Phytochemistry, Vol 24, No 5, pp 973–975, 1985
Printed in Great Britain

0031–9422/85 $3 00 + 0 00

INDOLE-METABOLIZING ENZYME SYSTEMS IN TROPICAL PLANTS

SATYA P KUNAPULI* and C S VAIDYANATHAN

Department of Biochemistry, Indian Institute of Science, Bangalore 560012, India

(*Revised received* 10 *September* 1984)

Key Word Index—Angiosperms, tropical plants, leaves, indole-metabolizing enzymes

Abstract—Extracts from the leaves of 60 plants from 33 families were screened for their ability to metabolize indole Of these plants, only 11 species were found to cause the rapid disappearance of indole There is no correlation between the family and indole disappearance While eight species can degrade indole anaerobically, *Tecoma*, *Mussaenda* and *Duranta* species require oxygen

INTRODUCTION

The indole nucleus as such is present in many biologically important compounds Indole was recognized as an essential constituent of jasmine oil as early as 1899 [1] Later indole and skatole were reported in *Celtis reticulosa* [2] and indole in citrus, coffee and mango plants [3] However, little work has been done on the biochemical aspects of biosynthesis and catabolism of indole in plants

Horvath *et al* studied the hydroxylation of indole in the Commelinaceae [4, 5], and also showed it is hydroxylated at the 4-position when incubated with cell-free extracts of pumpkin seedlings [6] Conversion of indole to 2,2′-*bis*(3-indolyl)indoxyl in isolated plant leaves was studied by Medvedev *et al* [7] Reports are also available on the oxidative ring cleavage of indole in a few plants [8–12] The purpose of the present study was to carry out such systematic investigation of the presence of such activities in a wide variety of tropical plants

RESULTS AND DISCUSSION

Sixty plants belonging to 33 families were screened for their ability to metabolize indole The disappearance of indole in the presence of a crude enzyme extract was used as criterion for activity The results are given in Table 1 As can be seen, there is no correlation between the ability to metabolize indole and the family of the plants examined Of plants screened, only 11 species were active in metabolizing indole Moreover, indole catabolism requires oxygen in *Tecoma stans*, *Mussaenda erythrophylla* and *Duranta ellisia*, while the other eight species do not require oxygen as evidenced by the lack of inhibition by dithionite (Table 2)

Since indole is present in jasmine flowers [13], it might be expected that indole-metabolizing enzyme systems are present Indeed, Divakar *et al* [9] purified an enzyme from *Jasminum grandiflorum* which requires 2 mol of oxygen for the oxidation of indole to anthranilic acid However, the enzyme system from *J flexile* does not require oxygen for degrading indole

The presence of various indole alkaloids [14] suggests that indole-metabolizing enzymes might occur in *Vinca rosea* However, crude extracts of neither *V rosea* nor *V major* caused indole disappearance Similarly, although indole is present in various citrus species [3] only cell-free extracts of leaves of *Citrus aurantium* and not *C medica*, caused the degradation of indole Similarly, although indole was detected in the fat from the seeds of *Mangifera indica* [3], no indole catabolism was detected in cell-free extracts of the leaves The metabolism of indole at some pH other than the one used can not of course be ruled out

Indole, skatole, tryptophan and tryptamine have been detected in the leaves of *Tecoma stans* [15] Here, indole might be formed from tryptophan by the well-known tryptophanase reaction [16, 17] and converted to anthranil by indole oxidase [8] and to anthranilic acid by indole oxygenase [11, 12] Indole oxidase is a cuproflavoprotein and atebrin sensitive, while indole oxygenase is not Further studies on the purification and characterization of the indole-metabolizing enzymes involved are in progress

EXPERIMENTAL

Preparation of crude enzyme preparations All operations were carried out at 0–5° unless otherwise stated Fresh leaves of each plant were washed thoroughly with cold H_2O, ground to a paste and extracted with citrate–Pi buffer, pH 5 0 The slurry was strained through cheesecloth and the extract centrifuged at 15 000 *g* for 20 min The supernatant was used as the crude enzyme preparation

Assay of indole-metabolizing enzyme systems The reaction mixture (2 0 ml) contained 100 nmol indole, 1 4 ml citrate–Pi buffer, pH 5 0, and enzyme preparation containing 500 μg protein was incubated at 30° for 20 min The reaction mixture was extracted with toluene (2 5 ml), 1 ml of the toluene layer used for the estimation of indole using the Ehrlich reagent [18]

One unit of the enzyme activity is defined as the amount which caused the disappearance of 1 nmol of indole in 1 min at 30°

*Present address Department of Human Biological Chemistry and Genetics, 504A, Basic Science F43, University of Texas Medical Branch, Galveston, TX 77550, U S A

Table 1 Distribution of indole-metabolizing enzymes in tropical plants

Plant name	Order	Activity
Thunbergia mysorensis T Anders	Acanthaceae	−
Alternanthera sessilis R Br	Amarantaceae	−
Gomphrena globosa L	Amarantaceae	−
Mangifera indica L	Anacardiaceae	−
Polyalthia longifolia B & H	Anonaceae	−
Allamanda cathartica L	Apocynaceae	−
Vinca major L	Apocynaceae	−
Vinca rosea L	Apocynaceae	−
Impatiens balsamina L	Balsaminaceae	−
Alnus maritima Muhlenb	Betulaceae	−
Bignonia magnifica Lodd	Bignoniaceae	−
Millingtonia hortensis L	Bignoniaceae	+
Spathodea campanulata Beauv	Bignoniaceae	+
Tababuia gravacan Hemsl	Bignoniaceae	−
Tecoma stans L	Bignoniaceae	+
Chrysanthemum indicum L	Compositae	+
Dahlia cactus	Compositae	−
Tagetes purpuria L	Compositae	−
Zinnia elegans Jacq	Compositae	−
Pelargonium zonale Willd	Geraniaceae	−
Psidium guajava L	Hyrtaceae	−
Gladiolus alatus L	Iridaceae	−
Salvia azuria Hort	Labiatae	+
Bauhinia acuminata L	Leguminosae	−
Bauhinia monandra Kurz	Leguminosae	−
Brownea ariza Benth	Leguminosae	−
Caesalpinia sepiaria Roxb	Leguminosae	−
Cassia spectabilis DC	Leguminosae	−
Delonix regia Raffin	Leguminosae	−
Mucuna pruriens DC	Leguminosae	−
Samanea saman L	Leguminosae	−
Stizolobium niveum Kuntze	Leguminosae	−
Tamarindus indica L	Leguminosae	+
Lagarstroemia indica L	Lythraceae	−
Galphimia glanca Cav	Malphighiaceae	−
Gossypium herbaceum L	Malvaceae	−
Hibiscus cannabinus L	Malvaceae	−
Hibiscus rosa sinensis L	Malvaceae	−
Callistemon pachyphyllus Cheel	Myrtaceae	−
Eucalyptus citriodora Hook	Myrtaceae	−
Bougainvillea brasiliensis Lund	Nyctaginaceae	−
Jasminum flexile Vahl	Oleaceae	+
Nyctanthes arbortristis L	Oleaceae	−
Pandanus odoratissimus Roxb	Pandanaceae	−
Plumbago capensis Thunb	Plumbaginaceae	−
Punica granatum L	Punicaceae	−
Rosa damascena Mill	Rosaceae	−
Rosa indica L	Rosaceae	−
Ixora coccinea L	Rubiaceae	−
Mussaenda erythrophylla Schum & Thonn	Rubiaceae	+
Pentas carnea Benth	Rubiaceae	−
Citrus aurantium L	Rutaceae	+
Citrus medica L	Rutaceae	−
Bassia latifolia Roxb	Sapotaceae	−
Antirrhinum majus L	Scrophulariaceae	−
Brunfelsia americana L	Solanaceae	−
Tropaeolum indica L	Tropaeolaceae	−
Clerodendron inerme Gaertn	Verbenaceae	+
Duranta ellisia Jacq	Verbenaceae	+
Verbena hybrida Hort	Verbenaceae	−

−, No activity, +, activity, i e the disappearance of indole was observed by crude enzyme preparations Only the leaves were used to prepare crude enzyme

Table 2 Effect of dithionite on indole-metabolizing enzymes

Species	Specific activity (units/mg protein)	% Activity relative to without dithionite* (1 mM)
Millingtonia hortensis	0 05	100
Spathodea campanulata	0 03	100
Tecoma stans	0 13	0
Chrysanthemum indicum	0 18	100
Salvia azuria	0 03	84
Tamarindus indica	0 04	100
Jasminum flexile	0 15	100
Mussaenda erythrophylla	0 21	10
Citrus aurantium	0 03	100
Clerodendron inerme	0 04	100
Duranta ellisia	0 08	20

*The enzyme was first incubated with dithionite for 10 min and assayed

Specific activity is expressed as units/mg protein estimated by the method of Lowry *et al* [19], using bovine serum albumin as the standard

Acknowledgements—We thank Ms Santhamani, Dr Chandrika, R and Mr D N N Murthy of Indian Institute of Science, Bangalore, India, for helping us in identifying the plants Financial assistance to one of us (S P K) from University Grants Commission, New Delhi, is gratefully acknowledged

REFERENCES

1 Hesse, A (1899) *Ber Dtsch Chem Ges* **32**, 2611
2 Herter, C A (1908) *J Biol Chem* **5**, 489
3 Sack, J (1911) *Pharm Weekbl* **48**, 307
4 Horvath, Maria, M, Guba, F, Hanusz, B and Kiraly, L (1975) *Acta Phyto Pathol Acad Sci Hung* **10**, 343
5 Horvath, Maria, M (1977) *Acta Biol (Szeged)* **23**, 69
6 Horvath, Maria, M (1977) *Bot Kozl* **64**, 117
7 Medvedev, V A, Korshikov, I I, Bashikatov, V G and Tarabrin, V P (1977) *Fiziol Rast (Moscow)* **24**, 858
8 Nair, P M and Vaidyanathan, C S (1964) *Biochim Biophys Acta* **81**, 496
9 Divakar, N G, Subramanian, V, Sugumaran, M and Vaidyanathan, C S (1979) *Plant Sci Letters* **15**, 177
10 Chauhan, Y S, Rathore, V S, Garg, G K and Arunbhargava (1978) *Biochem Biophys Res Commun* **83**, 1237
11 Kunapuli, S P and Vaidyanathan, C S (1982) *Plant Sci Letters* **24**, 183
12 Kunapuli, S P and Vaidyanathan, C S (1983) *Plant Physiol* **71**, 19
13 Divakar, N G and Bhupala Rao, N (1980) *Indian Perfum* **24**, 46
14 Hofmann, W, Kubeczka, K H and Czygan, Franz C (1983) *Z Naturforsch* **38C**, 201
15 Kunapuli, S P and Vaidyanathan, C S (1984) *Phytochemistry* **23**, 1826
16 Woods, D D (1935) *Biochem J* **29**, 649
17 Happold, F C and Hoyle, L (1935) *Biochem J* **29**, 1918
18 Stanley, A R and Spray, R S (1941) *J Bacteriol* **41**, 251
19 Lowry, O H, Rosebrough, N J, Farr, A L and Randall, R J (1951) *J Biol Chem* **193**, 265