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SHORT REPORTS

CIS-5-HYDROXY-L-PIPECOLIC ACID FROM *MORUS ALBA* AND *LATHYRUS JAPONICUS*

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Key Word Index—*Morus alba*; *Lathyrus japonicus*; Moraceae; Leguminosae; *cis*-5-hydroxy-L-pipecolic acid: *trans*-5-hydroxy-L-pipecolic acid.

Abstract—*cis*-5-Hydroxy-L-pipecolic acid was isolated and characterized from the leaves of *Morus alba* and the seeds of *Lathyrus japonicus*. The *trans*-form was also obtained from the former.

INTRODUCTION

5-Hydroxypipecolic acid has been found in several plants [1] and we have isolated this imino acid from a fungus, *Stereum ostrea* (Blume et Nees) Fr. [2]. The natural 5-hydroxypipecolic acid isolated from these sources was in the *trans*-L form [3].

Among the many non-protein amino acids which have been isolated from *Lathyrus* spp [4], 5-hydroxypipecolic acid has been detected by PC in the seeds of *Lathyrus japonicus* (=*L. maritimus*). [5] 5-Hydroxypipecolic acid may also occur in mulberry leaves [6]. The configuration of 5-hydroxypipecolic acid in these two plants, however, was not established. Dardenne *et al.* (personal communication) have recently isolated both isomers of 5-hydroxy-L-pipecolic acid from the legume, *Gymnocladus dioicus*.

RESULTS AND DISCUSSION

Two ninhydrin- and isatin-positive substances on 2 D-PC of the free amino acids of mulberry leaves, developed with solvents (a) and (b), (see Experimental), successively, were coincident with *trans*- and *cis*-5-hydroxypipecolic acid. All five cultivated forms of this species examined, Ichinose, Shin-ichinose, Kenmochi, Akameroso and Garyu, showed the same PC pattern of the free amino acids of their leaves.

These two amino acids could be separated from each other by fractionation on Dowex 50 (Na^+-form) with 0.2M citrate buffer, pH 3.3 [7]. Other amino acids were then destroyed by deamination with nitrous oxides and the mixture of the resistant imino acids were fractionated on Dowex 50 (H^+-form) with 1.5M HCl [3]. Identification was based on mp, optical rotation, elementary analysis, IR, colour reactions and R_f on PC. The IR spectrum of the *trans*-isomer (HCl-form) was superimposable on that of the authentic sample. Though the authentic *cis*-form could not be obtained, major bands in IR of the hydrobromide agreed well with those reported for that of *cis*-L-form, prepared from natural *trans*-L-isomer [3]. A bluish colour was given with ninhydrin and the products of both isomers were brick-red under UV at 365 nm. The *trans*-form moved on PC with the solvents (a), (b) and (c) at the same rates as those of the authentic specimen. Additionally, we obtained exactly the same R_{Ala} values for the *cis*- and *trans*-forms with solvent (c) as reported earlier [7].

Judging from the spot-size and the intensity of the ninhydrin-colouration, the concentrations of both isomers in mulberry leaves are similar, while the yields of the isolates were considerably different.

In contrast to mulberry leaves, we detected only one spot corresponding to 5-hydroxypipecolic acid on 2D-PC of the amino acids in the seeds of *Lathyrus japonicus*. It could be isolated by fractionation on Dowex 1 and 50, successively, without deamination. The result of the elementary analysis agreed with the formula. Mp of the hydrochloride, the value of the optical rotation and the colour reaction with ninhydrin and isatin were the same as those of the *cis*-isomer isolated from mulberry leaves. The comparison of the *cis*-form isolated from two plants by IR and PC was also satisfactory.

EXPERIMENTAL

General. Mps are uncorr. Solvents were evaporated *in vacuo* below 40°.

Solvents for PC. Solvents used were (a) *n*-BuOH–HOAc–H_2O (63:10:27), (*b*) PhOH–H_2O (in presence of NH_3 vapour) (25:9) and (c) *t*-AmOH-2,4-lutidine (1:1, satd with H_2O).

Plant material. The leaves of *Morus alba* L. cv Ichinose were harvested 22 July 1975 in Hino Mulberry Station of the Sericultural Experiment Station and stored for 3 days at 4° until extraction. Ripe seeds of *Lathyrus japonicus* Willd. were collected on 29 July 1973 on the seashore in Choshi, Chiba Prefecture and were extracted after 24 hr. The parent plants are deposited in the Herbarium of the University of Tokyo.

Isolation of trans- *and* cis-*5-hydroxy-L-pipecolic acid from mulberry leaves.* Leaves (15 kg) were crushed and extracted ×4 in a mixer with aq. EtOH and the filtered extract (125 l.) was passed through Amberlite IR-120 (H^+) (1 l.). After the resin was washed with aq. EtOH and H_2O, successively, the amino acids were eluted with 2M NH_4OH (15 l.). The eluate was concd to a small vol. (200 ml), applied to a column of Dowex 1 × 4 (200–400 mesh, OAc^-, 5 × 112 cm), and fractionated with 0.2 M HOAc. The neutral amino acid-fractions were combined (420 ml), concd and fractionated further on a column of Dowex 50 W × 8 (200–400 mesh, Na^+, 5 × 112 cm), which had been equilibrated with 0.2 M citrate buffer, pH 3.3. Fractionation was carried out with the same buffer (15 ml fractions). Fractions (83–88) containing the *trans*-form were desalted with Amberlite

IR-120 (H^+) (100 ml) and 2 M NH_4OH (1 l.). NH_3 was removed by concentration. 11 M HCl (1/10 vol.) was added to the resulting soln and oxides of nitrogen, generated from $NaNO_2$ and HCl, were bubbled in a stream of N_2 into the mixture until the ninhydrin-test was negative. The mixture was then extracted ×4 with Et_2O. The Et_2O layers were combined and evaporated to a syrup. 11M HCl (30 ml) was added and the mixture boiled under reflux for 45 min. HCl was reduced by concn. For the final purification, a column of Dowex 50W × 8 (200–400 mesh, H^+, 2.5 × 85 cm) and 1.5M HCl were used. The relevant fractions were combined and concnd twice with addition of small amount of H_2O. The crystals of hydrochloride separated were collected, washed with EtOH and dried (90 mg). They were recrystallized ×3 from H_2O and Me_2CO. Mp 216–20° (decomp), (lit. [3] 210–5° (decomp). $[\alpha]_D^{24} = -9.7°$ (c = 0.93, H_2O), (lit. [3] $[\alpha]_D^{20} = -10.9°$ (c = 0.92, H_2O). $C_6H_{11}NO_3$.HCl (Found: C 39.65; H 6.91; N 7.55; Cl 19.59. Calcd.: 39.68; H 6.66; N 7.71; Cl 19.52). *cis*-5-Hydroxy-L-pipecolic acid was eluted just after its *trans*-isomer from the system of Dowex 50 and 0.2M citrate buffer, pH 3.3. The fractions (92–200) were combined, desalted and treated with nitrous oxides as before. The nitrosoamino acids were extracted with Et_2O and reconverted to the free imino acids by refluxing in HCl. As a large amount of proline was present in the soln, it was removed using a Dowex 50W-column (H^+) and 1.5M HCl. The fractions containing only *cis*-5-hydroxypipecolic acid were combined (280 ml) and concnd. After the bulk of HCl had been removed, crystals of *cis*-5-hydroxy-L-pipecolic acid hydrochloride separated (470 mg). Recrystallization was repeated ×3 from aq. EtOH and Et_2O. mp 183–5° (decomp). $[\alpha]_D^{24} = -18.5°$ (c = 1.0, H_2O). Hydrobromide, mp 200–1°, (lit. [3] 205–7°). Free amino acid, mp 229–31° (decomp), (lit. [3] 255–8° (decomp)). $[\alpha]_D^{24}$ −32° (c = 0.8, H_2O), (lit. [3] $[\alpha]_D^{20}$ −31.1° ± 0.2 (c = 0.8, H_2O)). $C_6H_{11}NO_3$.HCl (Found: C 38.83; H 6.53; N 7.90; Cl 19.93. Calcd: C 39.68; H 6.66; N 7.71; Cl 19.52).

Isolation of cis-*5-hydroxy-L-pipecolic acid from seeds of* Lathyrus japonicus. Seeds (360 g) were powdered in a mill, defatted with Et_2O (3 l.) and dried. They were then extracted ×4 with 80% EtOH. The filtered extract (10.6 l.) was treated with Amberlite IR-120 (H^+) (300 ml) and the amino acids were eluted with 2M NH_4OH (3 l.). The NH_4OH eluate was concd to a small vol. and fractionated on a column of Dowex 1 × 4 (OAc^-, (4 × 90 cm)) and 0.2M HOAc. The fractions containing basic and neutral amino acids were combined, concnd to a small vol. and applied to a column of Dowex 50W (Na^+, 3.1 × 58 cm). Fractionation was achieved with 0.2M citrate buffer, pH 5. Neutral amino acids, which passed rapidly through the column, were, after desalting, fractionated further with Dowex 50W (Na^+, (3.1 × 58 cm)) and 0.2M citrate buffer, pH 3.3. During this procedure pure fractions of *cis*-5-hydroxy-L-pipecolic acid were obtained; they were desalted as before. The NH_4OH eluate was concnd to a small vol., taken up in aq. EtOH and Me_2CO was added. The separated free amino acid was recrystallized ×3 from aq. EtOH (192 mg). mp 230–5° (decomp). $C_6H_{11}NO_3$ (Found: C 49.00; H 7.28; N 9.59. Calcd: C 49.65; H 7.64; N 9.65). Hydrochloride, mp 184–7° (decomp), $[\alpha]_D^{24}$ −18.5° (c = 1.0, H_2O).

PC Data. Values of R_{Ala} of the isolated *trans*- and *cis*-form with solvent (c) were 1.31 and 1.0, respectively (descending, 75 hr) (lit. [7], 1.31 and 1.0) and with solvent (b) 1.22 and 1.38, respectively.

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13-HYDROXYHENTRIACONTAN-16-ONE FROM *NEOLITSEA SERICEA*

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Key Word Index—*Neolitsea sericea*; Lauraceae; γ-hydroxyketone; 13-hydroxyhentriacontan-16-one.

The essential oil of the leaves of *Neolitsea sericea* Koidz. (*Neolitsea sieboldii* Nakai, *Litsea glauca* Sieb.) from southern parts of Japan and China had been investigated to isolate sericealactone etc. [1]. In the course of our studies on the lignoids of the Lauraceae we examined a Me_2CO extract of the fresh leaves of this plant and isolated 13-hydroxyhentriacontan-16-one which has not been previously found in nature. No lignan could be isolated. In this paper we wish to report its isolation and structural determination.

The Me_2CO extract was concentrated to give a deposit, a mixture of glycosides of flavones, which is under investigation. The filtrate was then extracted with hexane. After concentration the hexane extract was steam-distilled to remove volatile components. The residue was dissolved in hexane and chromatographed on a neutral Al_2O_3 column with hexane–EtOAc (4:1); the eluates were further examined by chromatography on a Si gel column and/or preparative TLC to give a new hydroxyketone(1) in trace amounts, together with sericealactone and desoxysericealactone (previously isolated from this plant), a pentacyclic triterpene alcohol* 16-hentriacontanone, etc.

(1) mp 83°, gave one spot on TLC (R_f = 0.8 hexane–EtOAc, 17:3); $C_{31}H_{62}O_2$ [analysed and m/e 466 (M^+)]; IR (cm^{-1}) 3150 ~ 3300 (OH), 1700 (CO); PMR (δ ppm) 0.9 (6H, *t*, J = 7 Hz), 1.28 (48H, *br s*,), 2.41 (4H, *t*, J = 7 Hz), 3.6 (1H, *m*).