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METABOLISM OF PIPECOLIC ACID DERIVATIVES IN *CALLIANDRA* AND *ZAPOTECA*

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Key Word Index—*Calliandra*; *Zapoteca*; Mimosoideae; ^{14}C-label; pipecolic acid derivatives; amino acid metabolism.

Abstract—^{14}C-Labelled lysine, pipecolic acid and mono- and dihydroxy derivatives of pipecolic acid were administered to leaves of *Calliandra angustifolia* and *Zapoteca formosa*. Pipecolic acid was rapidly converted to the mono- and dihydroxy constituents, and both of the monohydroxy compounds were converted to the dihydroxy derivative as well as back to pipecolic acid.

INTRODUCTION

Pipecolic acid is widely distributed in plants and is particularly common among members of the Leguminosae [1]. Derivatives of this imino acid, however, are much more limited in their distribution. *Calliandra*, a tropical legume genus of the Mimosoideae, is rich in these compounds, containing no fewer than nine distinct pipecolic acid derivatives [2]. Four monohydroxylated derivatives, four dihydroxylated derivatives and an acetylamino compound display discontinuous distributions and are currently being employed in a monographic revision of *Calliandra* [3]. *Zapoteca*, a small genus that has only recently been separated from *Calliandra* [4], contains some of the same derivatives of pipecolic acid. *cis*-5-Hydroxypipecolic acid (C5), *trans*-4-hydroxypipecolic acid (T4), and 2,4-*trans*-4,5-*trans*-4,5-dihydroxypipecolic acid (TT) are present in all examined species [5].

It has been noted previously that in *Calliandra–Zapoteca*, a particular dihydroxypipecolic acid derivative usually co-occurs with the monohydroxy compounds that display the same absolute configuration about the 4- and 5-carbons [2]. This led us to believe that there is probably a distinct pathway leading to the formation of these compounds with some stereospecificity involved. Although the conversion of lysine to pipecolic acid in plants has been well documented, the formation of hydroxypipecolic acid derivatives is still under debate. Both the cyclization of hydroxylysine [6] and the direct hydroxylation of the pipecolic acid core [7–9] have been shown to lead to the formation of either the 4-hydroxy or the 5-hydroxy isomers of pipecolic acid. To date, the biosynthesis of any dihydroxylated pipecolic acid derivative has not been investigated.

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RESULTS AND DISCUSSION

The distribution of ^{14}C-labelled constituents from L-[U-^{14}C]-lysine fed leaves of *Zapoteca formosa* is displayed in Table 1. After 6 hr of incubation, pipecolic acid contained 18% of the total label incorporated into the aqueous soluble fraction of the leaves while 71% remained as lysine. After 48 hr, pipecolic acid made up 46% of the total and lysine dropped to 17%. Lysine was essentially unlabelled after 120 hr. A significant amount of the ^{14}C label was also detected in all three pipecolic acid derivatives. C5 and T4 reached values of 18 and 3.8% respectively after 48 hr, and TT reached a maximum of 8.1% after 72 hr. Data for ^{14}C-lysine fed *Calliandra angustifolia* (not shown), were similar to those of *Z. formosa*. A large percentage (44%) of the ^{14}C label was detected in pipecolic acid after only 24 hr. The pipecolic acid derivatives C5, T4 and TT all reached maximum amounts after 72 hr (14, 8.4 and 9.0% respectively). In neither genus was hydroxylysine detected, either labelled or otherwise.

Data for ^{14}C-pipecolic acid feeding experiments with both *Z. formosa* and *C. angustifolia* is shown in Table 2. In *Z. formosa*, the C5 monohydroxy compound composed 4.0% of the label after only 6 hr of incubation with the T4 isomer making up 1.5%. Within 72 hr, the percent of the total label in C5 was up to 10% and T4 up to 4.2%. The TT dihydroxy compound constituted 7.0% of the label after 72 hr. The 72 hr incubation data for *C. angustifolia* show that TT had the highest percent of label of the pipecolic acid derivatives (11%) followed by T4 (6.3%) and C5 (6.1%). In both species, large amounts of labelled proline and glutamine were also present after 72 hr.

Zapoteca formosa was fed quantities of ^{14}C labelled T4, C5, TT and a non-native pipecolic acid derivative, *trans*-5-hydroxypipecolic acid (T5). The 24 hr incubation data are summarized in Table 3. Pipecolic acid comprised 36% of the total label when fed labelled T4, 32% when fed labelled C5 and 27% when fed labelled TT. A substantial amount of radioactivity was detected in the TT isomer

Table 1. Distribution of aqueous soluble compounds of ^{14}C-lysine fed leaves of *Zapoteca formosa* over time (% radioactivity of the total aqueous fraction)

	Hr of incubation							
	6	12	18	24	48	72	96	120
Lys	71	55	43	32	17	9.1	4.2	1
Pip	18	27	35	42	46	31	29	12
T4	1.2	1.6	1.8	2.5	3.8	4.2	3.4	3.1
C5	5.1	8.5	12	15	18	14	15	11
TT	1	2.8	3.8	4.3	6.2	7.4	8.1	6.1
Pro	—	—	—	—	—	8.6	10	19
Glu	2.1	1.5	1.2	1	1.8	2.5	2.1	2.9
Gln	1.7	2.1	1.8	2.4	6.9	18	23	37

Table 2. Distribution of aqueous soluble compounds of ^{14}C-pipecolic acid fed leaves of *Zapoteca formosa* and *Calliandra angustifolia* over time (% total radioactivity of the aqueous fraction)

	Z. formosa			*C. angustifolia*
	Hr of incubation			
	6	24	72	72
Lys	1	1	1	1
Pip	89	60	27	31
T4	1.5	1.7	4.2	6.3
C5	4.0	9.0	10	6.1
TT	1	2.0	7.0	11
Pro	—	1.7	29	34
Glu	1	1.7	1.2	3.5
Gln	1.5	19	20	7.2

Table 3. Distribution of aqueous soluble compounds of ^{14}C-pipecolic acid derivative fed leaves of *Zapoteca formosa* after 24 hr incubation (% of the total radioactivity of the aqueous fraction)

	Radioactive label				
	Pip	T4	C5	TT	T5
Lys	1	1	1	1	1
Pip	60	36	32	27	—
T4	1.7	35	1.5	6.0	—
C5	9.0	9.1	45	31	—
TT	2.0	12	8.5	24	—
Pro	1.7	1.2	—	—	—
Glu	1.7	4.2	2.3	2.4	—
Gln	19	—	9.2	7.2	—

when fed either the T4 or C5 monohydroxy compounds (12% and 8.5). Labelled TT was also rapidly transformed to C5 (31%) and to a lesser extent to T4 (6.0%). It is noteworthy that essentially none of the label in any of the pipecolic acid compound experiments was detected in lysine. Labelled T5, the non-native monohydroxy isomer was apparently not metabolized.

While our data show that lysine is rapidly metabolized to pipecolic acid, this pathway is not readily reversible in either *Calliandra* or *Zapoteca*. Fowden [8] previously demonstrated a small (3.2%) conversion of labelled pipecolic acid to lysine in *Acacia*. These plants, however, contain only one additional pipecolic acid derivative in the free amino acid pool. Both *Calliandra* and *Zapoteca* on the other hand possess a number of these derivatized compounds. The metabolic emphasis is clearly on the conversion to other pipecolic acid compounds and not on a reversion to lysine. It is clear that the conversion of pipecolic acid to C5 and T4 is rapid, and also that both C5 and T4 can be converted back to pipecolic acid. C5 and T4 are more rapidly converted to TT than pipecolic acid or lysine. All of these data considered together support the hypothesis of hydroxylation of pipecolic acid to the monohydroxy derivatives followed by a subsequent hydroxylation of one or both monohydroxy compounds to form the dihydroxy isomer. The TT dihydroxy compound itself is also metabolically active and can be reconverted to the monohydroxy compounds. In *Z. formosa*, conversion of the TT dihydroxy isomer to C5 appears to be the preferred pathway.

A scheme for pipecolic acid metabolism is proposed in Fig. 1. The situation is clearly different from that found in the Caesalpinioideae species *Gleditsia triacanthos* [6]. In *Gleditsia*, both T5 and C5 are produced from 5-hydroxylysine and show no further metabolic transformations once formed. In both *Calliandra* and *Zapoteca*, pipecolic acid derivatives show significant conversion not only from one derivative to another, but eventually to pipecolic acid and proline, glutamine and other protein amino acids. Finally, the failure to detect any labelled products in the feeding experiment in which T5 was utilized demonstrates the apparent specificity of the enzymes involved.

EXPERIMENTAL

Labelled compounds. L-[U-^{14}C]-Lysine was obtained from Research Products, ^{14}C-labelled pipecolic acid, T4, C5 and TT were isolated from leaves of *Calliandra angustifolia* and *Zapoteca formosa* which had been fed L-[^{14}C]-lysine. Labelled T5 was isolated from L-[^{14}C]-lysine fed leaves of *C. haematocephala*. Purity of the compounds was confirmed by 2D separation followed by autoradiography.

Chemicals. Standards of pipecolic acid derivatives were isolated from *Calliandra* spp. [2, 10] and identities were confirmed by IR and NMR spectra. DL- and DL-*allo*-5-Hydroxylysine was obtained from Sigma.

Plant material. Viable seeds of *Zapoteca formosa* were collected in January 1986 along the northern border of Colombia. Seeds of *Calliandra angustifolia* were collected in Colombia in 1985. Voucher specimens are on deposit at the USF herbarium in Tampa, FL or the Missouri Botanical Gardens in St. Louis, MO. Seeds were germinated and grown in a 50/50 mixture of perlite and vermiculite in a growth chamber. Plants were watered daily, kept at 21–27°, and received 12 hr of light per day.

Application of labelled compounds. Leaves of *Calliandra* and *Zapoteca* were excised and their petioles immersed in solns containing the ^{14}C-labelled amino acids. They were placed in a humidified glass bell jar under a 75 W grow lamp for the allotted time interval after which the whole leaves were extracted.

Extraction of amino acids. Extraction of the free amino acids was according to ref. [22]. Incubated leaves were extracted × 3 with 2 ml of MCW (MeOH–$CHCl_3$–H_2O, 12:5:1). The com-

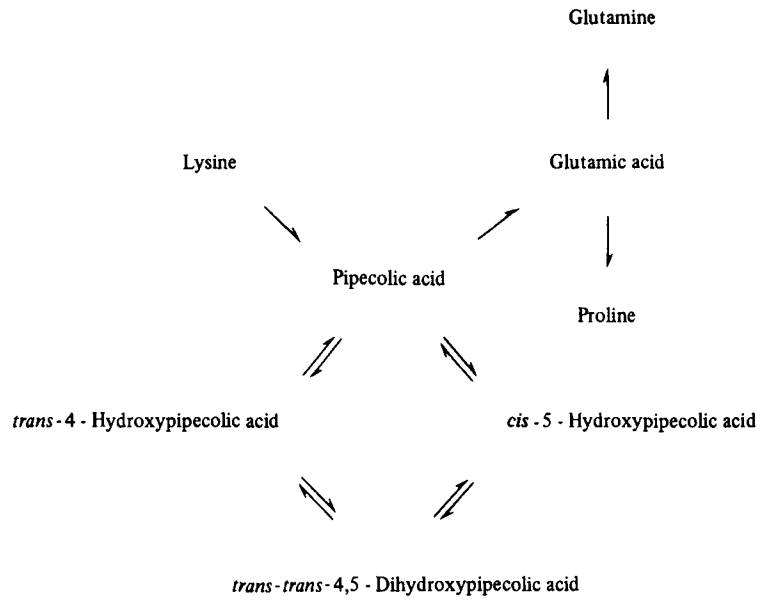

Fig. 1. Proposed metabolic pathway of imino acids in *Calliandra* and *Zapoteca*.

bined supernatant was separated into an upper aq. phase and a lower nonopolar phase with the addition of 1 ml of $CHCl_3$ and 1.5 ml of H_2O. The aq. phase was dried under an air jet and the residue was redissolved in 0.5 ml of 25% aq. EtOH.

Identification of labelled compounds. Labelled constituents were determined from 2D paper separation using high voltage paper electrophoresis (pH 1.9) followed by ascending chromatography in BuOH–HCO_2H–H_2O (12:3:2). Additional separation was done with 2D PC in BuOH–HOAc–H_2O (12:3:5) followed by 80% phenol–H_2O (w/v) in the presence of NH_3 vapour. Chromatograms were sprayed with an enhancing reagent, placed in a film cassette with Kodak XAR-5 X-ray film and developed at −70° for 7 days. Chromatograms were then developed with 2% ninhydrin to locate the amino acids. Individual ninhydrin-positive spots were cut from the paper and counted by liquid scintillation with a Beckman LS 200 liquid scintillation counter.

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