# Determination of Aglycone Chirality in Dihydroflavonol 3-*O*-α-L-Rhamnosides by $^1$H-NMR Spectroscopy[1)]

John DE BRITTO,[a] Visuvasam Soosai MANICKAM,[a] Subbarayan GOPALAKRISHNAN,[b] Toshiyuki USHIODA,[c] and Nobutoshi TANAKA*,[c]

*Department of Botany, St. Xavier's College (Autonomous),[a] Palayamkottai 627 002, Tamil Nadu, India, Department of Chemistry, Manonmaniam Sundaranar University,[b] Tirunelveli 627 007, Tamilnadu, India, and Faculty of Pharmaceutical Sciences, Science University of Tokyo,[c] Funakawara-machi, Ichigaya, Shinjuku-ku, Tokyo 162, Japan.* Received June 30, 1994; accepted September 26, 1994

**Three dihydroflavonol glycosides, 3-*O*-α-L-rhamnosides of (2*S*,3*S*)-, (2*R*,3*R*)- and (2*R*,3*S*)-5,7,3′,4′-tetrahydroxydihydroflavonol, were isolated from *Sphaerostephanos arbuscula* as the first example of dihydroflavonols from ferns. Aglycone chirality was determined by $^1$H-NMR data.**

**Key words** *Sphaerostephanos arbuscula*; dihydroflavonol; chirality determination; fern; $^1$H-NMR; astilbin

*Sphaerostephanos arbuscula* WILLD. (HOLTTUM) is a very common Thelypteridaceous fern of the Tirunelveli hills of the Western Ghats of South India, growing on partially or fully exposed stream banks in forests from 1000—1300 m. In the course of our chemical and chemotaxonomical studies of ferns,[2)] three diastereomers (**1**, **2**, and **3**) of astilbin [3-*O*-α-L-rhamnoside of (2*R*,3*R*)-5,7,3′,4′-tetrahydroxydihydroflavonol] were isolated from the fronds of this fern as the first example of dihydroflavonols from ferns. As all the diastereomers of astilbin had been isolated and their chirality had been proposed from circular dichroism (CD) data,[3)] the aglycone chirality of **1**, **2** and **3** was assigned as (2*S*,3*S*), (2*R*,3*R*) and (2*R*,3*S*), respectively, by comparison of the physical properties and spectral data, including CD spectra, with those reported.

Our detailed examination of their proton nuclear magnetic resonance ($^1$H-NMR) data also provided useful information on the aglycone chirality. The $^1$H-NMR spectra taken at 500 MHz defined all the signals as shown in Table I. It is noteworthy that each isomer has diagnostic signals. The signals of H-1″ and H-2″ of **1** and **3** occur in the relatively lower field and those of H-5″ and $H_3$-6″ in the relatively higher field. By contrast, the signals of H-1″ and H-2″ of **2** occur in the relatively higher field and those of H-5″ and $H_3$-6″ in the relatively lower field. Assuming that an anomeric proton and carbinyl proton at C-3 have a predominant conformation where they are *syn* to each other,[4,5)] anisotropy of the benzene ring (B-ring) causes upfield shifts of H-5″ and $H_3$-6″, and that of carbonyl group at C-4 causes downfield shifts of H-1″ and H-2″ in the case when both C-2 and C-3 have a (*S*) configuration (see Chart 1). In case of a (2*R*,3*R*) configuration, the contrary effects are expected to produce the signals shown in Chart 1 (compound **2**). With the *cis*-type compound (**3**), only a (2*R*,3*S*) configuration is reasonable to explain the chemical shifts.

These findings indicate that the aglycone chirality of a

TABLE I. $^1$H-NMR Data in DMSO-$d_6$

| | 1 | 2 | 3 |
|---|---|---|---|
| H-2 | 5.08 (d, 11.0) | 5.21 (d, 10.1) | 5.52 (d, 2.4) |
| H-3 | 4.70 (d, 11.0) | 4.62 (d, 10.1) | 4.20 (d, 2.4) |
| H-6 | 5.83 (d, 2.1) | 5.86 (d, 2.0) | 5.88 (d, 2.0) |
| H-8 | 5.88 (d, 2.1) | 5.89 (d, 2.0) | 5.92 (d, 2.0) |
| H-2′ | 6.91 (br s) | 6.89 (br s) | 6.86 (br s) |
| H-5′,6′ | 6.70—6.75 | 6.70—6.75 | 6.70—6.75 |
| H-1″ | 4.96 (d, 1.2) | 4.03 (d, 1.0) | 4.78 (d, 1.2) |
| H-2″ | 3.78 (dd, 3.1, 1.2) | 3.35 (dd, 3.7, 1.0) | 3.48 (dd, 3.1, 1.2) |
| H-3″ | 3.16 (dd, 9.2, 3.1) | 3.21 (dd, 9.5, 3.7) | 3.42 (dd, 9.4, 3.1) |
| H-4″ | 3.04 (t, 9.2) | 3.13 (t, 9.5) | 3.05 (t, 9.4) |
| H-5″ | 2.28 (dq, 9.2, 6.4) | 3.91 (dq, 9.5, 6.1) | 2.45 (dq, 9.4, 6.1) |
| H-6″ | 0.80 (d, 6.4) | 1.06 (d, 6.1) | 0.85 (d, 6.1) |

$\delta_{ppm}$ (coupling constants in Hz). Multiplicity: br s = broad singlet, d = doublet, t = triplet, q = quartet.

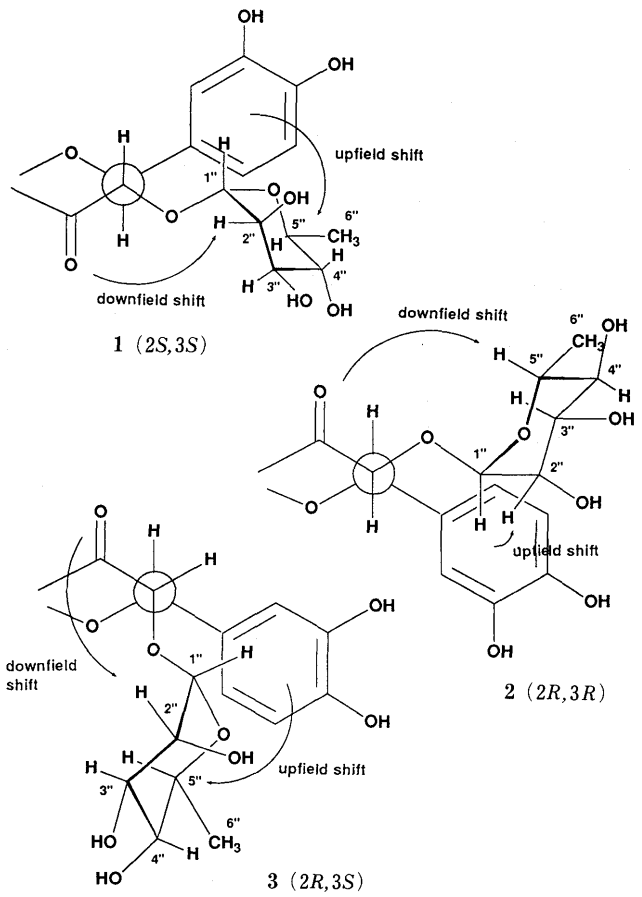


Chart 1. Anisotropic Effects in $^1$H-NMR

* To whom correspondence should be addressed.

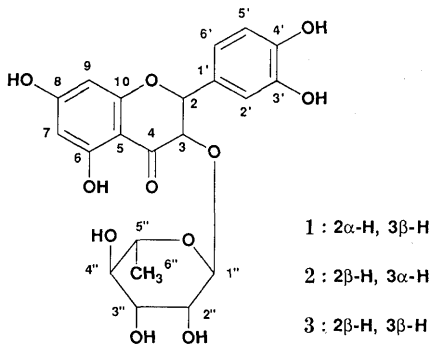


Chart 2

dihydroflavonol 3-*O*-glycoside is assignable by the well defined $^1$H-NMR data if the absolute structure of the sugar moiety has been determined.

## Experimental

Melting points were determined with a Yanagimoto micromelting apparatus and are uncorrected. Optical rotations were taken with a JASCO DIP-360 automatic polarimeter. The $^1$H- and $^{13}$C-NMR spectra were measured with a JEOL GSX-500 spectrometer. Ultraviolet (UV) spectra were recorded on a Hitachi 323 spectrometer and infrared (IR) spectra on a Shimadzu IR-460 spectrometer. CD spectra were recorded on a JASCO J-600 spectrometer. Mass spectra (MS) were measured with Hitachi M-80 and JEOL SX-102 spectrometers.

**Plant Material** Matured fronds of *Sphaerostephanos arbuscula* were collected from the Tirunelveli hills of Western Ghats of South India in October and identified by Prof. Manickam with the authentic specimens at the herbarium of St. Xavier's College.

**Isolation Procedure** Air-dried fronds (300 mg) of *Sphaerostephanos arbuscula* were extracted three times with 2 l of MeOH under reflux for 6 h. The extracts and 5 l of MeOH were passed over a column of activated charcoal (30 g) to obtain fraction M. The column was further eluted with 5 l of a mixture of $CHCl_3$ and MeOH (3 : 7) to obtain fraction C-M. Fraction M was concentrated to 200 ml, and 400 ml of acetone was added to precipitate sugars. The supernatant was concentrated and chromatographed on Sephadex LH-20 (90% MeOH) and silica gel ($CHCl_3$ : MeOH : $H_2O$ = 6 : 4 : 1) to yield 390 mg of a mixture of compounds **1**, **2** and **3** in a ratio of 6 : 4 : 3. The mixture was subjected to high performance liquid chromatography (TSK gel C-500, Tosoh Co., Ltd.; 90% $CH_3CN$) to get **1** (23 mg), **2** (15 mg) and **3** (12 mg). Fraction C-M was concentrated and chromatographed on Sephadex LH-20 (90% MeOH) to yield 55 mg of rutin [=quercetin 3-*O*-α-L-rhamnosyl-(1→6)-β-D-glucoside].

**Compound 1 (Neoastilbin)** An off-white powder, $[\alpha]_D^{22}$ −86.4° (*c* = 0.5, MeOH). UV $\lambda_{max}$ nm: (MeOH) 291 (log ε 4.34), 330 (log ε 3.66); (MeOH + MeONa) 246, 330; (MeOH + $AlCl_3$) 316, 380; (MeOH + $AlCl_3$ + HCl) 313, 380; (MeOH + NaOAc) 328; (MeOH + NaOAc + $H_3BO_3$) 292. CD: $[\theta]_{295}$ +39000° (MeOH). EI-MS *m/z*: 450 ($M^+$). $^{13}$C-NMR ($CD_3OD$) δ: 83.7 (C-2), 76.9 (C-3), 197.6 (C-4), 165.4 (C-5), 97.5 (C-6), 168.9 (C-7), 96.3 (C-8), 164.3 (C-9), 102.1 (C-10), 130.0 (C-1′), 116.3(C-2′), 146.7 (C-3′), 147.4 (C-4′), 115.5 (C-5′), 121.0 (C-6′), 102.8 (C-1″), 71.9 (C-2″), 72.0 (C-3″), 73.5 (C-4″), 70.4 (C-5″), 17.9 (C-6″).

**Acid Hydrolysis of 1** A mixture of compound **1** (10 mg) and 3% HCl (5 ml) was refluxed for 1 h. After cooling, the mixture was extracted with ethyl acetate and the remaining water layer was evaporated to yield 3 mg of L-rhamnose, $[\alpha]_D^{25}$ +12° (*c* = 0.3, $H_2O$). Its trimethylsilyl ether was identical with an authentic sample on GLC.

**Compound 2 (Astilbin)** An off-white powder, $[\alpha]_D^{25}$ −13.6° (*c* = 0.5, MeOH). UV $\lambda_{max}$ nm: (MeOH) 291 (log ε 4.28), 330 (log ε 3.65). CD: $[\theta]_{294}$ −43000° (MeOH). EI-MS *m/z*: 450 ($M^+$). $^{13}$C-NMR ($CD_3OD$) δ: 84.8 (C-2), 79.4 (C-3), 196.7 (C-4), 166.3 (C-5), 98.2 (C-6), 169.7 (C-7), 97.2 (C-8), 164.9 (C-9), 103.2 (C-10), 130.0 (C-1′), 117.1 (C-2′), 147.3 (C-3′), 148.1 (C-4′), 116.3 (C-5′), 121.3 (C-6′), 102.9 (C-1″), 72.6 (C-2″), 72.9 (C-3″), 74.6 (C-4″), 71.3 (C-5″), 18.6 (C-6″).

**Compound 3 (Neoisoastilbin)** An off-white powder, $[\alpha]_D^{22}$ +49.0° (*c* = 0.5, MeOH). UV $\lambda_{max}$ nm: (MeOH) 291 (log ε 4.35), 330 (log ε 3.75). CD: $[\theta]_{294}$ −38000° (MeOH). EI-MS *m/z*: 450 ($M^+$). $^{13}$C-NMR ($CD_3OD$) δ: 82.1 (C-2), 76.9 (C-3), 194.3 (C-4), 166.5 (C-5), 97.3 (C-6), 169.0 (C-7), 96.1 (C-8), 164.5 (C-9), 101.8 (C-10), 128.8 (C-1′), 116.3 (C-2′), 146.4 (C-3′), 146.7 (C-4′), 115.3 (C-5′), 119.4 (C-6′), 102.9 (C-1″), 72.0 (C-2″), 72.1 (C-3″), 73.4 (C-4″), 70.3 (C-5″), 17.8 (C-6″).

**Rutin** Yellow needles, mp 197—201°C, $[\alpha]_D^{25}$ +11° (MeOH, *c* = 0.5). IR (KBr) $\nu_{max}$ cm$^{-1}$: 3425, 1656, 1600, 1495, 1455, 1360, 1295,1203, 1062, 1014, 963, 807. UV $\lambda_{max}$ nm: (MeOH) 257 (log ε 4.19), 358 (log ε 4.07). $^1$H-NMR (DMSO-$d_6$) δ: 1.00 (3H, d, *J* = 6.1 Hz, Rha-6), 3.04—3.74 (10H), 4.40 (1H, d, *J* = 1.2 Hz, Rha-1), 5.35 (1H, d, *J* = 7.3 Hz, Glc-1), 6.20 (1H, d, *J* = 2.1 Hz, H-6), 6.39 (1H, d, *J* = 2.1 Hz, H-8), 6.85 (1H, d, *J* = 8.9 Hz, H-2′), 7.55 (2H, m). $^{13}$C-NMR (DMSO-$d_6$) δ: 156.4 (C-2), 133.3 (C-3), 177.4 (C-4), 161.2 (C-5), 98.7 (C-6), 164.1 (C-7), 93.6 (C-8), 156.6 (C-9), 104.0 (C-10), 121.2 (C-1′), 115.2 (C-2′), 144.7 (C-3′), 148.4 (C-4′), 116.3 (C-5′), 121.6 (C-6′), 101.2 (G-1), 74.0 (G-2), 76.4 (G-3), 70.6 (G-4), 75.9 (G-5), 67.0 (G-6), 100.7 (R-1), 70.4 (R-2), 70.0 (R-3), 71.8 (R-4), 68.2 (R-5), 17.7 (R-6). The structure was confirmed by direct comparison with an authentic sample.

## References and Notes

1) Part LXXXVI of the series, "Chemical and Chemotaxonomical Studies of Ferns."
2) Part LXXXV: H. Wada, K. Daidouji, H. Fuchino, J. Endo, T. Nakamura, N. Tanaka, T. Murakami, Y. Saiki, *Yakugaku Zasshi*, **114**, 27 (1994).
3) R. Kasai, S. Hirono, W.-H. Chou, O. Tanaka, F.-H. Chen, *Chem. Pharm. Bull.*, **36**, 4167 (1988).
4) R. U. Lemieux, S. Koto, *Tetrahedron*, **30**, 1933 (1974).
5) Supporting this assumption, an intense cross peak was observed in each compound between the anomeric proton signal and the H-3 signal in the nuclear Overhauser effect correlation spectroscopy (NOESY). Cross peaks were also observed between the B-ring proton signals and H-5 signals of **1** and **3** and between the B-ring proton signal and H-2″ signal of **2**.