrimidines in the melt. Analogous compounds, including uracil, pyrimethamine and its diacetyl derivative, gave similar thermograms presenting only endotherms alone or in admixture.

When pyrimidine derivatives containing bromine at the 5-position were subjected to DSC analysis it was immediately apparent that it was this substitution which was the cause of the anomalous thermal behaviour of the mixtures, although the exact behaviour of bropirimine was not duplicated. In the case of 5-bromo-1-methyluracil a sharp endotherm (273.3 °C) was immediately followed by an endothermic transition (278.5 °C) while with 5-bromocytosine only a strong endotherm (252.8 °C) was recorded (Fig. 4). A similar curve, with a minimum at 250.9 °C, was obtained from the 5-iodo analogue. It seems plausible to suggest, therefore, that the endotherm in the bropirimine mixtures is due to degradation caused by the incorporation of a labile C–Br bond in the molecule. Average bond dissociation energies for =C–H and =C–Br bonds in aromatic compounds are quoted as 460 and 335 $\mathrm{kJ \cdot mol^{-1}}$ (Kerr and Trotman-Dickenson, 1978). In an attempt to confirm this and to identify the degradation product(s) a 4 : 1 bropirimine–PEG mixture was heated in a DSC pan until the exotherm was just completed. The mixture, showing signs of a white sublimate, was extracted from the pan and was analysed by chromatography, by mass spectrometry and by ^{1}H-NMR spectroscopy. HPLC analysis, using two mobile phases, showed the presence of one major component only which, in both systems, co-eluted with 2-amino-6-phenylpyrimidin-4(3H)-one (II); the product derived from bropirimine by substitution of hydrogen for the bromine atom (Fig. 5). ^{1}H-NMR spectroscopy further supported this rationale with the presence of an absorption band at δ 6.11 ppm due to the new proton at the 5-position of the pyrimidine ring. Final confirmation of this assignment was provided by mass spectrometry which revealed a molecular ion at m/z 187 together with the absence of the ^{79}Br : ^{81}Br doub-

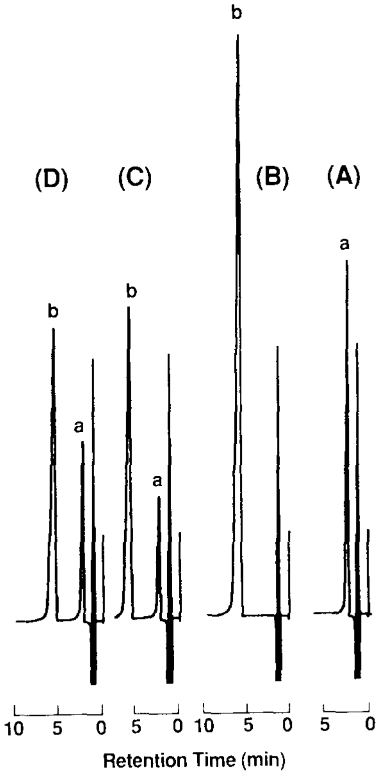
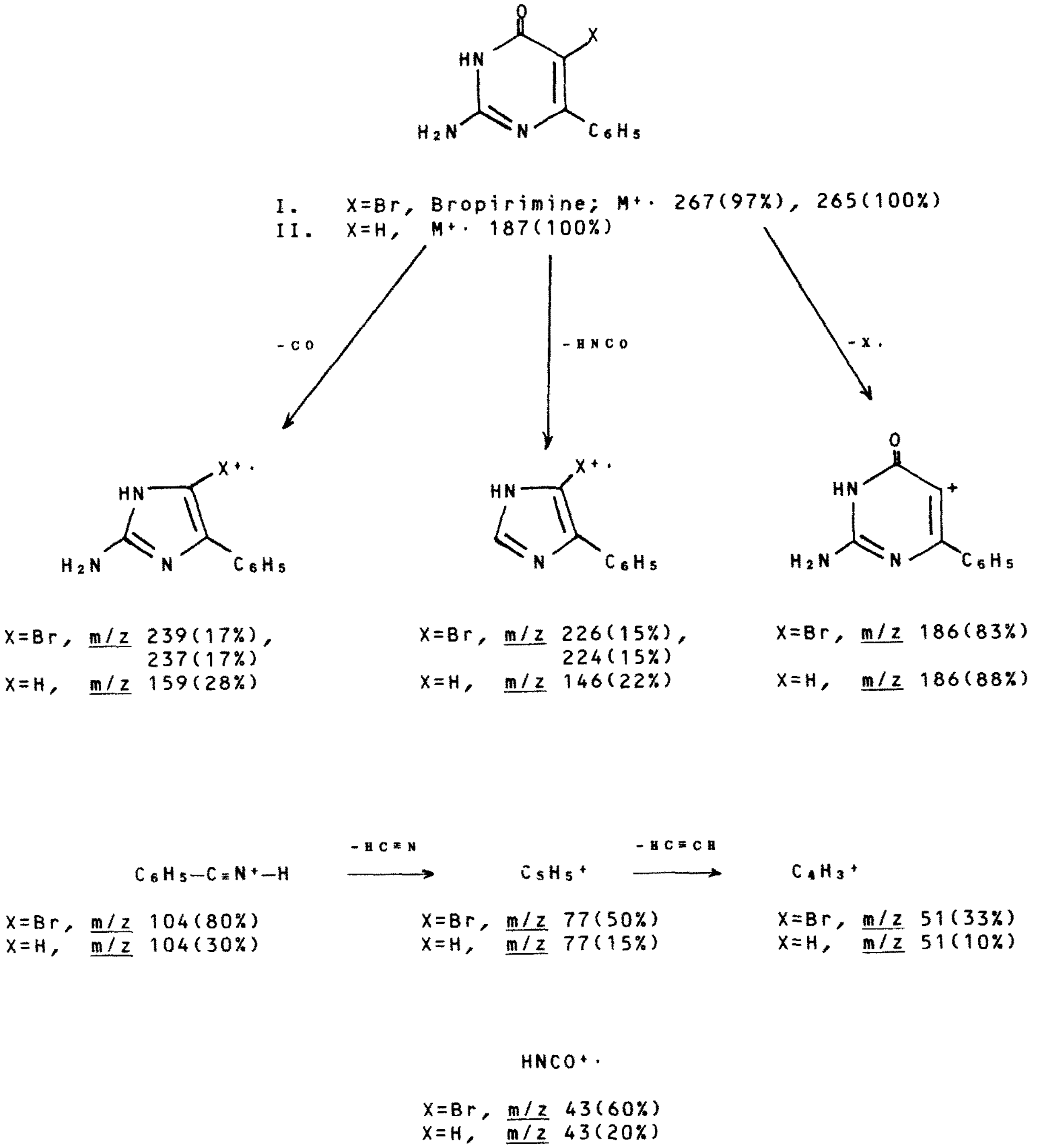


Fig. 5. HPLC trace showing: (A) 2-amino-6-phenylpyrimidin-4(3H)-one (a); (B) bropirimine (b); (C) a mixture of each obtained from partial thermal degradation of 5 mg bropirimine-PEG 20M mixture (10 : 1) at 210 °C for 5 min and dissolved in methanol (25 cm^3); and (D) a standard mixture of each component.



Scheme 1. Mass spectral assignments of bropirimine (I) and 2-amino-6-phenylpyrimidin-4(3H)-one (II), its debrominated product.

lets characteristic of the bromine-containing ions in the starting material (Scheme 1). 2-Amino-5-iodo-6-phenylpyrimidin-4(3H)-one (AIPP), the iodo analogue of bropirimine, reveals similar behaviour. Alone it shows an endotherm (285.6 °C) immediately followed by an exotherm whereas, on admixture with PEG 20M, both transitions are replaced by a single exotherm (257.6 °C) due to deiodination.

No trace of products incorporating oxyethylene chains, expected if reaction involved nucleophilic displacement of bromide by PEG hydroxyl groups, was detected. It is possible that thermal dissociation of the C-Br bond is followed by a hydrogen abstraction reaction involving the molten PEG 20M. The generality of this interaction may be demonstrated with the range of 5-halogenouracils. DSC analysis of these compounds shows that the fluoro, chloro and bromo compounds are characterised by a single endothermic transition (Table 1). This endotherm also begins to develop in the 5-iodo analogue but degradation overtakes melting and a strong exotherm immediately follows the initial transition. The DSC curves of these compounds have been reported previously during studies on nucleosides (Olafsson and Bryan, 1977; Olafsson et al., 1977) but thermograms with four (5-bromouracil) or five (5-chlorouracil) transitions were observed. In our hands a single endothermic transition was recorded for all but the 5-iodo compound. When each uracil is mixed with PEG 20M and the DSC analysis repeated, the 5-fluoro and 5-chloro derivatives appear as physical mixtures of the individual components. Some broadening of the endotherm was observed but disappearance did not occur nor were any new peaks introduced. In contrast, both 5-bromo and 5-iodo analogues reveal strong exothermic bands, due to the corresponding substitution reactions, as detailed in Table 1. The behaviour of this series is in accordance with predictions based on bond energies with the weakest bond (C–I) initiating reaction at the lowest temperature and parallels thermal degradation. To support this view, uracil has been detected as a thermolysis product of 5-bromo-2′-deoxyuridine and an exothermic transition at 345 ° C has been attributed to this reaction (Olafsson and Bryan, 1977).

TABLE 1

DSC onset transitions of 5-halogenouracils alone and in 1 : 1 admixture with PEG 20M

Uracil	Onset temperature of transition (° C)	
	Pure	In admixture
5-F	281.9	282.8
5-Cl	319.9	317.1
5-Br	310.4	296.5 *
5-I	285.3 292.6 *	266.6 *

* Signifies exothermic transitions.

TABLE 2

DSC onset transitions of bropirimine on 1 : 1 admixture with various excipients

Additive	Onset temperature of transition (° C)
β-Cyclodextrin	225.7 *
Hydroxypropyl-β-cyclodextrin	215.6 *
N-Methyl-D-glucamine (Meglumine)	241.4 *
Polyethylene glycol 1000	250.5 *
Polyvinylpyrrolidone	253.2 *
Urea	
urea melting	133.6
biuret formation	188

* Signifies exothermic transitions.

It is unlikely that the induction of this reaction is specific to PEG 20M; indeed, the dehalogenation of bropirimine in strongly alkaline aqueous solutions has been briefly mentioned (Wierenga, 1985). To investigate the generality of the observation, the interaction of bropirimine with a series of components which may be of use as additives to influence solubility profiles has been examined. Results are recorded in Table 2 which shows that several excipients share the potential exhibited by PEG 20M to facilitate thermal degradations of this type. With the exception of urea no melting endotherms are observed and all show a single exotherm for bropirimine degradation. Urea shows a melting endotherm (133.6 ° C) but on further heating a large endotherm develops from 188 ° C onwards. This signals the thermal degradation of urea ($H_2N–CO–NH_2$) to biuret ($H_2N–CO–NH–CO–NH_2$) with evolution of ammonia (Ford et al., 1979). On admixture with bropirimine the melting profile broadens and is depressed (116 ° C) while the degradation begins at 160 ° C and obscures any bropirimine transitions. That reaction has, indeed, occurred may be demonstrated by extraction of the melt from the DSC pan isolated after heating to: (i) 200 ° C; and (ii) 250 ° C. HPLC analysis of the extracts confirms that debromination is occurring over this range. At 200 ° C little

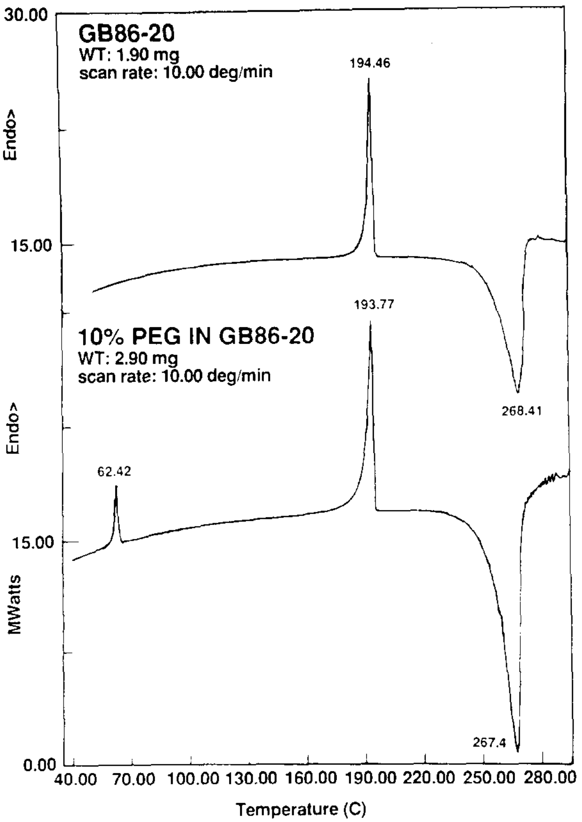


Fig. 6. DSC thermograms of CCRG GB86-20, alone and upon admixture with PEG 20M, showing the independence of the degradation exotherm at 267–268 °C once melting has been effected.

of the debrominated product is observed whereas at 250 °C considerable reduction in the intensity of the bropirimine peak, with a corresponding increase in that due to 2-amino-6-phenylpyrimidin-4(3H)-one, is apparent.

It is possible that the catalytic effect afforded by these additives is a consequence of the increased proportion of the aromatic halogeno compound in the liquid state at any fixed temperature. This may be due to the presence of the molten additive phase causing total or partial dissolution or else, for those additives such as PVP, which do not show a melting endotherm, a depression of melting point of the halogeno compound may occur which will similarly increase the proportion in the liquid state. It is well recognised that such factors significantly enhance the rates of reaction in the solid state (Mroso et al., 1982; Brown et al., 1980; Carstensen, 1977, 1980). Evidence for this interpretation may be gained from examination of a compound which clearly melts prior to decomposition. Such a compound is CCRG GB86-20, an analogue of bropirimine. The thermograms of this compound, alone and in admixture, are displayed in Fig. 6. The pure component shows successive melting (192.5 °C) and degradation (268.4 °C) behaviour. On admixture with PEG 20M the profile is retained. The melting transition is rather broader with a range of 173.9–197.3 °C in the presence of PEG, compared to 177.6–197.3 °C observed for the pure compound, which may be perhaps due to competing dissolution of the pyrimidine in the PEG melt. The degradation exotherm, however, is virtually unchanged showing a reaction range of 226.6–276.3 °C. This compares well with the range of 235.4–276.3 °C found in the pure state and confirms that degradation is largely unaffected by low amounts of the excipient in the liquid phase.

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