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2,4-*TRANS*-4,5-*TRANS*-4,5-DIHYDROXYPIPECOLIC ACID AND *CIS*-5-HYDROXYPIPECOLIC ACID FROM LEAVES OF *CALLIANDRA ANGUSTIFOLIA* AND SAP OF *C. CONFUSA*

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Key Word Index—*Calliandra angustifolia; C. confusa;* Mimosoideae; Leguminosae; imino acid; 2,4-*trans*-4,5-*trans*-4,5-dihydroxypipecolic acid; *cis*-5-hydroxypipecolic acid.

Abstract—2,4-*Trans*-4,5-*trans*-4,5-dihydroxypipecolic acid and *cis*-5-hydroxypipecolic acid have been isolated from the leaves of *Calliandra angustifolia* and the sap of *C. confusa*. Distribution of these and other non-protein amino acids is discussed.

INTRODUCTION

While L-pipecolic acid, *trans*-5-hydroxypipecolic acid and *trans*-4-hydroxypipecolic acid are relatively common constituents of the free amino acid pools of many legumes [1], the more complex pipecolic acid derivatives appear to have very restricted distributions. Previous chemical work has established that two unusual imino acid derivatives, 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-piperdine) are constituents of leaves of *Calliandra haematocephala* [2, 3]. The present paper describes the isolation of two additional pipecolic acid derivatives from leaves of *C. angustifolia* and sap of *C. confusa:* 2,4-*trans*-4,5-*trans*-4,5-dihydroxypipecolic acid (2*S*-carboxy-4*R*,5*R*-dihydroxypiperidine); a compound previously reported only from seeds of two species of *Julbernardia* and one of *Brachystegia* (Caesalpinoideae) [4]; and *cis*-5-hydroxypipecolic acid, previously known from seeds of *Gymnocladus* and *Gleditsia* spp. (Caesalpinoideae) [5, 6] and seeds of *Lathyrus japonicus* (Papilionoideae) [7].

RESULTS AND DISCUSSION

Two-dimensional paper chromatography of 50% ethanolic extracts of *C. angustifolia* leaves and *C. confusa* sap revealed small amounts of common amino acids and two major unidentified compounds, one of which gave a blue-green color with ninhydrin, the other a typical pipecolic acid violet color. Both compounds fluoresced red under UV and gave a positive isatin reaction. They were tentatively identified as 2,4-*trans*-4,5-*trans*-4,5-dihydroxypipecolic acid and *cis*-5-hydroxypipecolic acid on the basis of R_{ala} values and HVE [8]. Identity of the former was confirmed by comparison of NMR and IR spectra of the isolated compounds with those of a standard, and by synthesis. After isolation, *cis*-5-hydroxypipecolic acid was confirmed by cochromatography with an authentic standard in three solvent systems and HVE at pH 1.9.

These two compounds bring to four the number of rare imino acid derivatives reported from *Calliandra* spp. Neither the *trans*–*trans*-4,5-dihydroxy isomer nor *cis*-5-hydroxypipecolic acid has been previously reported from the Mimosoideae. Together with four other more common imino acids, proline, pipecolic acid, *trans*-4-hydroxypipecolic acid, and *trans*-5-hydroxypipecolic acid, these compounds may have chemosystematic significance within *Calliandra*. Twenty-two species were assayed for distribution of these eight imino acids. Several patterns were observed.

2,4-*Trans*-4,5-*trans*-dihydroxypipecolic acid was detected in seven central American spp. always accompanied by *cis*-5-hydroxypipecolic acid. Similarly the 2,4-*trans*-4,5-*cis*-4,5-dihydroxy isomer was usually accompanied by *trans*-5-pipecolic acid in those species in which it occurs. Two species had both 4,5-dihydroxy isomers and both *cis*- and *trans*-5-OH pipecolic acid. Acetylaminopipecolic acid was observed in two species. The most striking feature was the large amounts of proline, pipecolic acid and *trans*-4-hydroxypipecolic acid consistently found in xeric species and the virtual absence from these plants of the rarer derivatives. The uncommon imino acids appear to be restricted to the mesic species of central America. Whether or not the distribution patterns have taxonomic significance awaits the analysis of a greater number of species. Preliminary analysis indicates some correlation with the subgeneric classification of Britton and Rose [9].

EXPERIMENTAL

Chromatographic methods. Solvents for PC were: (1) *n*-BuOH–HOAc–H_2O (12:3:5), (2) 80% phenol–H_2O (w/v) in the presence of NH_3 vapor, (3) *n*-BuOH–HCO_2H–H_2O (15:3:2), (4) *t*-AmOH–2,4-lutidine–H_2O (178:178:114). HVE was carried out in buffer at pH 1.9, 60 V/cm, 45 min and the two dihydroxy isomers resolve into two spots whose R_{ala} mobilities are: 0.66 for the *trans–trans* and 0.61 for the *trans–cis* isomer. Similarly the *cis*- and *trans*-5-OH isomers give R_{ala} values of 0.74 and 0.68 respectively. Chromatograms and HVE papers were developed with ninhydrin and isatin.

Collection and documentation of material. Leaves of *Calliandra angustifolia* were collected in October 1978 from specimens growing at the Rio Palenque field station in Ecuador. A voucher specimen is on deposit at the University of South Florida in

Tampa, Florida. Sap of *Calliandra confusa* was provided by Merck, Sharp and Dohme Research Labs, Rahway, New Jersey from samples collected by Monie S. Hudson in Guatemala—identification No. L-259,218-00K01. 2 g of concentrated sap extracted from trees via the Prescap process was used for analysis.

Isolation of 2,4-trans-4,5-trans-4,5-*dihydroxypipecolic acid.* Finely ground dried leaf material (1.2 kg) was extracted 4 × with 50% EtOH. Twelve l of extract was placed on an ion exchange column (4.8 cm × 60 cm) containing Amberlite CG-120, H^+, 100–200 mesh, to remove uncharged macromolecules, pigments and anions. After washing with H_2O, amino acids were eluted with 2 N NH_3. The conc extract was taken up in 0.1 N HOAc and placed on a large column (4.8 × 95 cm) containing Amberlite CG-400, OAc^-, 100–200 mesh. Amino acids were eluted with 0.1 N HOAc and 8 ml fractions collected. Fractions 78–104 contained basic amino acids, fractions 105–116 neutral amino acids and fractions 117–210 acidic amino acids. The neutral fraction was evapd to a thick yellow syrup, taken up in 0.2 M sodium citrate buffer, pH 3.3, and placed on a column (2.6 × 110 cm) containing Dowex 50 × 8, Na^+, 200–400 mesh resin. Amino acids were eluted with the 0.2 M citrate buffer and 10 ml fractions collected. Fractions 37–53 contained only the *trans–trans*-dihydroxy isomer. Fractions 129–320 contained *cis*-5-OH-pipecolic acid and alanine. Fractions 311–350 had assorted neutrals and proline, and fractions 425–440 assorted neutrals and pipecolic acid.

The *trans–trans*-4,5-dihydroxypipecolic acid fraction was concentrated and placed on a Dowex 50 × 8, H^+, 100–200 mesh for desalting. After washing with H_2O, amino acids were eluted with 2 N NH_3. The compound was recrystallized from Me_2CO–H_2O. 780 mg pure compound was obtained. Identity was confirmed by IR and NMR spectra and by synthesis.

Isolation of cis-5-*hydroxypipecolic acid.* The purer fractions of *cis*-5-hydroxypipecolic acid, 241–320, were desalted, evapd to dryness and the compound crystallized from Me_2CO–H_2O. 270 mg of pure material was obtained. Identity was confirmed by cochromatography with an authentic sample. R_{ala} values in solvent system 4 match those previously reported [10].

Isolations from sap material. Ethanolic extracts (50%) were cleaned on an Amb. CG-120, H^+ ion exchange column. 2,4-*Trans*-4,5-*trans*-4,5-dihydroxypipecolic acid and *cis*-5-dihydroxypipecolic acid were isolated by prep. 1D-PC in solvent system 2 followed by prep. HVE pH 1.9. Elutions were purified on small ion exchange columns (Amb 120, H^+ form). A few mg of pure material was obtained and identities were confirmed by cochromatography and NMR.

Synthesis of 2,4-trans-4,5-trans-4,5-*dihydroxypipecolic acid.* The procedure of ref. [11] was followed for synthesis of the *trans* isomers. 39 mg L-Baikiain (Calbiochem) was added to 2.1 ml of 30% HCO_2H–H_2O_2 (3:7). This was heated at 40° for 1 hr with agitation and then evapd to a small vol. The resulting thick yellow liquid was applied to a CG 120, H^+ column (0.5 × 15 cm), washed with H_2O and eluted with 2N NH_3. After evapn to dryness, it was taken up in 50% EtOH and HVE performed. Two blue-green spots, the stronger of which migrated with the *C. angustifolia* isomer resulted. The second weaker spot is the *cis–trans* isomer. The expected ratio of the isomers is 10:1 in favor of the *trans–trans* using this procedure.

Spectral analysis. An authentic sample of 2,4-*trans*-4,5-*trans*-4,5-dihydroxypipecolic acid was not available for cochromatography. IR and NMR spectra of an authentic sample of this isomer as well as the other three possible isomers were, however, generously supplied by G. Dardenne (Faculté des Sciences Agronomiques, Gembloux, Belgium). The IR spectrum for the *trans–trans* isomer corresponded to that for the authentic *trans–trans* isomer.

NMR spectra were recorded at 100 MHz in D_2O with TSS as a standard. The spectra of the free and chlorhydrate forms were obtained and both corresponded closely to those provided by Dardenne. The chlorhydrate spectrum gave characteristic multiplets in the regions 2.2–2.4, 3.3–3.5, 3.9–4.1 and 4.2–4.4.

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