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SHORT REPORTS

L-*CIS*-5-HYDROXYPIPECOLIC ACID FROM SEEDS OF *GYMNOCLADUS DIOICUS*

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Key Word Index—*Gymnocladus dioicus*; Leguminosae; imino-acids; L-*cis* and *trans*-5-hydroxypipecolic acids; amino acids; 2-aminoadipic acid; 2(S),4(R)-4-methylglutamic acid.

INTRODUCTION

The isolation and the determination of the absolute configuration of two acidic amino acids from the seeds of *Gymnocladus dioicus* have been described in preceding papers [1–5]. The amino acids were shown to be: 2(S),3(S),4(R)-3-hydroxy-4-methylglutamic acid and 2(S),-3(R),4(S)-3-hydroxy-4-methylglutamic acid. The present paper describes the separation and characterization of two other acidic compounds,2-aminoadipic and 2(S),-4(R)-4-methylglutamic acids, and the isolation of L-*cis* and *trans*-5-hydroxypipecolic acids. Derivatives of pipecolic acid are widespread in living systems and *trans*-5-hydroxypipecolic acid [6, 7], *trans*-4-hydroxypipecolic acid [8, 9], 4-aminopipecolic acid [10], 4,5-dehydropipecolic acid [11], 2,4-*trans*-4,5-*cis*-4,5-dihydroxypipecolic acid [12, 13], 2,4-*cis*-4,5-*trans*-4,5-dihydroxypipecolic acid [14], 2,4-*trans*-4,5-*trans*-4,5-dihydroxypipecolic acid [15], S(2),5(S),6(S) and 2(S),5(R),-6(S)-5-hydroxy-6-methylpipecolic acids [16] and γ-glutamylpipecolic acid [17] occur in higher plants. L-*trans*-5-hydroxypipecolic acid has also been isolated from fungi [18].

RESULTS AND DISCUSSION

Analysis of a large quantity of seeds of *G. dioicus* revealed that they contained two other acidic compounds namely 2-aminoadipic acid and 2(S),4(R)-4-methylglutamic acid. The configuration of the Me group in this glutamic acid derivative was established by comparison of IR and NMR spectra with those of authentic material.

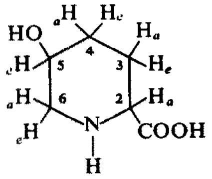
In addition to major concentrations of acidic compounds, large amounts of 5-hydroxypipecolic acid and pipecolic acid were detected. On 2D PC, 5-hydroxypipecolic appears as a partially resolved double spot but good resolution is obtained on HVE at pH 1.9. The two compounds give a blue colour with ninhydrin and a green colour with isatine. After reaction with ninhydrin, they give a red fluorescence in UV characteristic of pipecolic acid and its derivatives. One of the compounds moved like *trans*- 5-hydroxypipecolic acid added as a marker but the other moved faster and must therefore be *cis*- 5-hydroxypipecolic acid. The isolation was performed by use of ion exchange chromatography mainly on acid resins in the H^+ form and in the Na^+ form equilibrated with a buffer solution of pH 3.2 [2]. These methods in combination with preparative PC permitted the isolation of pure *cis* and *trans*-5-hydroxypipecolic acids. The imino-acids in the free form were recrystallized in H_2O–EtOH and the results of elementary analysis are in good agreement with the formula $C_6H_{11}NO_3$. Oxidation with $KMnO_4$ of both compounds yielded glutamic acid. These investigations suggest that the two imino acids are isomeric forms of 5-hydroxypipecolic acid. Their IR spectra, however, show important differences. The spectrum one of the compounds was identical to that of authentic L-*trans*-5-hydroxypipecolic acid. The NMR spectra in D_2O of the unknown compound was compared with those of natural 4-*trans*- and 5-*trans*-hydroxypipecolic acids [19]. From the values of chemical shifts and coupling constants, the *cis* configuration can therefore be assigned to this new pipecolic derivative (see Table 1 and formula). The values of optical rotation in water and in HCl show that the new natural imino-acid belongs to the L-series. The isolation from a natural source of L-*cis*-5-hydroxypipecolic acid or 2(S)-carboxy-5(R)-hydroxypiperidine has not previously been reported.

Metabolically related compounds of 5-hydroxypipecolic acid (lysine and 2-aminoadipic acid derivatives) have not been detected by 2D PC. The main report on *cis*-5-hydroxypipecolic acid is that of Thompson and Morris [20] where the administration of radioactive 5-hydroxylysine to *Gleditsia triacanthos* resulted in a nearly

Table 1. PMR chemical shifts and coupling constants of L-*cis*-5-hydroxypipecolic acid

Protons	5(1H)	2(1H)	6(2H)	3–4(4H)
δ°	4.14(*quint*)	3.61(*q*)	3.25(*o*)	1.75–2.25(*m*)
J°	$J_{6a-5e} = 3$	$J_{6e-5e} = 4$		$J_{6a-6e} = 13$
	$J_{2a-3a} = 9.5$		$J_{2a-3e} = 5.5$	

a = axial; *e* = equatorial; *quint* = quintuplet; *q* = quadruplet, *o* = octuplet; *m* = multiplet; ° first order approximation.



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quantitative conversion to 5-hydroxypipecolic acid. Strong but not conclusive evidence has been adduced that both the *cis* and *trans*-5-hydroxypipecolic acids are present in *G. triacanthos*.. Determination of the imino-acids was established by co-chromatography.

EXPERIMENTAL

Seeds of *G. dioicus* (L) Koch. were collected in October 1972 in Oklahoma (USA). Voucher specimens are deposited in the Department of Organic and Biological Chemistry, Faculté des Sciences Agronomiques, Gembloux.

Isolation of cis *and* trans *5-hydroxypipecolic acid*. (For extraction, purification and separation of acidic, neutral and basic amino acids, see ref. [2]). Neutral extract from 1.7 kg of seeds was dissolved in a min. vol. of 1.5 N NCl and passed through a column (150 × 5 cm) of Lewatit S 1080 in the H^+ form. The amino acids were eluted with 1.5 N HCl (12 ml fractions). 5-Hydroxypipecolic acid is eluted in fractions 80–140 (9 g) and pipecolic acid in fractions 160–195. *Cis* and *trans*-5-hydroxypipecolic were then separated on Dowex 50 W × 8 (50 × 4 cm) in the Na^+ form, equilibrated with a buffer sol. of pH 3.2 [2]. *Cis*-5-hydroxypipecolic acid is eluted in fractions 235–250 and *cis* + *trans*-5-hydroxypipecolic acid in fractions 251–335 (10 ml fractions). From the latter fractions, *cis* and *trans*-5-hydroxypipecolic acids were separated using preparative PC (*tert* amyl alcohol–2,4-lutidine–H_2O; 89:89:57). Elution was performed during 5 days. $R_{cis/trans}$ = 0.84 (Piez *et al.*: 0.83) [21]. *Cis*-5-hydroxypipecolic acid recrystallized (170 mg): C, 49.65; H, 7.64; N, 9.65 calculated for $C_6H_{11}NO_3$: C, 49.47; H, 7.61; N, 9.62%. $[\alpha]_{589}^{22^\circ}$ nm = −29.5° (*c*, 2; H_2O), $[\alpha]_{589}^{22^\circ}$ nm = −17.3° (*c*, 1; HCl 6 N). IR(KBr) ν_{max}cm^{-1}: 3400 (large), 3000 (large), 1625 (*s*), 1440(*w*), 1400(*s*), 1340(*w*), 1315(*w*), 1210(*w*), 1165(*w*), 1130(*w*), 1090(*m*), 1040(*m*), 980(*m*), 935(*w*), 850(*w*), 770(*m*).

General instrumentation. PC was performed in *n*-BuOH–HOAc–H_2O (12:3:5) and phOH at pH 4.2. HVE was carried out in buffer at pH 1.9; 60 V/cm; 90 min. The mobilities of the hydroxypipecolic acids in cm were *cis* form = 13.6; *trans* form = 12.0 PMR spectra were measured at 100 MHz and chemical shift are in ppm downfield from 2,2,3,3,-tetradeuterio-3-(tris-TMSi)propionate in D_2O.

From a personal communication, we have just heard that Hatanaka has isolated *cis*-5-hydroxypipecolic acid from *Lathyrus japonicus* (seeds) and *cis* and *trans*-5-hydroxypipecolic acid from *Morus multicaulis* (leaves).

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SOLUBLE SUGARS OF MAIZE SEEDLINGS*

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Key Word Index—*Zea mays*; Gramineae; maize seedlings; soluble sugars.

Abstract—The soluble sugars were determined in different parts of maize seedlings (seeds, roots and shoots), 0, 2, 4 and 6 days after sowing.

INTRODUCTION

Although germination, as a series of biochemical processes, has been extensively studied, information on such 'simple' biochemical events as the hydrolysis of seed polymers, is still lacking [1]. The present work approaches the chemical differences during early stages