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CONVERSION OF DOPA TO TETRAHYDROISOQUINOLINES AND STIZOLOBIC ACID IN A CALLUS CULTURE OF *STIZOLOBIUM HASSJOO*

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Key Word Index—*Stizolobium hassjoo*; Leguminosae; Yokohama velvet bean; tetrahydroisoquinolines; stizolobic acid; DOPA; callus culture.

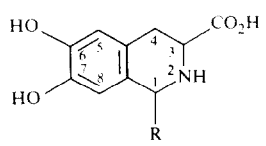
Abstract—Isolation and identification of L-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline and L-1-methyl-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline from seeds and callus of *S. hassjoo* are described. Administration of [β-^{14}C]-labelled DOPA to a callus culture of this legume resulted in the incorporation of radioactivity into L-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, L-1-methyl-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline and stizolobic acid, which was confirmed by constant specific radioactivity after co-crystallization with authentic samples of each compound.

INTRODUCTION

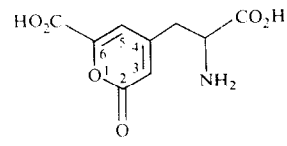
L-3-Carboxy-7, 7-dihydroxy-1, 2, 3, 4-tetrahydroisoquinoline (**1**) and its C-1 methylated analogue, L-1-methyl-3-carboxy-6, 7-dihydroxy-1, 2, 3, 4-tetrahydroisoquinoline (**2**) have been isolated from seeds of *Mucuna mutisiana* [1] and *M. deeringiana* [2], respectively. On the basis of their structures it can be supposed that these imino acids may arise from DOPA (**3**) by cyclization of the alanyl side-chain to an alicyclic hetero form. As the first step in studying the synthetic mechanism of bicyclic ring systems it is necessary to examine the possibility that these tetrahydroisoquinolines are directly formed from **3**. *Stizolobium hassjoo* is a closely related species to *Mucuna* and also accumulates a large quantity of **3** in seeds and seedlings [3], while only a small amount of **3** was found in a callus culture of *S. hassjoo*. In a previous communication [4], the suppression mechanism for **3** accumulation was investigated in the callus culture of this legume and the active catabolism of **3** was suggested as one possible mechanism. We showed previously that stizolobic acid (**4**) was formed from **3** through an extradiol ring fission followed by recyclization to an α-pyrone ring system [5, 6]. The transformation of **3** to **4** was also reported in a suspension culture of *M. deeringiana* [7]. It is interesting to know whether or not **3** is converted to tetrahydroisoquinolines as well as to **4**. In the present paper, identification of two tetrahydroisoquinolines and the conversion of **3** to these compounds in the callus culture of *S. hassjoo* are described.

RESULTS AND DISCUSSION

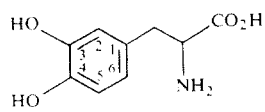
2-D PC of EtOH extracts from seeds and callus of *S. hassjoo* revealed the presence of two uncommon ninhydrin-reacting substances, which were isolated by ion-exchange CC. One gave a bright yellow colour with ninhydrin and the other showed a reddish-brown spot on cellulose TLC. The compounds resembled one another in giving a green colour with $FeCl_3$, blackening with $K_3[Fe(CN)_6]$ and darkening rapidly in alkaline solution. The elution patterns from a Dowex 1 column were very similar to those of authentic **1** and **2**. Comparison of their chromatographic behaviour on cellulose TLC developed with several different solvents, and their ninhydrin and $K_3[Fe(CN)_6]$ coloration, were also in good agreement with those of authentic samples. ^{1}H and ^{13}C NMR spectrum of **2** isolated from the seeds was coincident with structure **2**. In addition to these tetrahydroisoquinolines a trace amount of **4** was detected by 2-D PC from an EtOH extract of the callus culture.



1 R = H
2 R = Me



4



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Preliminary feeding experiments with labelled **3** resulted in radioactive **1**, **2** and **4**. The incorporation of radioactivity was confirmed by co-crystallization with authentic samples (Tables 1–3). All compounds showed constant specific radioactivities after recrystallizing three times. These findings suggest the incorporation of radioactivity of labelled **3** into **1**, **2** and **4** in callus of *S. hassjoo* and the possibility that **3** is a precursor of the 4,5-dihydroxy-β-phenethylamine moiety of **1** and **2**. Müller and Schütte showed that *m*-tyrosine was a precursor of 1-methyl-3-carboxy-6-hydroxy-1, 2, 3, 4-tetrahydroisoquinoline in *Euphorbia myrsinites* [8]. By analogy with evidence obtained from tetrahydroisoquinoline alkaloid biosyntheses in peyote cacti and other plants [9–14], the C-1 carbon unit of **1** and **2** would be derived from α-keto acids or related substances in the course of the synthetic reaction leading to these two tetrahydroisoquinolines. The process, by which **3** cyclizes to tetrahydroisoquinoline, remains to be solved. Biosynthesis of **4** was shown in etiolated seedlings of *S. hassjoo* in our previous papers [5, 6, 15]. It was confirmed that **4** was also synthesized from **3** in a callus culture in the present study.

Table 1. Recrystallization of radiolabelled **1**

Recrystallization no.	Crystal wt (mg)	Sp. act. (dpm/μmol)
1	39.4	3100
2	34.8	2560
3	27.0	2640
4	19.7	2510
5	16.9	2420
6	3.4	2530

Before the first recrystallization, sp. act. was 8931 dpm/μmol.

Table 2. Recrystallization of radiolabelled **2**

Recrystallization no.	Crystal wt (mg)	Sp. act. (dpm/μmol)
1	20.6	527
2	13.9	315
3	7.5	211
4	4.1	174
5	1.5	205

Before the first recrystallization, sp. act. was 5635 dpm/μmol.

Table 3. Recrystallization of radiolabelled **4**

Recrystallization no.	Crystal wt (mg)	Sp. act. (dpm/μmol)
1	9.9	528
2	4.9	120
3	1.8	80
4	1.1	71

Before the first recrystallization, sp. act. was 11600 dpm/μmol.

EXPERIMENTAL

Materials. Seeds of *S. hassjoo* were harvested in a local field during Oct.–Nov. 1978 and 1979. A callus culture was derived from the segments of the etiolated seedlings and subcultured every 4 weeks for 6 years on an agar medium of ref. [16] supplemented with 1 ppm 2,4-D and 0.1 ppm kinetin.

Identification of **1** *and* **2** *from seeds and callus.* Ground seeds (1 kg) were extracted × 4 with 80% EtOH. The combined extract (11.3 l.) was passed through a column of DAIAION SK-1B (Mitsubishi Chemical Industries) (500 ml). After the resin was washed with 80% EtOH and H_2O, successively, amino acids were eluted with 2 M NH_4OH (5 l.). The conc eluate was loaded onto a column of Dowex 1 × 4 (OAc^-, 2.3 × 93 cm) and then eluted with 0.05 M HOAc. Crude crystalline **2** was separated immediately in the eluate of some fractions which were kept standing at 4°, collected by filtration (39 mg), and recrystallized from 10% HOAc; mp 240–264° (decomp.). ^1H NMR, (D_2O + DCl/DSS); 1.7(3H, *d*, J = 8 Hz), 3.2(2H, *m*), 4.3(2H, *m*), 6.70(1H), 6.78(1H). ^{13}C NMR, (D_2O + DCl/DSS); 21.1(*q*, –Me), 31.4(*t*), 56.0(*d*), 58.2(*d*), 115.4(*d*), 118.5(*d*), 125.1(*s*), 127.2(*s*), 145.9(*s*), 146.4(*s*), 173.4(*s*). MS *m/z*: 223(H^+), 208, 162, 123, 107. Although **1** was detected on cellulose TLC in the eluate using 0.05 M HOAc, the isolation was unsuccessful. Comparison of the chromatographic behaviour on cellulose TLC developed with *n*-BuOH–HOAc–H_2O (64:10:27), *n*-BuOH–HOAc–*n*-PrOH–H_2O (4:1:2.5:3.5), PhOH–H_2O (25:8), coloration with ninhydrin and $K_3[Fe(CN)_6]$ of natural **1** and **2** with those of the authentic samples was satisfactory.

41–45-day-old callus (4.5 kg) was blended in cold 99.5% EtOH and filtered. The residue was extracted additionally × 2 with 80% EtOH. The combined extract (18 l.) was passed through a column of Dowex 1 × 4 (OH^-, 50–100 mesh, 3.4 × 142 cm) and amino acids were eluted with 1.7 M HOAc. The conc eluate was treated again on a column of Dowex 1 × 4 (OAc^-, 200–400 mesh, 1.3 × 143 cm) with 0.02 M HOAc. The relevant fractions were conc to a syrup. Further fractionation was carried out repeatedly on a cellulose column (1.3 × 131 cm) with *n* - BuOH–HOAc–*n* - PrOH–H_2O (4:1:2.5:3.5). The conc fraction containing **1** and **2** was separately applied to a column of Dowex 1 × 4 (OAc^-, 200–400 mesh, 0.5 × 50 cm) and displaced with 0.05 M HOAc. Small amounts of **1** (1.3 mg) and **2** (3.3 mg) were obtained. To compare their elution patterns from the Dowex 1 column exactly, amounts of each natural **1** and **2** were dissolved in 0.05 M HOAc and applied to a column of Dowex 1 × 4 (OAc^-, 200–400 mesh, 0.5 × 47 cm) which had been equilibrated previously with 0.05 M HOAc. The fractionation was carried out with the same solvent (40 drops/fraction). Fraction nos. 35–54, **1**; 33–49, **2**. A mixture of the authentic **1** (3 mg) and **2** (3 mg) behaved in the same way on the same column under the same conditions. Fraction nos. 35–49, **1**; 34–44, **2**. R_f values of the natural and synthetic **1** and **2** in *n*-BuOH–HOAc–H_2O (63:10:27) were 0.20 and 0.43, and those in PhOH–H_2O (25:8) 0.53 and 0.74, respectively.

Synthesis of **1** *and* **2**. The method for **1** described in ref. [1] was slightly modified. **3** (2 g) was dissolved in hot H_2O (150 ml) and 37% HCHO (1.9 ml) was added to the cooled soln. The mixture was put in a brown-coloured bottle and

the atmosphere changed to N_2. After 72 hr at room temp. separated crystals (790 mg) were collected and recrystallized from 10% HOAc; mp 250–282° (decomp.). Found: C, 56.79; H, 5.28; N, 6.50. calc. for $C_{10}H_{11}NO_4$: C, 57.43; H, 5.30; N, 6.70%. **2** was synthesized by the method of ref. [2]: mp 240–269° (decomp.). Found: C, 58.58; H, 5.69; N, 6.57. Calc. for $C_{11}H_{13}NO_4$: C, 59.19; H, 5.87; N, 6.27%.

Feeding of radioactive **3**. *Ca* 1 g fr. wt of 31 day-old callus was incubated in 1.7 ml of culture medium containing DL-[β-^{14}C]DOPA (10 μCi, 54 mCi/mmol, NEN, Boston) for 24 hr at 27° in a flask with a centre well in which a small tube containing 0.5 ml scintillamine-OH was placed to trap evolved CO_2. After incubation, the sample was washed × 3 with dist. H_2O and extracted × 4 with hot 80% EtOH. The EtOH extracts were conc and subjected to cellulose TLC developed with PhOH–H_2O (25:8). Authentic samples of **1**, **2** and **4** were co-chromatographed and the spots were detected with ninhydrin. Cellulose powder of each area corresponding to **1**, **2** and **4** on the chromatograms was collected and extracted × 4 with 80% EtOH. The radioactivity of a portion of extracts was counted in Tritosol scintillator [17] or ACS-II (Amersham). Radiolabelled **1**, **2** and **4** were co-crystallized with authentic specimens (61, 60 and 22.3 mg, respectively) from 10% HOAc for **1** and **2**, and from *n*-PrOH–H_2O (3:2) for **4** [5]. The recrystallization was repeated until the crystals showed constant sp. act.

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LIGNANS OF *HORSFIELDIA IRYAGHEDHI*

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Key Word Index—*Horsfieldia iryaghedhi*; Myristicaceae; (+)-asarinin; (−)-dihydrocubebin; lignans; dodecanoylphloroglucinol.

Abstract—A detailed chemical investigation of extracts of the bark, leaf and timber of *Horsfieldia iryaghedhi* collected in Sri Lanka, led to the isolation of (+)-asarinin, dodecanoylphloroglucinol and (−)-dihydrocubebin.

INTRODUCTION

Horsfieldia iryaghedhi Warb. (= *Myristica horsfieldia*, *M. iryaghedhi*; *Sinhala*, Iryaghedhi), a plant indigenous to Sri Lanka is a member of the nutmeg family (Myristicaceae) [1]. The chemical constituents of nutmeg (*Myristica fragrans*) have been studied extensively due to their pharmacological properties [2]. Previous chemical investigation of *H. iryaghedhi* seeds has resulted in the isolation of (+)-asarinin **1** and dodecanoylphloroglucinol **2** [3]. However, no detailed

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