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KETO AND IMINO ACIDS IN *DELONIX REGIA* FLOWERS

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Key Word Index—*Delonix regia*; Leguminosae; gold mohur; biosynthesis; keto acids; imino acids.

Abstract—Floral parts and buds of *Delonix regia* contain α-ketoglutaric acid, oxaloacetic acid, pyruvic acid and glyoxylic acid. Proline and hydroxyproline biosynthesis has been demonstrated. The calyx and androecium accumulate glyoxylic acid, in amounts greater than those reported for other plants.

INTRODUCTION

Extensive research has been carried out in the field of amino acids related to the metabolic changes during growth and development [1]. Keto acids provide the carbon skeletons for the synthesis of amino acids and proteins [2, 3], but very few attempts have been made to correlate changes in keto acids with those of amino acids. Previous reports have dealt mainly with the occurrence of different keto acids in different plant tissues [4–10], and some studies have been made during the growth of seedlings [11–13]. Changes in keto acids of the corolla of *Madhuca* have been investigated [14], but there has been little attempt to show the metabolic changes and biosynthesis of these and related metabolites in the individual floral parts, i.e. calyx, corolla, androecium and gynoecium. The present study was therefore made to correlate changes in keto acids with free amino and imino acids in different floral parts of *Delonix regia*.

RESULTS

Hydroxyproline, U_{10}, U_{11} and U_{12} are the major amino acids found in leaves (Table 1). The presence of α-ketoglutaric acid (α KGA), glyoxylic acid, pyruvic acid and oxaloacetic acid (OAA) has been established. The concentration is greater than 160 mg/kg except in the case of OAA which is found in trace amounts (Table 2).

A wider range and greater concentration of amino acids appear in floral buds than in leaves. However, during the development of flowering buds, in stage I, the occurrence of only few amino acids has been observed, notable among them are hydroxyproline, γ-aminobutyric acid, glutamic acid, asparagine and the spot no. U_{10}. Besides these amino acids, α-alanine and aspartic acid are present in traces (Table 1). Levels of leucine, phenylalanine, proline, arginine, glycine, serine, U_{11} and U_{12} were low at this stage. These compounds appear in the later stages of the bud development. The content of hydroxyproline, aspartic acid, glycine and serine increases gradually during the maturation process while the amount of asparagine declines by about 48% at stage II as compared to stage I.

The level of the keto acids in the different stages of floral buds is much lower than that in the leaves (Table 2). Glyoxylic acid exhibits a low and constant value throughout the stages of floral bud development. OAA also appears in stage I followed by only trace amount of this metabolite in the subsequent stages. Pyruvic acid is absent at stage I but appears in stage II, and the content increases in stage III. The amount of α-KGA is comparatively higher than other keto acids in all the bud stages.

Like the stages of floral buds, the different foral parts also show the presence of a large number of amino acids. A marked increase in the con-

Table 1. R_f values in two solvents (see Experimental) and amounts of free amino and imino acids (mg/5 g fr. wt) in leaves, flower buds and different floral parts of *Delonix regia*

	HO-Pro	γ-ABA	Pro	Ala	Glu	Arg	Asp	Gly and Ser	$AspNH_2$	$GluNH_2$	U_{10}*	U_{11}*	U_{12}*
R_{f1}/R_{f2}	0·58	0·61	0·70	0·50	0·24	0·83	0·17	0·30	0·38	0·70	0·60	0·32	0·25
	0·18	0·34	0·29	0·21	0·19	0·13	0·16	0·16	0·12	0·17	0·30	0·46	0·52
1. Leaf	1·25	0·53			0·35		0·20	0·35	0·75		1·40	1·35	1·40
2. Flower bud Stage I	0·90	0·60		0·10	0·20	Trace	0·10	Trace	1·50		0·50		
3. Flower bud Stage II	1·00	0·10	0·80	0·15	0·50	0·35	0·35	0·35	0·80	Trace	1·40		
4. Flower bud Stage III	1·10	0·20	0·60	1·30	0·40	0·35	0·40	0·75	1·20	0·10	1·60	1·00	1·15
5. Peduncle	1·10	0·70	1·25		Trace	2·40	0·40	0·70	2·95	0·35	1·50		
6. Calyx	1·00	0·50	0·95		0·50	0·70	0·40	Trace	1·35		1·00		
7. Corolla	1·00	0·30		0·53	0·30	0·70	0·40	0·50	1·75	0·10	0·90	0·80	1·10
8. Androecium	1·00	0·53	1·20	1·20	0·30	0·95	0·75	1·15	1·75	0·10	0·60	1·20	
9. Gynoecium	1·20	0·53	0·90	0·30	0·35	1·50	0·60	0·10	2·55	0·35	1·20		

* Unidentified spots.

observed in these parts compared with leaves and floral buds (Table 1). The amount of hydroxyproline, γ-aminobutyric acid, proline, arginine, asparagine and U_{10} is much higher in the peduncle than in the calyx, corolla, androecium or gynoecium. The level of hydroxyproline in the calyx, corolla and androecium is similar, having a slightly lower amount than in the peduncle and gynoecium.

Table 2 shows that glyoxylic acid has been found in all the floral parts but the corolla. The calyx and androecium accumulate this compound. The value is as high as about 0·6 and 0·5% respectively of the fr. wt of the plant material; in the peduncle and gynoecium, however, it is much lower. Among other keto acids, OAA, α-KGA and pyruvic acid are present in small amounts. A number of unidentified spots have been observed (0·1–1 g/kg) in different floral parts.

DISCUSSION

The level of almost all keto acids in the leaves is either higher or equal to that of the floral buds. However, the calyx and androecium are unique in having a large accumulation of glyoxylic acid. In these parts glyoxylic acid constitutes about 88 and 70% of the total concentration of keto acids respectively. Glyoxylic acid has been reported from many plants. Krupka and Towers[15] have reported this compound from *Cucurbita pepo* roots, *Nicotiana tabacum* and *Mentha* leaves, potato tubers and in *Oxycoccus*, *Vaccinium*, *Arachis*, *Tulipa* and wheat. It has been also reported

Table 2. Amounts of various keto acids (mg/5 g fr. wt) in terms of 2,4-dinitrophenyl hydrazone of α-ketoglutaric acid in leaves, flower buds and different floral parts of *Delonix regia*.

Keto Acids	Leaf	Flower Buds Stage I	Stage II	Stage III	Peduncle	Calyx	Corolla	Androecium	Gynoecium
1. α-Keto-glutarate	2·00	1·60	1·60	1·20	0·80	0·40		1·20	0·80
2. Oxalo-acetate	Trace	0·80	Trace	Trace	0·80	0·80	0·80	0·80	1·60
3. Glyoxylate	2·00	0·80	0·80	0·80	0·80	28·80		24·80	1·60
4. Pyruvic acid	0·80		Trace	0·80	Trace		0·80	1·20	
5. Un_1*	0·80	Trace	0·80	Trace		0·40	0·80	0·80	0·40
6. Un_2*	3·20	0·80		1·20	Trace	1·20	0·40	1·20	0·40
7. Un_3*		Trace	0·80		0·80	1·20	0·40	5·20	2·00
8. Un_4*	3·20			1·60					

Table 3. R_f and colour reactions of various keto acid hydrazone spots (2,4-dinitrophenyl hydrazones) of *Delonix regia* on paper chromatograms after spraying with ethanolic NaOH.

R_f 1st/2nd Direction	Colour reaction	Identification
1. 0·24/0·32	Brick-red	Glyoxylic acid
2. 0·41/0·36	Chocolate	Pyruvic acid
3. 0·11/0·30	Brown	Oxaloacetate*
4. 0·10/0·25	Olive-green	α-Ketoglutarate
5. 0·50/0·40	Yellow	Unidentified$_{(Un_1)}$
6. 0·49/0·45	Yellow	Unidentified$_{(Un_2)}$
7. 0·07/0·48	Light brown	Unidentified$_{(Un_3)}$
8. 0·03/0·12	Brown	Unidentified$_{(Un_4)}$

* Fast-moving isomer.

from the bleeding sap of pumpkin [10] as well as in some halophytes [9]. Glyoxylic acid and OAA are the major components of the keto acid pool in the *Madhuca* corolla [14]. However, the amount of glyoxylate found in *D. regia*, is greater than that reported for other plants. Among the organic acids the content of citric and malic acids is also very high. The reasons for these high levels are not known.

The amount of glycine, serine, aspartic acid and glutamic acid is much lower than their corresponding keto acids. Proline, hydroxyproline and asparagine are of common occurrence in the different parts. Proline is commonly present as a free amino acid in plants [16]. However, the occurrence of free hydroxyproline is more rarely recorded in plants. Tyankova [17] has reported the distribution of the free hydroxyproline in the separate organs of wheat plants during drought. The biosynthesis of imino acids (L-azetidine-2-carboxylic acid and trans-3-hydroxy-L-proline) has been studied in *D. regia* seedlings by labelled precursor feeding techniques [18]. Results of Sung and Fowden [18] indicate a continuous turnover of the hydroxyimino acid in seedlings. From the available data it appears that imino acids also actively metabolized during the process of flowering in *Delonix regia*.

EXPERIMENTAL

Delonix regia (gold mohur) is a medium-sized tree having large, crimson–red flowers. Different floral parts were collected in May and June when flowering takes place.

Keto acids were extracted as their 2,4-DNPHs [14]. 5 g fr. wt was kept at −15° for 24 hr. It was then homogenized with 0·25 ml cold H_2SO_4, 50 mg of 2,4-dinitrophenyl hydrazine (2,4-DNPH) and 60 ml of 80% EtOH. The extract was kept at room temp. with occasional stirring for 1 hr. It was centrifuged and 20 ml of cold EtOAc was added to the ppt. The ppt was further re-extracted 3 × with EtOAc. Extracts were mixed and evaporated to a vol of 2–3 ml. This fraction was extracted 3 × with 2 ml 10% Na_2CO_3. The soln of keto acid hydrazones (Na salts) was diluted to a final vol of 10–15 ml with H_2O. The combined extracts (which also contained some free hydrazones) were then re-extracted twice with 2 ml aliquots of EtOAc. The uncombined hydrazones which dissolved in the EtOAc were discarded. The carbonate soln was cooled and adjusted to pH2 with ice cold conc H_2SO_4 and extracted 3 × with 2 ml of EtOAc. The combined extracts were evaporated to dryness under a current of cold air. Hydrazones were dissolved in 80% EtOH to give a concn of 5 ml/5 g fr. wt in the soln.

Two-dimensional PC was effected on Whatman No. 1 paper using *n*-BuOH–NH_3–H_2O (80:15:5) and *n*-BuOH–EtOH–H_2O (5:1:4) for the 1st and 2nd direction resp. Keto acids were detected on the chromatograms by their yellow spots and identified by their R_f values. Their characteristic colour reactions and R_f values were noted after spraying with NaOH in EtOH (Table 3). For quantitative analysis, keto acid spots after a 2-dimensional run, were eluted with 5 ml of 0·2 M Na_2CO_3. The amounts of the keto acids were calculated in terms of the 2,4-DNP of αKGA at 420 nm.

Amino acids were extracted as described in ref [19] and analyzed by PC [20]. The amounts of proline and hydroxyproline were estimated using authentic compounds as standards, while amounts of the other amino acids were calculated in terms of glycine. R_f values of the different amino acids are shown in Table 1.

The presence of proline and hydroxyproline which give weak reactions with ninhydrin were further confirmed by spraying the chromatograms with a 0·2% soln of isatin in Me_2CO containing 4% HOAc. Chromatograms were dried at the room temp. and on heating at 100° for 10 min, development of blue colours confirmed the presence of proline and hydroxyproline.

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