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SHORT REPORTS

3,4-DIHYDROXY-2-HYDROXYMETHYLPYRROLIDINE FROM *ARACHNIODES STANDISHII*

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Key Word Index—*Arachniodes standishii*; Pteridophyte; 3,4-dihydroxy-2-hydroxymethylpyrrolidine.

Abstract—3,4-Dihydroxy-2-hydroxymethylpyrrolidine, which has not been encountered naturally before, was isolated from the Pteridophyte *Arachniodes standishii*. Its configuration was determined as 2,3-*cis* and 3,4-*trans* from NMR spectra.

INTRODUCTION

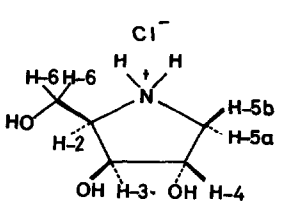
A PC survey of the non-protein amino acids in Pteridophytes revealed that *Arachniodes standishii* contains an unusual substance which gave an yellowish colouration with ninhydrin. This paper reports its isolation and structural studies.

RESULTS AND DISCUSSION

The substance under investigation was isolated as the corresponding hydrochloride by ion exchange and cellulose CC and was found to be basic. Elementary analysis and its electron impact mass spectrum agreed with the formula $C_5H_{11}NO_3 \cdot HCl$. The molecular ion at m/z 133, $[C_5H_{11}NO_3]^+$ was accompanied by a peak m/z 134, $[M+1]^+$, which is typical of an amine hydrochloride. One unsaturation of the molecular formula should be attributed to a ring structure, because of the absence of an sp^2 carbon in the ^{13}C NMR spectrum. A yellowish ninhydrin colouration suggested a pyrrolidine ring and this was consistent with the base peak at m/z 102, $[M-CH_2OH]^+$, in its mass spectrum. The structure **1** was, therefore, proposed. The ^1H NMR spectrum confirmed this and the assignments were made by decoupling experiments. A W-shape long-range coupling was observed between H-3 and H-5a, $J_{3,5a} = 0.5$ Hz [1]. The stereochemical requirement for the long-range coupling is to fix H-3 and H-5b in pseudo-equatorial positions. Under these conditions flexibility of the pyrrolidine ring would be reduced and the dihedral angles between C-2–H-2 and C-3–H-3, C-3–H-3 and C-4–H-4, and C-4–H-4 and C-5–H-5a were fixed around $\theta = 30°$ or 90° for *cis* or *trans* configurations, respectively. The vicinal coupling constant for $\theta = 90°$ was estimated at a small value (i.e. $J = 0$–3 Hz) from the Karplus equation, $J = 7 - \cos\theta + 5\cos 2\theta$ [2], and this assumption for $\theta = 90°$ would be valid even when the effect of electronegative groups (N^+ and hydroxyls of **1**) was taken into account [3]. In several related compounds reported earlier [1, 4–6] a coupling constant less than 3 Hz was always attributed to the *trans* configuration ($\theta = 90°$). From the above considerations, two hydroxyls on C-3 and C-4 ($J_{3,4} = 2.5$ Hz), and H-4 and H-5a ($J_{4,5a} = 2.6$ Hz) were assigned to be *trans* ($\theta = 90°$). On the other hand, the coupling constant $J_{2,3} = 4.1$ Hz was appreciably larger than those values for $\theta = 90°$ or the *trans* relationships. In addition, the comparable value of the *cis* coupling constant, $J_{4,5b} = 4.6$ Hz, suggests the 2,3-*cis* structure of **1**. The relatively small vicinal coupling constant, $J_{2,3} = 4.1$ Hz, for $\theta = 30°$ would be attributed to the electronegative substituents (N^+ and hydroxyl on C-3) which were suitably orientated to produce the maximum decreasing effects [3] in the magnitude of $J_{2,3}$.

Consequently, the structure of the compound was concluded to be **1** and the probable conformation in water was thought from ^1H NMR analysis, to be a half-chair in which the C_2-axis passed through the nitrogen atom, the CH_2OH group was pseudo-equatorial, and the two hydroxyls on C-3 and C-4 were in pseudo-axial environments.

Some related compounds containing pyrrolidine or piperidine ring are known as natural products. For example 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine [6], 2*S*-carboxy-4*R*,5*S*-dihydroxypiperidine and 2*S*-carboxy-4*S*,5*S*-dihydroxypiperidine [7] from *Derris elliptica* and 4,5-dihydroxy-L-pipecolic acid from *Calliandra haematocephala* [8] have been reported previously.



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EXPERIMENTAL

General. Evaporation of solvent or concn were carried out in a rotary evaporator below 40°. The 100 MHz ^{13}C NMR and 400 MHz ^{1}H NMR spectra were measured with dioxane (δ67.4) and DSS (δ0.0) as references, respectively. Assignments of the ^{13}C signals were made by selective proton decouplings and J_{CH} values were obtained by gated non-decouplings with NOE.

Plant material. Fronds of *A. standishii* (Moore) Ohwi were collected on 15 October 1981, in Oguri-mura, Iruma-gun, Saitama Prefecture, Japan and subjected to extraction after a few days. A voucher has been deposited in the Herbarium of the Faculty of Science, The University of Tokyo (TI).

Isolation. Fronds (4.8 kg) were extracted twice in a mixer with EtOH and the combined filtrate (75 l.) was passed through a column of Diaion SK-IB (H^+) (1 l.). After the resin was washed successively with 80% EtOH and H_2O amino acids and related compounds were eluted with 2 M NH_4OH (10 l.). NH_3 was removed and the syrup obtained was applied to a column of Dowex 50 × 8 (H^+) (82 × 3.4 cm). After washing with H_2O, neutral and acidic amino acids were displaced with 1 M pyridine. The basic substances were then fractionated with 1 M NH_4OH. The relevant fractions were combined, evaporated and the syrup was purified further on a cellulose column (90 × 3.4 cm) by fractionating with BuOH–pyridine–H_2O (1:1:1). The pure fractions were combined and concentrated, followed by treatment with a small amount of activated charcoal. To the filtrate an adequate amount of 1 M HCl was added to make it weakly acidic. After addition of small amounts of EtOH and Et_2O, the mixture was concd again, yielding crude crystals (1.38 g). Recrystallization was carried out from EtOH–Et_2O or EtOH–Me_2CO. Mp 115° (uncorr.) $[\alpha]_D^{25}$ +4.7° (H_2O; *c* 0.09). The compound showed a positive plain curve in ORD: $[\alpha]_{250}^{25}$ +27.8°, $[\alpha]_{300}^{25}$ +18.1°, $[\alpha]_{350}^{25}$ +13.9°, $[\alpha]_{450}^{25}$ +7.2°, $[\alpha]_{550}^{25}$ +4.7°. (Found: C, 35.13; H, 7.53; N, 8.31; Cl, 20.61. $C_5H_{11}NO_3 \cdot HCl$ requires: C, 35.20, H, 7.68; N, 8.21; Cl, 20.78%.) ^{13}C NMR (100 MHz, D_2O): δ67.4 (*d*, *J* = 145 Hz, C-2), 76.4 (*d*, *J* = 150 Hz, C-3), 75.1 (*d*, *J* = 150 Hz, C-4), 50.8 (*t*, *J* = 148 Hz, C-5), 59.7 (*t*, *J* = 142 Hz, C-6). ^{1}H NMR (400 MHz, D_2O): δ3.62 (1H, *ddd*, *J* = 4.1, 4.6, 8.4 Hz, H-2), 4.10 (1H, *dd*, *J* = 2.5, 4.1 Hz, H-3), 4.34 (1H, *ddd*, *J* = 2.5, 2.6, 4.6 Hz, H-4), 3.37 (1H, *dd*, *J* = 2.6, 12.7 Hz, H-5a), 3.58 (1H, *dd*, *J* = 4.6, 12.7 Hz, H-5b), 3.84 (1H, *dd*, *J* = 8.4, 12.3 Hz, H-6a), 3.96 (1H, *dd*, *J* = 4.6, 12.3 Hz, H-6b).

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