Phytochemistry, Vol. 25, No. 6, pp. 1469–1470, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00

SHORT REPORTS

BIOSYNTHESIS OF PIPECOLIC ACID AND 4-HYDROXYPIPECOLIC ACID

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(*Received* 22 *October* 1985)

Key Word Index—*Acacia mellifera* subsp. *detinens*; Mimosaceae; biosynthesis; pipecolic acid; 4-hydroxypipecolic acid.

Abstract—Pipecolic acid is formed from lysine, and 4-hydroxypipecolic acid is formed from pipecolic acid in the leaves of *Acacia mellifera* subsp. *detinens*.

Pipecolic acid appears to be universally derived from lysine ostensibly through the oxidative deamination of either the alpha [1–3] or the epsilon [4] amino group followed by reductive cyclization. The biosynthesis of 5-hydroxypipecolic acid is claimed to follow an analogous pathway [5–7] with 5-hydroxylysine as the substrate. However, an attempt to biosynthesize 4-hydroxypipecolic acid from 4-hydroxylysine was unsuccessful [4]. In this case pipecolic acid was found to serve as a suitable substrate for the biosynthesis of 4-hydroxypipecolic acid [4].

In the present study, pipecolic acid, 4-hydroxypipecolic acid and alanine were critically identified as major constituents of the free amino acid pool of the leaves of *Acacia mellifera* (Vahl) Benth. subsp. *detinens*. Support for the biosynthesis of 4-hydroxypipecolic acid by the hydroxylation of pipecolic acid was obtained by feeding L-[U-^{14}C]lysine to detached leaves of *A. mellifera* subsp. *detinens* through their petioles and analysing the leaves for ^{14}C-labelled amino acids after 2, 4 and 24 hr of incubation. After 2 and 4 hr, pipecolic acid was the only amino acid that became labelled. The incorporation of the label into pipecolic acid proceeded at a constant rate throughout the 24 hr experimental period. 4-Hydroxypipecolic acid also became labelled, but only after 24 hr of incubation.

When [U-^{14}C]pipecolic acid was fed to fresh *A. mellifera* subsp. *detinens* leaves, ^{14}C-labelled 4-hydroxypipecolic acid was detected in the leaves 4 hr after the start of the experiment.

Although an earlier comprehensive survey of the free amino acids of the seeds of *Acacia* species did not reveal either pipecolic acid or 5-hydroxypipecolic acid in *A. mellifera* subsp. *detinens* [8], the present study provides conclusive evidence for the occurrence of the former and tentative evidence for the occurrence of the latter amino acid in the leaves of this species.

EXPERIMENTAL

Fresh leaves (1.9 kg) of *A. mellifera* subsp. *detinens* were macerated in 21 l. 70% EtOH. After shaking for 24 hr at room temp. the homogenate was filtered and concd below 55° to *ca* 1 l. Samples of the concentrate were subjected to two-dimensional PC on Whatman no. 1 filter paper using PhOH–H_2O (2.6:1) and *n*-BuOH–HOAc–H_2O (9:1:2.9) as solvents and the PC treated with a 0.2% soln of ninhydrin in MeOH containing 1% HOAc, before heating at 80°.

The bulk of the concentrate was filtered and its amino acids fractionated by ion exchange chromatography [9]. Pure crystalline isolates of alanine, pipecolic and 4-hydroxypipecolic acid were obtained and critically identified by co-chromatography with authentic standards as well as by comparative MS and ^{1}H NMR.

L-[U-^{14}C]Lysine, contained in horizontally-held glass capillary tubes, was fed to the cut surface of the petioles of detached young but mature leaves of *A. mellifera* subsp. *detinens*. The leaves were maintained at 27° and a white light irradiance of 170 to 200 μE/m^2/s at leaf level throughout the incubation period. *Ca* 40 μg lysine in 50 μl H_2O was used per 0.25 g fresh leaf material. The sp. act. of the soln was 9.25×10^4 Bq/ml. After the lysine soln was absorbed, the capillary tubes were filled with H_2O. Leaves were harvested 2, 4 and 24 hr after the start of the experiment.

The leaves were homogenized in 70% EtOH and the homogenate filtered. Aliquots of the concd filtrates were subjected to PC and a radioautogram of each chromatogram was prepared. The radioactivity of the pipecolic acid and 4-hydroxypipecolic acid spots of the 24 hr treatment, as measured with a liquid scintillation counter were 2003 and 331 dpm respectively.

The remainder of those extracts which contained [^{14}C]pipecolic acid were used for the PC isolation of the [^{14}C]pipecolic acid which was then fed to leaves of *A. mellifera* subsp. *detinens* are described above for [^{14}C]lysine.

REFERENCES

1. Grobbelaar, N. and Steward, F. C. (1953) *J. Am. Chem. Soc.* **75**, 4341.
2. Meister, A., Padhakrishnan, A. N. and Buckley, S. D. (1957) *J. Biol. Chem.* **229**, 789.
3. Gupta, R. N. and Spencer, I. D. (1970) *Phytochemistry* **9**, 2329.
4. Fowden, L. (1960) *J. Exp. Botany* **11**, 302.
5. Cohen, L. A., Irreverre, F., Piez, K. A., Witkop, B. and Wolf, H. L. (1956) *Science* **123**, 842.
6. Lindstedt, S. and Lindstedt, G. (1959) *Arch. Biochem. Biophys.* **85**, 565.
7. Thompson, J. F. and Moore, D. P. (1968) *Biochem. Biophys. Res. Commun.* **31**, 281.
8. Evans, C. S., Qureshi, M. Y. and Bell, E. A. (1977) *Phytochemistry* **16**, 565.
9. Hirs, C. H. W., Moore, S. and Stein, W. H. (1952) *J. Biol. Chem.* **195**, 669.

Phytochemistry, Vol. 25, No. 6, pp. 1470–1471, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00

A 2-OXO-3-PYRROLINE DIMER FROM *MERCURIALIS LEIOCARPA*

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(*Revised received* 11 *November* 1985)

Key Word Index—*Mercurialis leiocarpa*; Euphorbiaceae; 2-oxo-3-pyrroline derivative.

Abstract—A neutral component has been isolated from *Mercurialis leiocarpa* and its structure determined by X-ray analysis as 3,3′-bis-(1,1′-dimethyl-2,2′-dioxo-4,4′-dimethoxy-5,5′-dihydroxy-5,5′-dimethoxycarbonyl-3-pyrroline).

Mercurialis leiocarpa Sieb. et Zucc. [1], was used as an indigo dye in Japan between the 8th and 12th centuries. A neutral component has been isolated in crystalline form, and the structure determined by a single crystal diffraction analysis. The neutral component (**1**) (mp 266–268°, $[\alpha]_D^{20} = 0$), was isolated in 0.03% yield as colourless needles from the methanolic extract of the fresh herb of *Mercurialis leiocarpa*.

The molecular formula $C_{16}H_{20}N_2O_{10}$ was determined on the basis of the mass spectrum (*m/z* 400.1131) and elemental analysis. The IR spectrum showed the presence of a hydroxyl group (3475 and 3300 cm^{-1}), carbonyls (1760, 1745, 1700 and 1685 cm^{-1}) and an enol double bond (1645 cm^{-1}). The UV absorption spectrum gave no characteristic maxima. The ^1H ($CDCl_3$) and ^{13}C (DMSO-d_6) NMR spectra were rather simple, which suggested **1** to be a dimer of a $C_8H_{10}NO_5$ unit. In addition to the carbonyl absorptions in the IR spectrum, the appearance of the skeletal carbons as singlets in the ^{13}CNMR spectrum made the structural assignment difficult.

The correct structure was established by a single crystal X-ray diffraction study as 3,3′-bis-(1,1′-dimethyl-2,2′-dioxo-4,4′-dimethoxy-5,5′-dihydroxy-5,5′-dimethoxycarbonyl-3-pyrroline). The reddish colour of **1** in sodium methoxide–benzene and its decoloration on exposure to air were very similar to those of crysohermidine, isolated from *Mercurialis perennis* L. [2]. This seems to suggest the possibility of the transformation of **1** to crysohermidine.

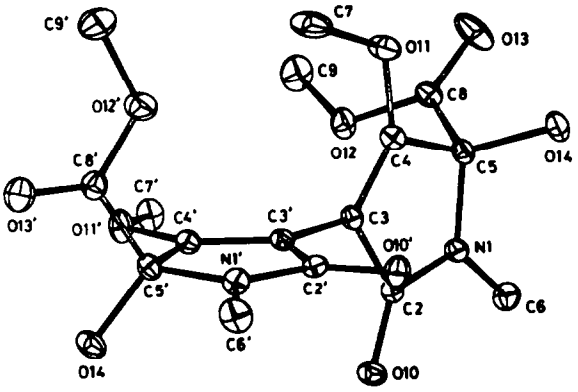

Fig. 1. Computer generated perspective drawing of **1**.