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DSC study of a metabolic pathway (biosynthesis of pineal indoles)

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Abstract

By means of a DSC study of the indoles that form part of the pineal metabolism, it is possible to verify the parallelism between the sequences of stability deduced from the peak temperatures of the melting/decomposition effects and those established through biochemical methods.

INTRODUCTION

In order to demonstrate the potential of differential scanning calorimetry (DSC) in research on metabolic pathways, a study of the thermal behaviour of the organic compounds involved in pineal metabolism has been carried out.

Pineal metabolism starts from tryptophan and, through a series of stages indicated in Fig. 1, yields a variety of interesting substances, including melatonin or *N*-acetyl-5-methoxytryptamine, which is of interest in the neuroendocrine–gonadal and the hypothalamic–hypophysary–adrenal axes [1].

Over recent years we have seen significant progress in the field of pineal research. Up to the present, the existence of circadian rhythms in various pineal metabolism products has been recognized and the hormonal function of melatonin [2] is also admitted for the 5-methoxytryptamine, 5-methoxyindole-3-acetic acid and 5-methoxytryptophol terminal indoles [3].

In parallel with such studies on the knowledge of the precise nature and function of all chemical species segregated by the gland, it was thought necessary to undertake work that might elucidate the structure–activity relationships by new methodologies.

EXPERIMENTAL

DSC curves were used for the study of eight pineal indoles [L-tryptophan, 5-hydroxy-L-tryptophan, 5-hydroxytryptamine (or serotonin), 5-methoxytryptamine, *N*-acetyl-5-methoxytryptamine (or melatonin), 5-hydroxyindole-3-acetic acid, 5-methoxyindole-3-acetic acid and 5-methoxytryptophol) as well as a non-pineal indole (tryptamine) which was included for comparison.

The compounds were obtained from Sigma Chemical Co. in the crystalline state.

A Perkin-Elmer DSC 7 differential scanning calorimeter equipped with a data station was used. Experimental conditions were: 10 μl aluminium pans, sample weights of about 5 mg, heating rate 10 °C min^{-1}, nitrogen as the purge gas.

RESULTS AND DISCUSSION

The DSC curves of the different species involved in the pineal metabolism are shown in Fig. 2a–h. The data from each curve (peak temperatures and enthalpies) together with those given in the Merck Index are summarized in Table 1.

Additionally, and in order to allow both the detection of thermal similarities among the substances involved and a reasonable discussion, the peak temperatures of the most characteristic thermal effects are included in Fig. 3.

The first of the results shown is the good correspondence between the data of our curves, those observed in capillaries and those referred to in the Merck Index (Table 1), although the thermal information derived from the DSC curves is considerably greater and, therefore, lends itself to a better analysis.

The thermal patterns of these substances permit the recognition, for the parent compound of the series (L-tryptophan), of a single decomposition effect; for the species 5-hydroxy-L-tryptophan a phase transition followed by a decomposition effect; for the compounds 5-hydroxyindole-3-acetic acid, 5-methoxyindole-3-acetic acid, 5-methoxytryptophol and serotonin a melting effect followed by a decomposition effect (in the case of serotonin, the two effects overlap) and, for the compounds tryptamine hydrochloride, 5-methoxytryptamine and melatonin, a melting effect only. The nature of such effects is clearly discerned by DSC: the sharp and well defined peaks associated with melting are perfectly distinguishable from the multiple and poorly defined peaks that correspond to thermal decomposition processes. It may be noted here that a thermal technique more suitable for the study of the decomposition process is DTG. Working with a Perkin–Elmer TG-2 apparatus (nitrogen flow 20 cm^3 min^{-1}, rate of heating 10 °C min^{-1})

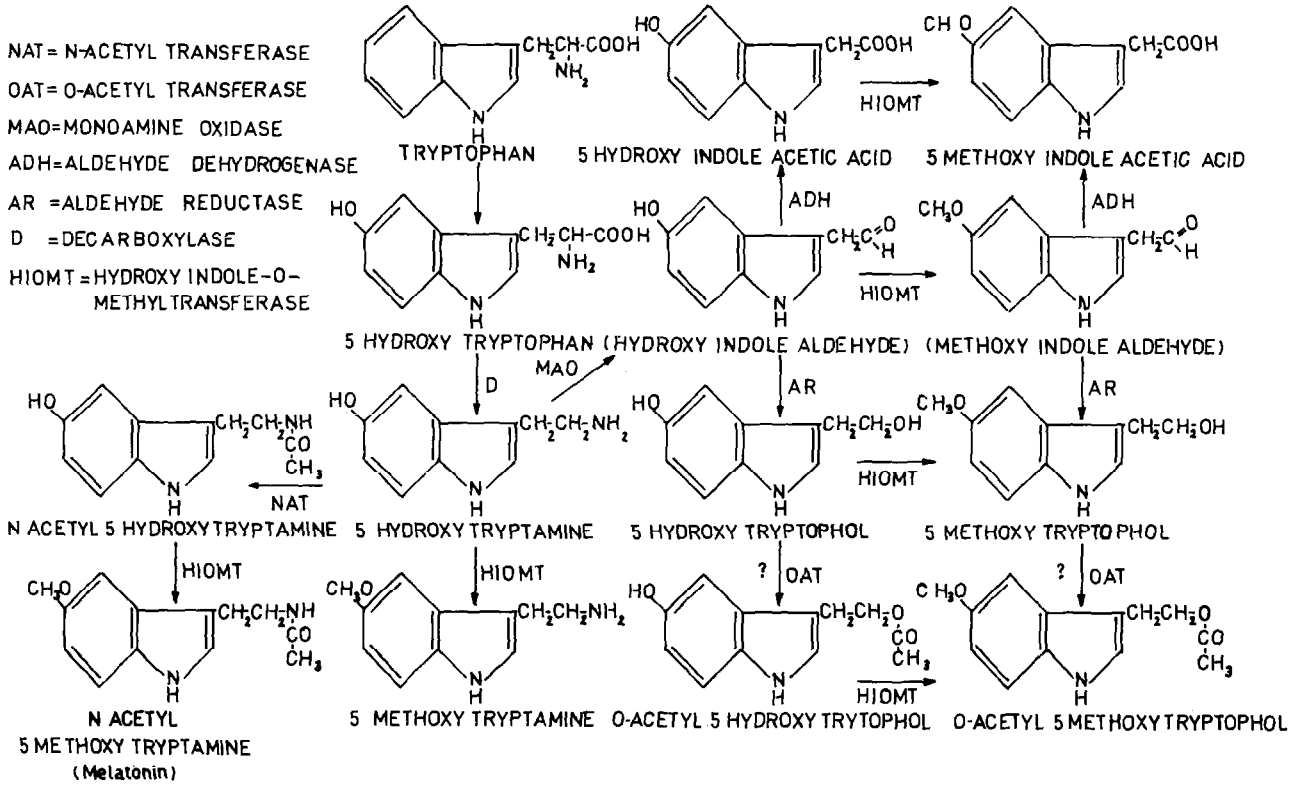


Fig. 1. Classical scheme of pineal indole metabolism.

decomposition of even melatonin and 5-methoxytryptamine was detectable. The DTG peak temperatures for the reported compounds were: 242 °C for 5-methoxytryptophol; 284 °C for melatonin; 220 °C for 5-methoxytryptamine; 210 °C for 5-methoxyindole-3-acetic acid; 210 °C for 5-hydroxyindole-3-acetic acid, 215 °C for serotonin (as oxalate); 278 °C for 5-hydroxy-L-tryptophan; 281 °C for L-tryptophan; 258 °C for tryptamine (as hydrochloride).

Adopting as the criterion of stability the peak temperature of the melting effect with priority over that of the decomposition effect, and

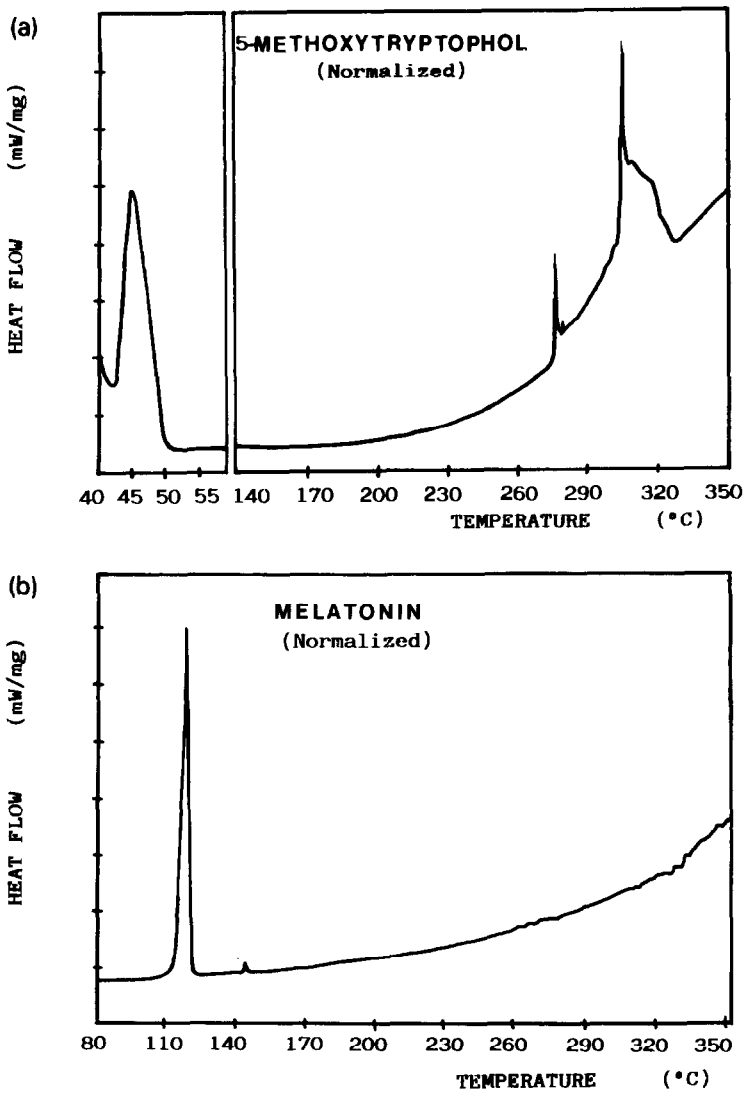


Fig. 2a–h. DSC curves of the different species involved in the pineal metabolism.

considering the data shown in Fig. 3, we have found a trend towards lowered thermal stability as one progresses through the sequence of reactions of the pineal metabolism. This feature applies for the three metabolic pathways that, starting from tryptophan, lead to the biosynthesis of the respective hormones.

With respect to the influence of the substituents on the thermal stability of the indole nucleus, it can be stated that the methoxy radical in position 5 reduces the thermostability as compared with the hydroxyl radical in the same position, and that this effect is greater when the substituent in

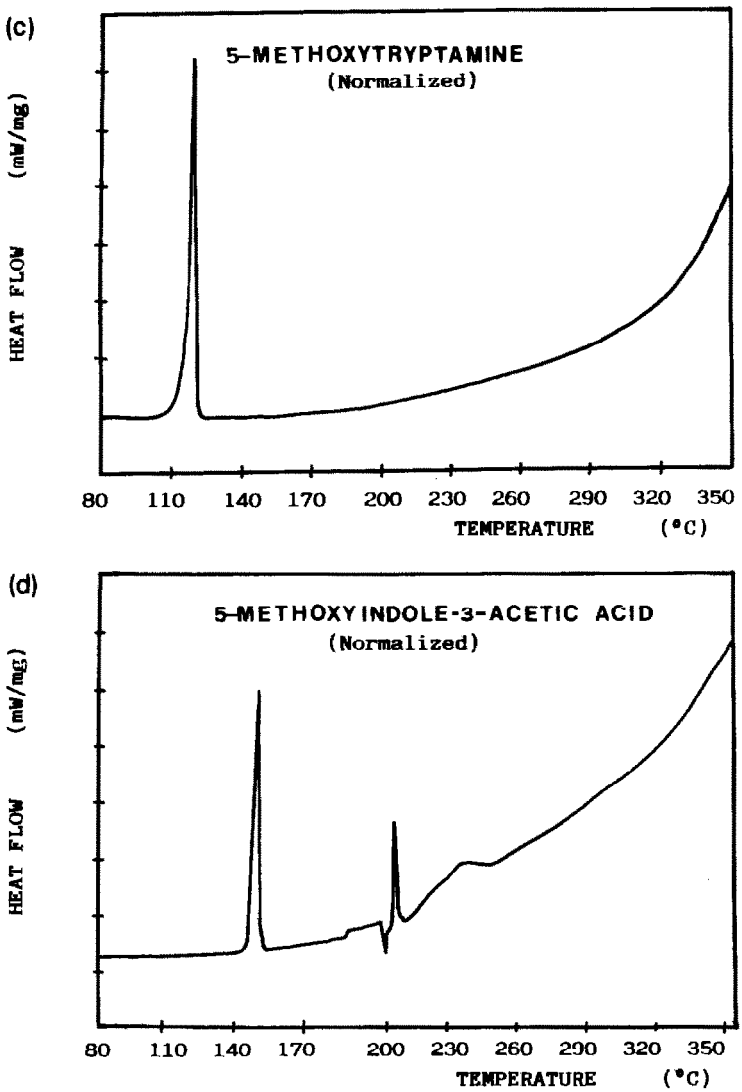
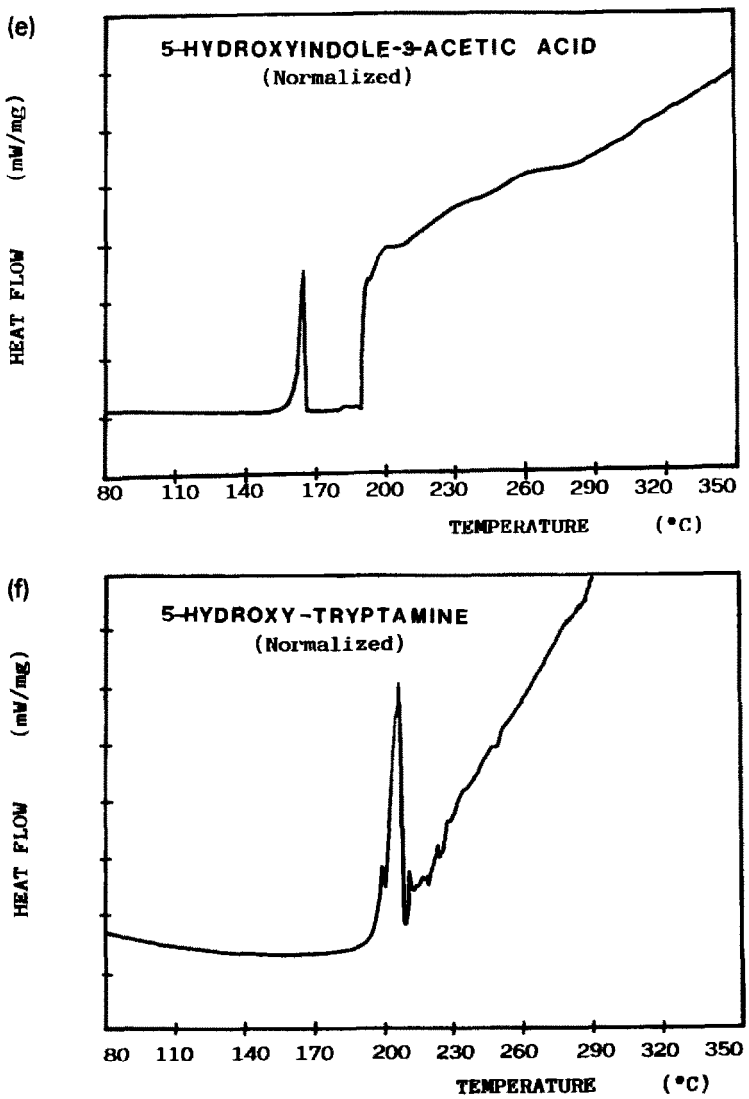


Fig. 2 (continued).

position 3 is ethanamine rather than acetic acid. This latter observation can be explained by the greater facility of hydrogen bridge formation (a stabilizing factor) by the acid function than by the amino function [4,5].

It should be made clear that the reasons that the enthalpies have not been taken as markers of stability are (a) the different values of the melting and decomposition effects of the studied metabolites, which cause difficulties in an overall interpretation, and (b) the impossibility of accurately determining such a thermodynamic parameter when multiple decomposition peaks occur. Nevertheless, the enthalpy mixed data of Table 1 appear



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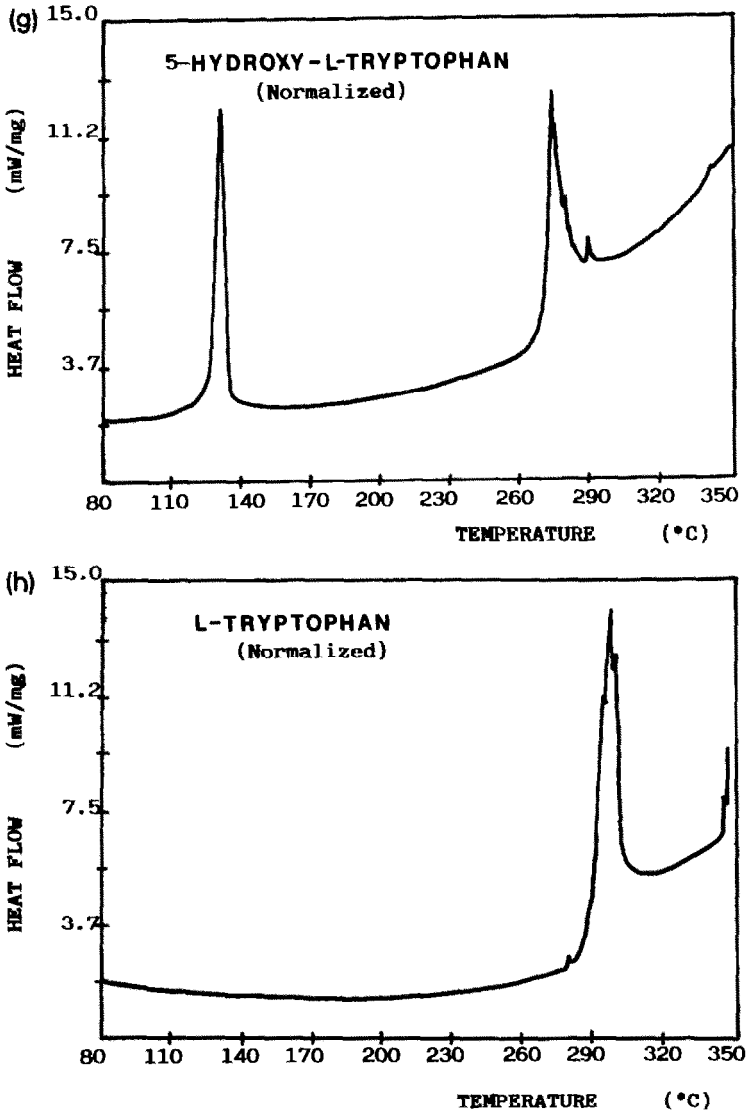

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to show the same sequence of stability as has been observed with the chosen criterion of temperature.

In conclusion, the possibility of establishing, through sequences of thermal stability, thermal relationships among the different species involved in metabolic pathways (which parallel those established by biochemical methods) allows the DSC technique to be considered as a new investigative tool in the field of biochemistry.

TABLE 1
Thermal data of pineal indoles

Indole	Thermal effect	DSC data		Capillary (°C)	Merck index (°C)
		ΔH(J g^{-1})	T_{peak} (°C)		
5-Methoxytryptophol	m.p.	–	45.0	44	–
Melatonin	m.p.	131	118.7	118	116–118
5-Methoxytryptamine	m.p.	166	120.6	117	121–122
5-Methoxyindole-3-acetic acid	m.p.	160	149.1	146	–
5-Hydroxyindole-3-acetic acid	m.p.	176	164.2	161	–
Serotonin (oxalate)	m.p.	(166)	205.2	205	–
5-Hydroxy-L-tryptophan	dec	(401)	273.7	253	298–300
L-Tryptophan	dec	(437)	296.8	260	289
Tryptamine (hydrochloride)	dec	180	258.6	251	248

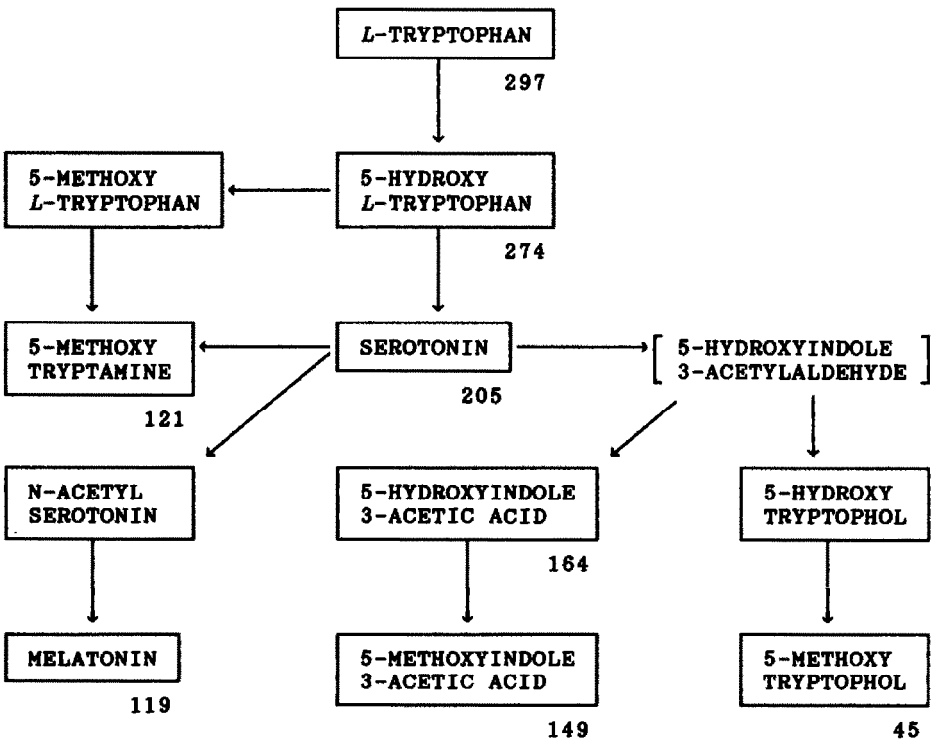

Fig. 3. Peak temperatures (°C) of the most characteristic DSC thermal effects in pineal indole metabolism.

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