STEREOCHEMISTRY OF THE SPIROBIFLAVONOID GENKWANOL B FROM DAPHNE GENKWA*

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Key Word Index—Daphne genkwa; Thymelacaceae; spirobiflavonoid; genkwanol B; stereochemistry; X-ray analysis; modified Mosher's method.

Abstract—The absolute configuration of genkwanol B isolated from Daphne genkwa was studied and assigned as C-2 (R), C-3 (S), C-8 (S), C-2" (S) and C-3" (R) by modified Mosher's method and the X-ray analysis of a pentamethyl ether acetate.

INTRODUCTION

In our preceding paper [1], we reported the isolation of a spirobiflavonoid, genkwanol B (1), from the roots of *Daphne genkwa* Sieb. et Zucc., and we elucidated its structure including the relative configuration. In the present paper, we report the absolute configuration of 1.

RESULTS AND DISCUSSION

The relative configuration between C-2 and C-3 in 1 was concluded to be trans-catechin type, from the characteristic feature of the 2-H signal ($\delta 4.56$, d, J = 8.4 Hz) in ¹HNMR spectrum, and the analysis of the NOESY spectrum of 1 hexamethyl ether. Hundt et al. [2] had reported that ¹H NMR analysis of $R_{-}(+)-\alpha$ -methoxy- α trifluoromethyl phenylacetic acid (MTPA) ester of different sets of enantiomeric flavan-3-ols and their derivatives permits assessment of the absolute configuration at C-3 position of the condensed tannin structural units. That is, the comparison of the ¹HNMR data at 300 MHz in $CDCl_3$ of the (+)-catechin acetyl and R-(+)-MTPA ester reveals a conspicious shielding of the B-ring protons in the R-(+)-MTPA ester relative to the chemical shifts of those protons in the acetyl derivatives [$\Delta \delta - 0.10$, 2-H(B); -0.07, 5-H(B); -0.03, 6-H(B)]. In the (-)-catechin derivatives, the B-ring protons are de-shielded in the R-(+)-MTPA ester relative to those of the acetate $\Delta \delta + 0.04$, 2-H(B); +0.04, 5-H(B); +0.08, 6-H(B)]. In the case of pentamethyl ether (2), the B-ring protons are shielded in the R-(+)-MTPA ester (4) relative to those of the acetate (3) $[\Delta \delta - 0.17, 2'-H(B), 6'-H(B); -0.12, 3'-H(B), 5'-H(B)]$ similarly to those of (+)-catechin. Thus the absolute configurations of C-2 and C-3 positions in 1 were assigned as R and S, respectively. In order to confirm the absolute configuration at C-8, C-2" and C-3" positions, X-ray analysis of 3 was carried out. The X-ray diffraction intensities from transparent, plate-like crystal of 3 were measured on a Rigaku AFC automatic four-circle diffractometer equipped with a graphite monochrometer. The atomic parameters of crystal data are presented in the Experimental. Since the absolute configurations at the C-2 and C-3 positions of 1 were decided to be R and S as described above, from this X-ray analysis the absolute configurations of C-8, C-2" and C-3" were concluded to be S, S and R, respectively (Fig. 1).

EXPERIMENTAL

General. Mps: uncorr; EIMS: 70 eV; ¹H NMR: 200 MHz with TMS as int. standard; CC: Merck silica gel 60 (70–230 mesh), Merck silica gel 60H. TLC and prep. TLC: Merck silica gel 60 F_{254} plate (0.25 mm) and Whatman silica gel 150A PLK 5F(1 mm). Spots and band were detected by UV irradiation (254 and 365 nm).

 α -Methoxy- α -trifluoromethylphenylacetyl chloride. (R)-(+)-MTPA (1.0 g), SOCl₂ (10 ml) and NaCl (0.08 g) were refluxed together for 50 hr. The reaction mixt. was treated by literature procedures [3], to give (+)-MTPA Cl (1.02 g).

Pentamethyl ether of 1 (2). Pale yellow viscous oil. HRMS m/z: 628.1946 [M]⁺ (calcd for $C_{35}H_{32}O_{11}$, 628.1942). ¹H NMR (CDCl₃): δ 7.12 (2H, d, J = 8.8 Hz), 6.77 (2H, d, J = 8.8 Hz), 6.72 (2H, d, J = 8.8 Hz), 6.62 (2H, d, J = 8.8 Hz), 6.14 (1H, d, J = 2.2 Hz), 6.09 (1H, d, J = 2.2 Hz), 5.83 (1H, s), 5.77 (1H, s), 4.60 (1H, d, J = 6.7 Hz), 3.81, 3.71 (each 3H, s), 3.70 (1H, m), 3.69 (6H. s), 3.31 (3H, s), 3.80 (1H, br, s). 2.72 (1H, dd, J = 17.3, 5.0 Hz), 2.28 (1H, dd, J = 17.3, 7.5 Hz).

(R)-(+)-MTPA ester of 2 (4). Compound 2 (85 mg) was dissolved in pyridine (1 ml) and (+)-MTPA Cl

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Fig. 1. Stereoscopic diagram of 3.

(1 mm mol) in CCl₄ (1 ml) was added. The mixt. was allowed to stand for 17 hr at room temp. N,N-Diethylethylendiamine (2 ml) was added to the reaction mixt. and stirred, allowed to stand for 10 min and diluted with Et₂O (40 ml), washed with dil. HCl, satd Na₂CO₃ and H₂O, dried. The filtered ether soln was concd, the residue was purified by prep. TLC with hexane-EtOAc (2:1) to afford 4 (85 mg). Compound 4: colourless viscous oil, HRMS m/z: 844.2343 [M]⁺ (calcd for C₄₅H₃₉F₃O₁₃, 844.2341). ¹H NMR (CDCl₃): δ 7.17 (2H, d, J = 8.8 Hz), 6.86 (2H, d, J = 8.8 Hz), 6.81 (2H, d, J = 8.8 Hz), 6.63 (2H, d, J = 8.8 Hz), 6.17 (1H, d, J = 2.1 Hz), 6.10 (1H, d, J = 2.1 Hz), 5.88 (1H, d, J = 5.5 Hz), 3.87, 3.80, 3.78, 3.76, 3.39, 3.14 (each 3H, s), 2.82 (1H, dd, J = 17.6, 4.7 Hz), 2.66 (1H, dd, J = 17.6, 5.8 Hz).

Acetate of 2 (3). Colourless prisms, mp 166–167°, HRMS m/z: 670.2018 [M]⁺ (calcd for C₃₇H₃₄O₁₂, 670.2047), ¹H NMR (CDCl₃): δ 7.19 (2H, d, J = 8.8 Hz), 6.98 (2H, d, J = 8.8 Hz), 6.87 (2H, d, J = 8.8 Hz), 6.75 (2H, d, J = 8.8 Hz), 6.14 (1H, d, J = 2.2 Hz), 6.11 (1H, d, J= 2.2 Hz), 5.94 (1H, s), 5.87 (1H, s), 5.22 (1H, m), 5.10 (1H, d, J = 3.4 Hz), 3.85, 3.82, 3.80, 3.78, 3.51 (each, 3H, s), 2.58 (1H, dd, J = 18.0, 3.8 Hz), 2.45 (1H, dd, J = 18.0, 4.4 Hz), 1.88 (3H, s).

X-Ray analysis of 3. X-Ray analysis of 3, transparent, plate-like crystals were obtained from hexane-EtOAc soln. Crystal data: $C_{37}H_{34}O_{12}$, monoclinic, space group P2₁, a = 22.423 (8), b = 20.849 (2), c = 8.305 (3) Å, β = 111.71°, V = 3607 (2) Å³, Z = 2, Dc = 1.246 gcm⁻³, μ (CuK_a)=6.85 cm⁻¹, F (000)=1408. In total, 6351 independent observed reflections [$F^2 \ge 2\sigma(F^2)$] were measured on a Rigaku AFC automatic four-circle diffractometer using graphite-monochromated CuK_a radiation ($\lambda = 1.5418$ Å). Crystals were too weak for X-ray radiation and physical action. Therefore crystals were sealed in thin-walled glass capillaries. Integrated intensities were measured by the continuous $2\theta - \omega$ scan method: scan speed 6° min⁻¹, scan width 1.15–1.45°. Backgrounds for each reflection were measured at the beginning and the end for 3.0 sec. Observed data were corrected for Lorentz and polarization effects and for absorption using the empirical ϕ scanning method at $\chi = 90^{\circ}$. The structure was solved by the direct method using program MULTAN87. Anisotropic refinement was carried out for non-hydrogen atoms and included only the calculation of structure factors. The final R value was 0.0938. This value is rather high, because of the relatively small crystal size, much delay for X-ray radiation and too weak intensity data. [Supplementary publication. For 3, full crystal data have been deposited at Cambridge Crystallographic Data Centre]

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