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Note

Stability of hydralazine pyruvate hydrazone

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Recent papers by Haegele *et al.*[1], Ludden *et al.*[2] and O'Donnell *et al.*[3] have indicated interest in the hydrazones of hydralazine, particularly the pyruvate hydrazone, which Haegele *et al.*[1] indicated may be unstable under certain conditions. We have investigated the stability of hydralazine pyruvic acid hydrazone under various conditions of pH and also its disposition *in vivo*. We have found that the pyruvate hydrazone breaks down under acidic conditions to yield another metabolite, methyltriazolophthalazine (MTP). As MTP may also arise by acetylation of the parent drug its origin is of some interest. We have also found that the hydrazone is not metabolically inert.

The pyruvate hydrazone decarboxylates to yield the acetylated metabolite, MTP (Fig. 1) on gas chromatography (GC) and GC–mass spectrometry (MS) as previously described by Facchini *et al.*[4]. It now appears that this may occur in aqueous solution.

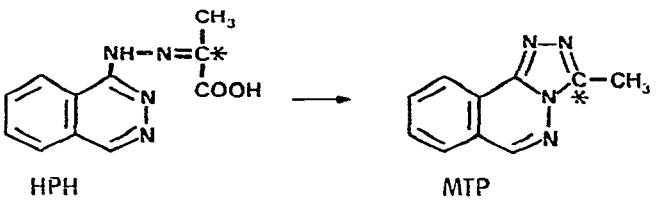

Fig. 1. Breakdown of hydralazine pyruvic acid hydrazone (HPH) to methyltriazolophthalazine (MTP). Asterisk indicates position of ^{14}C label.

EXPERIMENTAL AND RESULTS

Hydralazine [2-^{14}C]pyruvate hydrazone (HPH) was synthesised using sodium [2-^{14}C]pyruvate and hydralazine hydrochloride and GC–MS analysis carried out as previously described by Facchini *et al.*[4]. Thin-layer chromatography (methanol; R_F(HPH), 0.1; R_F(MTP), 0.44) indicated that the synthetic compound was contaminated with MTP. This was removed by extraction of an aqueous solution (200 mg/ml) at alkaline pH (9.5) with methylene chloride immediately prior to the study. MTP is quantitatively extracted under these conditions. Duplicate aliquots of this

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extracted solution were adjusted to various pH values with 2 *M* HCl and each aliquot incubated for 1 h at room temperature. After this time each solution was readjusted to pH 9.5 and extracted with methylene chloride and filtered through phase-separating paper. The extracts, reduced to dryness and taken up in methanol were analysed by liquid scintillation counting and high-performance liquid chromatography (HPLC). HPLC was carried out on a Shandon Hypersil ODS reversed-phase column, mobile phase methanol–water (50:50), flow-rate 1.5 ml/min.

Eluent from the column was monitored by UV absorption (254 nm) and scintillation counting of aliquots. A single radioactive peak was detected with the retention time of MTP. The amount of MTP was determined by scintillation counting, and each incubation mixture was analysed in this way. The effect of pH on the breakdown of the pyruvic acid hydrazone to MTP can be seen in Fig. 2. Production of MTP clearly occurs, particularly under acidic conditions. Haegele *et al.*[1] also report the production of a tricyclic dehydration product but indicate that this is not formed from the salt form of the pyruvate hydrazone under acidic conditions. This other tricyclic product is unlikely to be present and mistaken for MTP in the study as the alkaline conditions of the extraction (pH 9.5) would reconvert it into the salt form of the pyruvate hydrazone, as described by Haegele *et al.*[1].

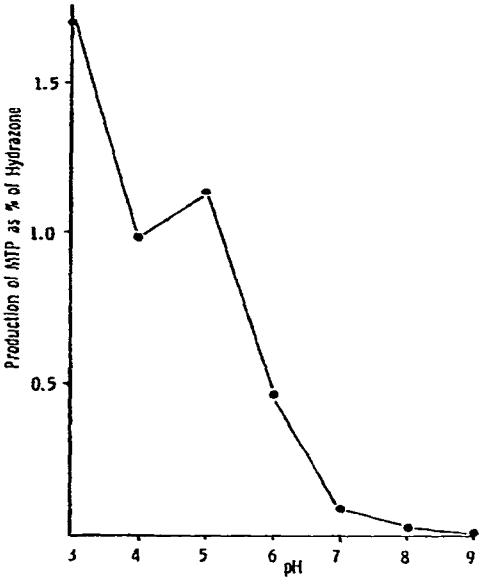

Fig. 2. The effect of pH on the production of methyltriazolophthalazine from hydralazine pyruvic acid hydrazone. Incubations were for 1 h at room temperature.

Preliminary investigations of the *in vivo* disposition of the pyruvate hydrazone in rats were also carried out. A solution of hydralazine [2-^{14}C]pyruvic acid hydrazone (5 mg/ml) adjusted to pH 9.5, was extracted with methylene chloride prior to administration to the animals. Radioactivity in the dose solution was determined immediately prior to dosing and purity was checked by HPLC. Sprague-Dawley rats

given the radio-labelled hydrazone (5 mg/kg), intraperitoneally, were housed in metabolic cages and urine collected frozen over dry ice and expired $^{14}CO_2$ collected as previously described by Timbrell and Wright[5]. Radioactivity was determined by liquid scintillation counting. The results of this experiment are shown in Table I.

TABLE I

IN VIVO DISPOSITION OF HYDRALAZINE [2-^{14}C]PYRUVIC ACID HYDRAZONE IN RATS

Results are means ± standard deviations of three animals.

Time after dosing (h)	*% of Dose*		
	Urine	*$^{14}CO_2$ expired*	*Total*
0–9	17.8 ± 6.2	16.5 ± 7.9	
9–24	17.4 ± 8.5	—	
0–24	35.2 ± 11.2	—	51.7 ± 4.1

It can be seen that considerable $^{14}CO_2$ is expired indicating that either pyruvate is lost and metabolised via intermediary metabolism to $^{14}CO_2$ or that the hydrazone rearranges to MTP *in vivo* and this is then further metabolised to $^{14}CO_2$. Analysis of the 0–9-h urine revealed 5.3 ± 1.6% of the dose as MTP. Therefore it can be seen that under acidic conditions *in vitro*, the pyruvate hydrazone decarboxylates to MTP, but at physiological pH little breakdown occurred. The hydrazone was not unchanged *in vivo*, a considerable amount being metabolised to $^{14}CO_2$.

These preliminary findings indicate that MTP, the product of metabolic acetylation of hydralazine, could arise chemically by decarboxylation of another metabolite, the pyruvate hydrazone. However, the acidic conditions required and the metabolic data obtained from humans given hydralazine, indicate that this is most likely to occur in urine rather than *in vivo*. The *in vivo* disposition of the hydrazone reveals that it is metabolised to a considerable extent, either by loss of the pyruvate moiety or by breakdown to MTP and then further metabolism of the triazolo ring to $^{14}CO_2$. Previous studies by Lesser *et al.*[6] have shown that MTP is metabolised in the rat. The presence of radioactive MTP in the urine however, indicates that this metabolite probably arises, at least partially, by chemical breakdown and rearrangement. Hydrolysis of the hydrazone to hydralazine and pyruvate and acetylation of the former by [^{14}C]acetate derived from the latter is another possibility, however. Much further work remains to be done to resolve the question of whether in man MTP is produced by acetylation or chemical breakdown of the pyruvate hydrazone.

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