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BIOSYNTHESIS OF STIZOLAMINE IN *STIZOLOBIUM HASSJOO*

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Key Word Index—*Stizolobium hassjoo*; Leguminosae; stizolamine; 1-methyl-3-guanidino-6-hydroxymethyl pyrazin-2-one; seeds; biosynthesis.

Abstract—In *Stizolobium hassjoo*, stizolamine is located in seeds and pericarps and is not found in mature leaves, stems and roots. The content of stizolamine in seeds and the fruits increases with their maturation but do not correlate with their dry weights. A feeding experiment showed that guanosine triphosphate [U-^{14}C] is effectively incorporated into stizolamine in pericarps.

INTRODUCTION

Stizolamine has been isolated from the seeds of *Stizolobium hassjoo* and its structure shown to be 1-methyl-3-guanidino-6-hydroxymethylpyrazin-2-one by chemical, physical and X-ray diffraction analyses [1]. It has also been shown that stizolamine is detected widely in seeds of Leguminosae, especially of the subfamily Lotoideae [2]. The present investigation was undertaken to study the biosynthetic pathway of stizolamine.

RESULTS AND DISCUSSION

Change of stizolamine content

Stizolamine has been previously detected in the seeds and in the etiolated whole plants of *S. hassjoo* [1], but not in the mature plants. In the present work stizolamine could not be detected in any organs except fruits (Table 1). It is also shown that the content in seeds increases with maturation. This result is shown more distinctly in the next experiment, in which the contents of stizolamine in pericarps and in seeds were measured. The content in seeds gradually increased until the 4th week, remained constant until the 8th week and then increased (Fig. 1). The latter increase did not correspond to the change in dry wt of the seeds (Fig. 2). In pericarps and fruits the change of stizolomine content did not correlate with the change in dry wt. The maximum content is obtained in the 2-week-old pericarps but the maximum dry wt was found at the 4th week.

To investigate the possibility that stizolamine can be synthesized in pericarps and in seeds, suggested from the results described above the 8-week-old fruits were detached to prevent import of stizolamine and the stizolamine was measured two weeks later. Fruits of the same stage were also halved longitudinally and the contents in both halves were measured. The results are summarized in Table 2. An apparent increase in pericarps and seeds was observed, but the stizolamine contents in those seeds and fruits did not reach the level found normally. The results described above show that stizolamine is synthesized in pericarps and seeds, and

Table 1. Localization of stizolamine

Organ	Content 10^{-8} mol/g
Stem	[<0.06]*
Root	[<0.34]
Mature leaf	[<0.60]
Petal	[<0.94]
Calyx	[<4.19]
Stamen	[<1.92]
Pistil	0.25
Immature pericarp	6.54
Immature seed	175
Mature seed	697

* Stizolamine could not be detected.

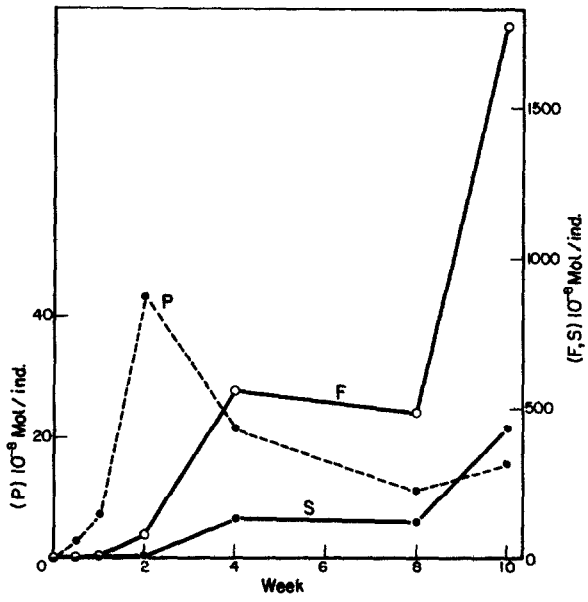

Fig. 1. Change of stizolamine content in pericarp, seed and fruit. ●·····● P; pericarp, ●——● S; seed, ○——○ F; fruit.

suggest that the amine is accumulated in the seeds at the latest stage of their maturation.

Precursor of stizolamine

Few pyrazine compounds have been found in nature and their biosynthesis has not been studied previously. Aspergillate and related compounds isolated from *Aspergillus* species seem to be synthesized from the appropriate amino acids [3]. According to this serine would be expected to be a precursor of stizolamine. On the other hand, the pyrazine ring systems of

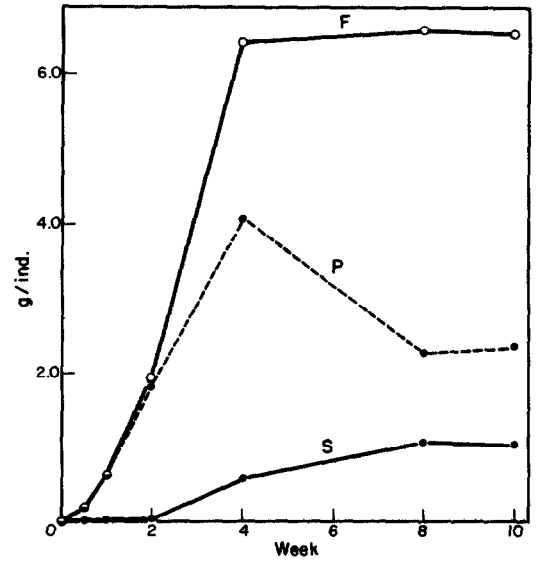

Fig. 2. Change in dry weight of pericarp, seed and fruit. ●·····● P; pericarp, ●——● S; seed, ○——○ F; fruit.

Table 2. Change of stizolamine content on some treatments

Treatment Organ	Content (10^{-8} mol/individual)	
	8-week-old	10-week-old
Normal		
Pericarp	11.2	15.8
Seed	117	429
Fruit	(483)	(1770)
Detached		
Pericarp	0.54	12.3
Seed	163	256
Fruit	(653)	(1040)
Halved		
Pericarp	9.8	15.7
Seed	93.8	173
Fruit	(385)	(706)

folates and pterins are synthesized from guanosine triphosphate (GTP) [4–6]. To obtain some information on the biosynthesis of stizolamine, three ^{14}C-labelled compounds DL-serine[3-^{14}C], folate-[2-^{14}C] and GTP-[U-^{14}C] were administered to the slices of the 2-week-old pericarps.

The radioactivity in stizolamine was not detectable when serine and folate were fed, but a considerable incorporation of radioactivity was found when GTP-[U-^{14}C] was administered. The results show that GTP is an effective precursor of stizolamine (dilution value of 1970 ± 100 and incorporation rate of 1.23 ± 0.11%).

It is unlikely that stizolamine is synthesized from GTP via the folate pathway, because folate-[2-^{14}C] was a poor precursor of stizolamine in the slices of pericarps. However, the reduced form of folate-[^{14}C] which is a member of the folate pathway and seems to be the active form in metabolism, was not tested in this experiment.

EXPERIMENTAL

Plant material. Seeds of *S. hassjoo* were sown in May. In Tokyo the flowers bloom from September till October and the seeds mature during November to December. One to 6 seeds were present in a fruit. In these expts, fruits containing 3–5 seeds were used. The fruit is therefore composed of pericarp and 4 seeds.

Chemicals. DL-Serine-[3-^{14}C] (56 mCi/mmol) was obtained from the Radio Chemical Centre, Amersham, England. Na folate [2-^{14}C] (54.3 mCi/mmol) and tetra-Na guanosine triphosphate-[U-^{14}C] (GTP; 360 mCi/mmol) were purchased from New England Nuclear Co. Stizolamine was extracted and isolated by the method of ref. [2].

Treatments of the fruits. Fruits (8-week-old, of which colour was turning from green to yellow with white spots and shape was changing from swollen to shrunk), were harvested to prevent import of stizolamine. One group of the fruits was extracted immediately and the other was extracted after being kept for 2 weeks at room temp. ('detached 8- and 10-weeks', respectively). The latter seeds showed no difference in the germination to the 'normal' seeds. In the other expt the 8-week-old fruits were halved longitudinally. One of the halved pericarps was extracted immediately and the other was extracted after two weeks ('halved pericarp 8- and 10-weeks', respectively). The half numbers of seeds which were present in the fruits described above were extracted immediately and the remaining seeds were extracted after two weeks ('halved seed 8- and 10-weeks', respectively).

Feeding experiments. Fruits (2 weeks old) were harvested and halved longitudinally. The halved pericarps were cut transversely into slices (0.2–0.5 mm thick) and rinsed twice into 2×10^{-4} M $CaSO_4$ soln. The slices (1.5 g) were put into a test tube (1.5 × 3.0 cm), dipped into 3 ml of 10^{-2} M Na Pi buffer (pH 6.8) containing 10^{-3} M Na ascorbate and then the radioactive compound (2 μCi) was added. CO_2 generated from the slices was trapped with 10 ml of N NaOH. After incubation for 24 hr in the light the radioactive stizolamine was extracted and isolated by the column of the Amberlite IRA 410 (OH^- form) described previously [2]. Carrier (10 mg) was added to the isolated samples and the amine was recrystallized from boiling H_2O.

Analyses. Stizolamine was measured spectrophotometrically (330 nm in MeOH; $\varepsilon = 13600$) and fluorometrically (Exc., 350 nm, Anal., 390 nm; pH 7). Radioactivities were measured using a 2,5-diphenyl oxazole (PPO)-dioxane scintillator.

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BIOSYNTHESIS OF CYANOGENIC LIPID IN *CARDIOSPERMUM GRANDIFLORUM* FORMA *HIRSUTUM* SEEDS

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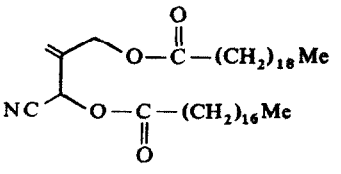
Key Word Index—*Cardiospermum grandiflorum* forma *hirsutum*; Sapindaceae; cyanolipids; biosynthesis; origin from leucine,

Cyanolipid **1** has been reported from a number of Sapindaceous plants [1–8].



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In addition, 3 other cyanolipids have been isolated, all from the seed oils of members of the Sapindaceae. Two of these, which occur in the seeds of *Koelreuteria paniculata*, have recently been shown to be derived from leucine [9]. The aglycones of several cyanogenic glycosides have also been shown to the synthesised from amino acids [10]. One of these glycosides, acacipetalin, is found in several species of *Acacia* [11]. It is a glucoside of the same α-hydroxynitrile found in the cyanolipid of *Ungnadia speciosa* [4]. The aglycone portion of acaci petalin has been shown to be derived from leucine in *Acacia sieberiana* [12, 13]. Another cyanogenic glucoside, cardiospermin has also been isolated from *Cardiospermum grandiflorum* forma *hirsutum* [14] which possesses an identical aglycone portion to that of cyanolipid **1**, but its biosynthetic origin has not been examined. Because the structure of **1** suggests that leucine is a logical biosynthetic precursor and all compounds of this type possess similar structures and occur in related plants, we initiated the present study.

We have not made comparisons of the effectiveness of labelling by other possible precursors as the fruiting period of the plant limits the number of experiments possible as well as the availability of materials.

Labelling data for samples 1, 2 and 3 are presented in Table 1. The samples were obtained by feeding 20, 20, and 50 μCi respectively of L-leucine-[U-^{14}C] as previously described. Percentage incorporation was calculated assuming a MW of 782 based on the relative percentages of the cyanogenic compound and the fatty acid composition of the whole oil. As previously observed in *Koelreuteria paniculata* [9], the radioactive label does not appear to 'turn over' rapidly in seeds of *C. grandiflorum*.

Cyanolipid (**1**) and the co-occurring glycerides were labelled but label is predominatly found in **1** (Table 2). A lesser degree of incorporation into the fatty acid portions of the molecules suggests that a certain amount of leucine is converted to acetyl CoA and this compound is subsequently incorporated into the fatty acids of both types of compounds. This hypothesis was confirmed by transesterification of the cyanolipid and of glycerides followed by measurement of label in the derived Me

Table 1. Labelling data for *Cardiospermum grandiflorum* forma *hirsutum* seed oil. Sample 1 (138 mg), Sample 2 (83.4 mg) and Sample 3 (181 mg) were fed 20,20, and 50 μCi of L-leucine-[U-^{14}C] respectively

Sample	Mg counted	dpm/mg	dpm/μmol	%Incorporation
1	26.4	199	155	0.1
2	20.9	288	225	0.1
3	30.4	9870	7700	1.6