Phytochemistry, Vol. 20, No. 8, pp. 2017–2018, 1981.
Printed in Great Britain.

0031-9422/81/082017-02 $02.00/0

SHORT REPORTS

EFFECT OF pH AND TEMPERATURE ON THE DEGRADATION OF MIMOSINE AND 3-HYDROXY-4(1 H)-PYRIDONE

R. B. H. WILLS and B. TANGENDJAJA

School of Food Technology, University of New South Wales, Kensington, NSW 2033, Australia

(*Received* 30 *October* 1980)

Key Word Index—*Leucaena leucocephala*; Leguminosae; mimosine; 3-hydroxy-4(1 H)-pyridone; degradation.

Abstract—Mimosine and 3-hydroxy-4(1 H)-pyridone were extensively degraded in solutions at pH > 9.5 when held at temperatures of 80–120° for 1–2 hr. Mimosine was also degraded at pH 1.

INTRODUCTION

Mimosine, β-[*N*-(3-hydroxy-4-oxopyridyl)]-α-amino propionic acid occurs in the tropical legume *Leucaena leucocephala* in sufficient amounts to prevent the widespread use of the plant for animal feed. Mimosine has been found to be toxic to ruminants [1], to cause sheep to shed their wool [2] and induces alocepia in non-ruminants [3]. Matsumoto *et al.* [3] examined various methods of reducing the quantities of mimosine in the plant before the preparation of animal meals and found that holding the moist leaf at elevated temperatures was partially effective e.g. after 72 hr at 100° only 35% of the original mimosine remained. More rapid degradation of mimosine was found by Hegarty *et al.* [4], who in an *in vitro* study reported substantial reductions of mimosine by heating at 100° in 0.1 M HCl with the maximum effect being a 60% reduction in 3 hr. However, there was a simultaneous production of 3-hydroxy-4(1 H)-pyridone (DHP) during the reaction and DHP has since been found to be a toxic goitrogen in ruminants [5]. Any method for degrading mimosine needs therefore to result also in the degradation of DHP. In this paper we have examined the stability of mimosine and DHP *in vitro* over the pH range 1–12 at elevated temperatures.

RESULTS AND DISCUSSION

Preliminary studies showed that temperatures in the order of 100° were necessary to produce degradation of mimosine and DHP in a reasonably short time. Solutions of mimosine and DHP were therefore heated at 118° for 2 hr and the resulting solutions analysed by HPLC (Fig. 1). At pH < 2 and pH > 9.5 mimosine was extensively degraded. It was relatively stable at intermediate pH with the greatest stability occurring at pH 4–7. DHP was relatively stable at pH < 9.5 but virtually all degraded at pH ≥ 11. Degradation of mimosine at low pH (1–2) resulted in a corresponding increase in DHP but at high pH the loss of mimosine was not accompanied by an increase in any detected component. Presumably DHP was produced but then degraded. No other compound was detected in the incubated DHP solutions.

While the extent of destruction of mimosine and DHP at 118° was adequate, the use of lower temperatures would probably be more desirable *in vivo* to minimise changes in other nutrients in the plant. Table 1 shows that for all pH values examined, degradation of mimosine after incubation for 2 hr was greatest at 120° and decreased as the temperature was reduced. While the losses of mimosine at 120° were > 80% at all the pHs examined, in the temperature range 60–100°, pH 11.5 was more effective than pH 10 and pH 12.5; at 100° the loss of mimosine at pH 11.5 was almost as great as at 120°.

Since the degradation of DHP at pH > 10 was more rapid than that of mimosine, the effect of temperature on DHP degradation was examined using an incubation period of 30 min. Degradation of DHP was found to be related to temperature with the effect of pH being pH 12.5 > 11.5 > pH 10 although at 100° and 120°, pH 12.5 and pH 11.5 were similarly effective, resulting in a loss of *ca* 80% of the initial DHP (Table 1). pH 12.5 was also highly effective in degrading DHP at 80°.

In highly alkaline solutions both mimosine and DHP are degraded but because the rate of degradation of mimosine is slower than that of DHP, it becomes the limiting factor in the elimination of these compounds

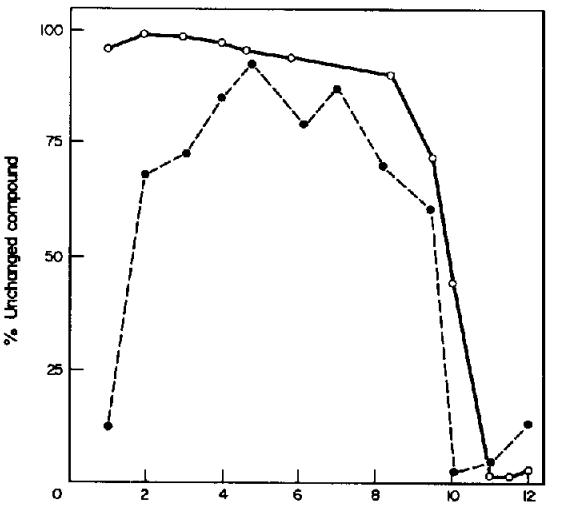

Fig. 1. Stability of mimosine (●---●) and DHP (○——○) in solutions at different pH after heating at 118° for 2 hr.

Table 1. Stability of mimosine and DHP after heating in alkaline conditions at different temperatures

	40°	60°	80°	100°	120°
% Mimosine unchanged after heating 2 hr					
pH 10	95	86	79	39	5
pH 11.5	85	65	33	9	4
pH 12.5	89	80	78	59	17
% DHP unchanged after heating 30 min					
pH 10.0	99	94	91	82	41
pH 11.5	100	89	63	20	10
pH 12.5	98	67	14	14	15

from solution. For solutions in the pH range 11–12, reducing the incubation period at 100° to 90 min was found to give *ca* 80% loss of mimosine while for an incubation period of 60 min a loss of *ca* 60% mimosine was obtained. Hence at 100°, an incubation period of 90–120 min is required to remove most of the mimosine from solution. *In vivo* degradation of mimosine and DHP will no doubt be influenced by other components in the plant but alkaline degradation at elevated temperatures would appear to be worthy of further investigation.

EXPERIMENTAL

Aliquots (1 ml) of solns of mimosine (15.4 mg/l) and DHP (19 mg/l) were placed in a small tube (1.5–2 ml capacity), the pH of the soln varied with 10 M HCl or 5 M NaOH, the tube was sealed and then held at a specified temp. in a H_2O or oil bath. Mimosine and DHP were analysed after incubation by HPLC [6] using a C_{18} column and UV detector at 280 nm.

REFERENCES

1. Hegarty, M. P., Schinckel, P. G. and Court, R. D. (1964) *Aust. J. Agric. Res.* **15**, 153.
2. Reis, P. J., Tunks, D. A. and Hegarty, M. P. (1975) *Aust. J. Biol. Sci.* **28**, 495.
3. Matsumoto, H., Smith, E. G. and Sherman, G. D. (1951) *Arch. Biochem.* **33**, 201.
4. Hegarty, M. P., Court, R. D. and Thorne, P. M. (1964) *Aust. J. Agric. Res.* **15**, 168.
5. Hegarty, M. P., Court, R. D., Christie, M. D. and Lee, C. P. (1976) *Aust. Vet. J.* **52**, 420.
6. Tangendjaja, B. and Wills, R. B. H. (1980) *J. Chromatogr.* **202**, 317.