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SHORT REPORTS

2,4-*CIS*-4,5-*CIS*-4,5-DIHYROXYPIPECOLIC ACID—A NATURALLY OCCURRING IMINO ACID FROM *CALLIANDRA PITTIERI*

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Key Word Index—*Calliandra pittieri*; Mimosoideae; Leguminosae; imino acid; pipecolic acid; 2,4-*cis*-4,5-*cis*-4,5-dihydroxypipecolic acid.

Abstract—2,4-*cis*-4,5-*cis*-4,5-Dihydroxypipecolic acid has been isolated from the leaves of *Calliandra pittieri*. This is the third dihydroxypipecolic acid isomer isolated from *Calliandra* and the first report of this compound from a natural source.

INTRODUCTION

Pipecolic acid derivatives have restricted distributions in plants [1]. The genus *Calliandra*, however, has a wide array of these imino compounds. 2,4-*trans*-4,5-*cis*-4,5-Dihydroxypipecolic acid (2*S*-carboxy-4*R*,5*S*-dihydroxypiperidine) and 2,4-*trans*-acetylaminopipecolic acid (2*S*,4*R*-carboxy-2-acetylamino-4-piperidine) have been isolated from leaves of *Calliandra haematocephala* [2, 3]; 2,4-*trans*-4,5-*trans*-4,5-dihydroxypipecolic acid (2*S*-carboxy-4*R*,5*R*-dihydroxypiperidine) and *cis*-5-hydroxypipecolic acid have been isolated from leaves of *C. angustifolia* and sap of *C. confusa* [4]; and the more common compounds, pipecolic acid, *trans*-4-hydroxypipecolic acid, and *trans*-5-hydroxypipecolic acid are found in several North American species occupying xeric habitats [5]. This paper describes the isolation from leaves of *C. pittieri* of 2,4-*cis*-4,5-*cis*-4,5-dihydroxypipecolic acid (2*S*-carboxy-4*S*,5*R*-dihydroxypiperidine), the third dihydroxy stereoisomer from this genus and a compound not previously reported from a natural source.

RESULTS AND DISCUSSION

An ethanolic extract of *C. pittieri* revealed a compound which gave the typical blue-green color with ninhydrin characteristic for dihydroxypipecolic acid isomers. The compound fluoresced brick-red under UV and gave a positive reaction with isatin. Subjected to high voltage paper electrophoresis (HVE) (pH 1.9), the compound co-migrated with a standard of the *trans–trans*-4,5-dihydroxypipecolic acid isomer (R_{ala} 0.66). The compound also co-eluted with this isomer at 13 min on the amino acid analyser. The compound, however, was distinguished from its *trans–trans* isomer on the basis of 1D chromatography in solvent systems 1 (R_{ala} 0.37 vs 0.59 for the *trans–trans* isomer) and 2 (R_{ala} 0.36 vs 0.97 for the *trans–trans* isomer). The compound is distinguished from the *trans–cis* isomer by HVE (pH 1.9) (R_{ala} 0.61) and in solvent system 3 (R_{ala} 0.87 vs R_{ala} 0.78 for the *trans–cis* isomer). The compound was tentatively identified as the *cis–cis* isomer from R_{ala} values previously reported for the synthetic compound [6]. After isolation, identity was confirmed by comparison of IR and NMR spectra with a synthesized authentic sample of this isomer.

The isolation of this compound brings to three the number of dihydroxypipecolic acid stereoisomers reported from *Calliandra* and to eight the number of non-protein imino compounds variously distributed in this genus. In addition to *C. pittieri* the *cis–cis* isomer was also consistently detected as a major compound in the leaves of six other Central and South American species of *Calliandra*: *C. formosa*, *C. lambertiana*, *C. tenuiflora*, *C. deamii*, *C. purdiaei*, *C. carbonaria* and in lesser amounts in 11 others. It is also found in the seeds of the above species which have been examined. The concentration of the *cis–cis* isomer in those species containing it ranged between 0.02 and 0.25% dry wt. The distribution pattern of this and the other imino compounds is expected to be useful in the systematics of *Calliandra*, a genus presently undergoing morphological revision.

EXPERIMENTAL

Chromatographic methods. Solvents for PC were: (1) *n*-BuOH–HCO_2H–H_2O (15:3:2), (2) *t*-AmOH–2,4-lutidine–H_2O (178:178:114), (3) 80% PhOH–H_2O (w/v) in the presence of NH_3 vapor. HVE was carried out in buffer at pH 1.9, 60 V/cm, 35 min. Automated amino acid analysis was performed using a modified Dionex amino acid analyser adapted for fluorometric detection [7]. Leaf extracts were prepared using MeOH–$CHCl_3$–H_2O (12:5:1) [8].

Collection and documentation of material. Leaves of *Calliandra pittieri* were collected in July 1982 from a tree growing in Medellin, Colombia. A voucher specimen is on deposit at the University of South Florida, Tampa, U.S.A.

Isolation of 2,4-cis-4,5-cis-4,-5-*dihydroxypipecolic acid.* Oven dried leaves (400 g) were ground to a powder and extracted × 3 with 50% EtOH. The combined extract (8 l.) was passed through a column (3 × 50 cm) filled with Amberlite CG-120, cation exchange resin (H^+), 100–200 mesh. After washing with several vols. EtOH and H_2O, amino acids were eluted with 4 H NH_3. Ninhydrin positive effluent was evaporated to a small vol., placed on a column (2 × 100 cm) of Amberlite CG-400, anion exchange resin (Cl^-) 100–200 mesh and eluted with H_2O at a flow rate of

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1 ml/min. 10 ml fractions were collected and fractions 35–47 containing neutral amino acids were combined and evaporated to a thick yellow syrup. The syrup was diluted with a small vol. of 1.5 N HCl and run through a column (2 × 100 cm) of Dowex 50 × 8, cation exchange resin (H^+), 200–400 mesh. Amino acids were eluted with 1.5 N HCl at a flow rate of 1 ml/min with 10 ml fractions collected. Fractions 54–59 contained 2,4-*cis*-4,5-*cis*-4,5-dihydroxypipecolic acid and a second uncharacterized imino compound which gave a blue ninhydrin color. These fractions were combined, evaporated to a small vol. and desalted on a small CG-120, (H^+) column. The imino acids were eluted from the column with 2 N NH_3 and concd. The two compounds were separated by ascending PC on Whatman 3MM paper using solvent system 3. The *cis–cis* dihydroxy isomer migrated as the upper band. This was cut from the paper, extracted with a large vol. of H_2O and placed on a small CG-120, (H^+) column to remove residual PhOH. The pure *cis–cis* isomer was eluted from the column with 2 N NH_3, evaporated to dryness and recrystalized twice in aq. EtOH. 270 mg of compound were obtained.

Spectral analysis. Spectral data for both the free and chlorohydrate forms of the *cis–cis* isomer were obtained. IR and NMR spectra of synthesized samples of all four 4,5-dihydroxypipecolic streoisomers were provided by G. Dardenne (Faculté des Sciences Agronomiques, Gembloux, Belgium). The IR spectra for the *cis–cis* isomer showed the appropriate functional group absorptions and the fingerprint region also matched exactly that of the synthetic *cis–cis* compound [6]. NMR spectra (recorded at 100 MHz in D_2O with TSS as a standard) also corresponded closely to those of the synthetic compound. The chlorhydrate spectrum gave characteristic multiplets in the regions 1.9–2.4, 3.1–3.6 and 3.9–4.2 [6].

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N-ACETYL-6-HYDROXYTRYPTOPHAN A NATURAL SUBSTRATE OF A MONOPHENOL OXIDASE FROM *ASPERGILLUS NIDULANS*

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Key Word Index—*Aspergillus nidulans*; mutant; conidiophore pigment; monophenol oxidase; enzyme substrate; *N*-acetyl-6-hydroxytryptophan.

Abstract—An *ivo B* mutant of *Aspergillus nidulans*, deficient in conidiophore pigment has been shown to accumulate *N*-acetyl-6-hydroxytryptophan. This acts as a substrate for a specific monophenol oxidase present in the wild type but absent in the mutant.

INTRODUCTION

Many species of *Aspergillus* have a grey-brown pigment in the upper parts of the conidiophore, including the metulae and phialides [1]. Studies with conidiation mutants of *A. nidulans* [2] have used aconidial mutants, such as *brl A42*, in which these parts of the mycelium are exposed at the colony surface, to select 'ivory' mutants lacking this pigment. These mutants fall into two groups, genetically distinguished as belonging to *ivo A* and *ivo B* loci. Mutants of the latter type are deficient in a specific phenol oxidase [3] and accumulate, at the time of conidiophore formation, a pigment precursor which is the subject of this paper. The *ivo A* mutants possess the phenol oxidase and are assumed to be unable to synthesize the precursor.