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# Pyridine-Induced Deshielding of 4-Methylene Protons for the Determination of C-6 Stereochemistry of Sterols Having a 5α,6-Diol Moiety. Revision of the C-6 Stereochemistry of Marine Sterol Isolated from a Sponge, *Dysidea* sp.

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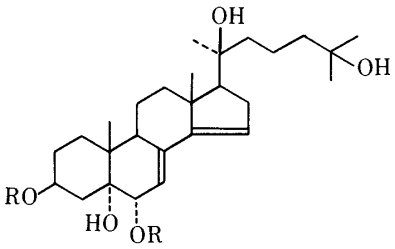
The utility of the proton nuclear magnetic resonance method involving pyridine-induced deshielding was demonstrated for stereochemical assignment at the C-6 position of sterols having a 5α,6ξ-diol moiety. Thus, the 4α-hydrogen resonance of 6α-isomers such as cholest-7-ene-3β,5α,6α-triol (**8**) is observed at *ca.* 3.0 ppm, whereas the 4β-hydrogen resonance of 6β-isomers such as cholest-7-ene-3β,5α,6β-triol (**10**) is observed at *ca.* 3.0 ppm. This method was applied to a marine sterol (**4**) isolated from a sponge, *Dysidea* sp., and it was concluded that the structure should be revised to the 6α-isomer (**5**) rather than the reported 6β-isomer (**4**).

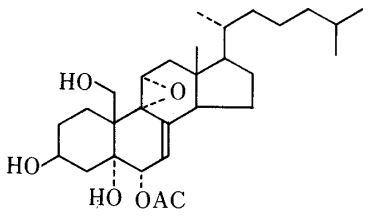
**Keywords**——pyridine-induced deshielding; bombycosterol; cholest-7-ene-3β,5α,6α-triol; cholest-7-ene-3β,5α,6β-triol; cholestane-3β,5α,6α-triol; cholestane-3β,5α,6β-triol; cholesta-7,14-diene-3β,5α,6α-triol

Recently we have isolated a unique ecdysteroid, named bombycosterol, from the pupal ovaries of the silkworm, *Bombyx mori*, and have established the structure as (20*S*)-cholesta-7,14-diene-3β,5α,6α,20,25-pentol (**1**).[1] The last stage of the structure elucidation of bombycosterol was focussed on the assignment of its C-6 stereochemistry. We initially thought that this problem could be easily solved by measuring the $J_{6,7}$ value in the proton nuclear magnetic resonance ($^1$H-NMR) spectrum, since the C-6 stereochemistry of the polyoxygenated sterol **4** isolated from a sponge, *Dysidea* sp., was deduced based on this value.[2] However, during the course of the study, including inspection of the original $^1$H-NMR spectra of **4** and the corresponding 6-ol **3**, the C-6 stereochemistry reported for the marine sterol **4** became doubtful. Therefore, we have synthesized a series of sterols with a 5α,6-diol moiety, **6**—**15**, and compared their $^1$H-NMR spectra. We have now found an alternative and more reliable method for the determination of the C-6 stereochemistry of these sterols, based on the pyridine-induced chemical shift of the 4-methylene protons. It was concluded that the C-6 stereochemistry of the marine sterol should be revised to that shown in the structure **5**. In this paper we describe this new method of assigning the C-6 stereochemistry of 5α,6ξ-sterols.

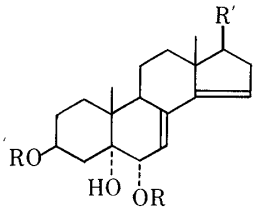
First we would like to discuss the previously described method based on the $J_{6,7}$ value. The authors in ref. 2 stated that the $J_{6,7}$ values of **4** ($CDCl_3$) was 3.5 Hz, whereas the isomeric 6α-acetate would have nearly zero $J_{6,7}$ value. In our study[4] on the isomeric 5α,6-dihydroxy-7-enes **8** and **10**, the $J_{6,7}$ value of the 6β-isomer **10** was 5 Hz while that of the 6α-isomer **8** was too small to be accurately measured (the $W_{1/2}$ values of the 6- and 7-hydrogens were each 5.7 Hz). Parallel data were obtained in the corresponding 3,6-diacetates **9** and **11** as well. Further, the 5α,6α-dihydroxy-7,14-diene **6** exhibited broad singlet-like 6- and 7-hydrogen signals (the $W_{1/2}$ values of the 6- and 7-hydrogens were 7 and 5 Hz, respectively). Bombycosterol (**1**) also showed a small $J_{6,7}$ value (*ca.* 1.5 Hz) (the $W_{1/2}$ values of the 6- and 7-hydrogens were 6.2 and 5.6 Hz, respectively). The bombycosterol 3,6-diacetate (**2**) showed analogous data. However,



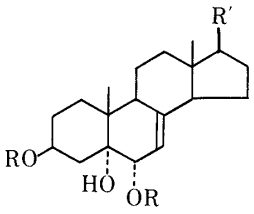
1 : R=H
2 : R=Ac



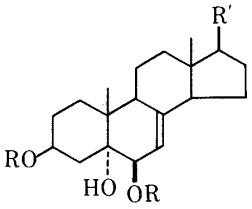
5
4 : 6β-OAc instead of 6α-OAc
3 : 6β-OH instead of 6α-OAc



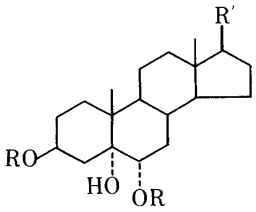
6 : R=H
7 : R=Ac



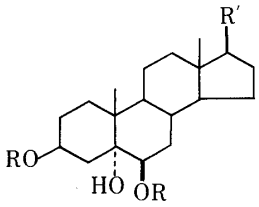
8 : R=H
9 : R=Ac



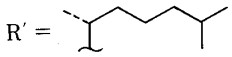
10 : R=H
11 : R=Ac



12 : R=H
13 : R=Ac



14 : R=H
15 : R=Ac



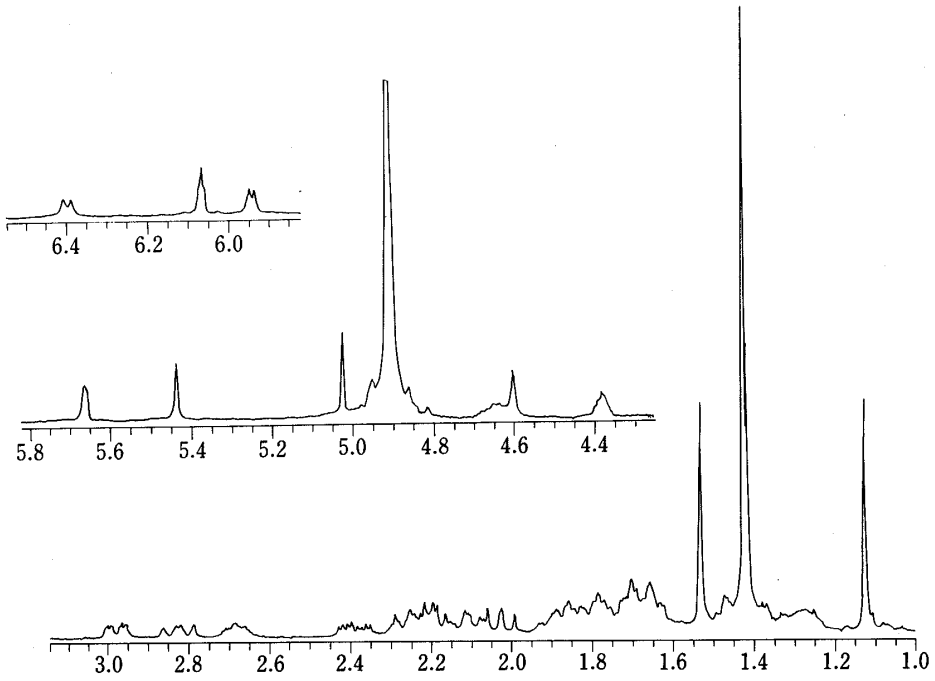



Fig. 1. $^1$H-NMR Spectrum of Bombycosterol (**1**) (360 MHz in Pyridine-$d_5$)

The resonances at 6.40 (6-OH), 5.95 (3-OH), 5.44, 5.03 and 4.60 ppm are due to alcoholic hydrogens. For assignments of other signals, see Table I.

the $^1$H-NMR spectrum of **1** (shown in Fig. 1) recorded for another sample (also in pyridine-$d_5$) exhibited $W_{1/2}$ values of 13.7 Hz (6-H) and 5.6 Hz (7-H). Thus it appears that the $W_{1/2}$ value of CHOH (such as at the 6-position) is changeable, probably due to the presence of trace amounts of water in the solvent.

According to our inspection of the original spectra of **3** and **4** provided by Professor Schmitz, the $W_{1/2}$ values of the 6- and 7-hydrogens of **3** (pyridine-$d_5$) were 12.8 (evidently OH coupling was involved) and 4.7 Hz, respectively, and the corresponding values of **4** were each 5.0—5.5 Hz (pyridine-$d_5$). In these two spectra, the 6- and 7-hydrogen signals were observed as rather sharp singlets and the $J_{6,7}$ values appeared to be much less than 3.5 Hz. The results of our studies in terms of the $J_{6,7}$ values can be summarized as follows. The general trend that the 6$\beta$-isomer has a larger $J_{6,7}$ value than the 6$\alpha$-isomer is substantiated, but application of this coupling value for C-6 stereochemical assignment should be done cautiously particularly when the $J_{6,7}$ value is in the range of 2 to 4 Hz. The use of *J*-resolved two-dimensional (2DJ) $^1$H-NMR of **1** revealed the presence of homoallylic coupling between 6-H and 9-H (*ca.* 1.0 Hz) and allylic coupling between 7-H and 9-H (*ca.* 1.5 Hz). Similar coupling was also reported for the marine sterol **4**. These couplings, in addition to the aforementioned OH coupling, make it difficult to get an accurate $J_{6,7}$ value.

The alternative method described below is very simple and free from such difficulties. The $^1$H-NMR data obtained in the present study are listed in Tables I and II. The chemical shifts of either 4$\alpha$- or 4$\beta$-hydrogen of these sterols are of importance (Table I, measured in pyridine-$d_5$). The assignment of the 4-methylene protons is based on their coupling pattern: 4$\alpha$-hydrogen appeared as a doublet of doublet ($J$ = *ca.* 13 and 5 Hz), whereas 4$\beta$-hydrogen gave a

TABLE I. $^1$H-NMR Data for Hydroxysterols (360 and/or 400 MHz in Pyridine-$d_5$)

| Compound | **1** | **3**[a] | **4**[a] | **6** | **8** | **10** | **12** | **14** |
|---|---|---|---|---|---|---|---|---|
| 3$\alpha$-H | 4.64 | 4.61 | 4.75 | 4.64 | 4.65 | 4.83 | 4.72 | 4.87 |
| 4$\alpha$-H | 2.98 | 3.04 | 2.77 | 2.98 | 2.985 | | 3.03 | |
| 4$\beta$-H | 2.02 | | | 2.02 | | 3.01 | 2.11 | 2.97 |
| 6-H | 4.38 | 4.49 | 5.67 | 4.38 | 4.385 | 4.32 | 4.00 | 4.17 |
| 7-H | 6.06 | 5.72 | 5.86 | 6.06 | 5.43 | 5.74 | | |
| 9$\alpha$-H | 2.68 | | | 2.68 | | | | |
| 15-H | 5.66 | | | 5.57 | | | | |
| 16-H | 2.82 | | | 2.49 | | | | |
| 16-H′ | 2.39 | | | 2.30 | | | | |
| 19-Me | 1.12 | | | 1.12 | 1.12 | 1.53 | 1.10 | 1.67 |
| 18-Me | 1.42 | 0.87 | 0.82 | 0.64 | 0.64 | 0.66 | 0.70 | 0.75 |
| 21-Me | 1.54 | 0.97 | 0.87 | 0.97 | 0.97 | 0.975 | 0.97 | 1.00 |
| 26,27-Me | 1.425 | 0.87 | 0.87 | 0.89 | 0.88 | 0.886 | 0.895 | 0.894 |

a) From ref. 2.

TABLE II. $^1$H-NMR Data for Sterol Acetates (100 MHz in $CDCl_3$)

| Compound | **2**[a] | **7** | **9** | **11** | **13** | **15** |
|---|---|---|---|---|---|---|
| 3$\alpha$-H | 5.09 | 5.08 | 5.0 | 5.1 | 5.0 | 5.1 |
| 6-H | 5.24 | 5.24 | 4.90 | 4.83 | 5.0 | 4.70 |
| 7-H | 5.47 | 5.45 | 5.24 | 5.27 | | |
| 15-H | 5.68 | 5.65 | | | | |
| 19-Me | 1.03 | 1.03 | 1.03 | 1.05 | 1.03 | 1.16 |
| 18-Me | 1.03 | 0.84 | 0.55 | 0.55 | 0.64 | 0.67 |
| 21-Me | 1.23 | | | | | |
| 26,27-Me | 1.23 | 0.89 | 0.86 | 0.85 | 0.85 | 0.85 |
| 3-OAc | 2.02 | 2.03 | 2.02 | 2.02 | 2.01 | 2.02 |
| 6-OAc | 2.14 | 2.15 | 2.11 | 2.05 | 2.06 | 2.07 |

a) Recorded in 400 MHz.

triplet-like signal ($J=ca.$ 12 and 13 Hz). In the case of bombycosterol this assignment was firmly established by a 2DJ $^1$H-NMR study.[1] All the compounds in the present work meet the following criteria: the 4α-hydrogen resonance of the 6α-isomer is observed at *ca.* 3.0 ppm, whereas the 4β-proton resonance of the 6β-isomer appears at *ca.* 3.0 ppm. The origin of this characteristic chemical shift can be ascribed to the influence of the 6-hydroxyl group through 1,3-diaxial (with the 6β-isomer) or 1,3-diequatorial (with the 6α-isomer) interaction which is intensified by pyridine-induced deshielding. Mitsuhashi *et al.*[5] have recently isolated several sterols with a 1α,3β,5α,6β-tetraol system from the soft coral, *Sarcophyton glaucum*. The chemical shift of 4β-hydrogen in these sterols was reported to be 2.95—3.05 ppm (br t, $J=$ 12 Hz), which is in good agreement with our observation.

Compound **3** exhibited a signal at 3.04 ppm (dd, $J=13$ and 4.6 Hz) assignable to 4α-hydrogen (no signal having the coupling pattern of 4β-hydrogen could be found in this region). Therefore, it is concluded that the structure of the marine sterol isolated by Schmitz *et al.* should be revised to the 6α-isomer **5**.

Finally we would like to describe the chemical shift of 19-methyl signal. The 19-Me signal of the 6β-isomer is expected to be observed at lower field because of pyridine-induced deshielding. Indeed, the signals of **10** and **14** appeared at 1.53 and 1.67 ppm, respectively, whereas those of the 6α-isomers **8** and **12** appeared at *ca.* 1.10 ppm (see Table I). This difference also has diagnostic value.[6]

## Experimental

Melting points were determined on a Yazawa hot stage microscope and are uncorrected. $^1$H-NMR spectra were recorded on a JEOL FX-100 (100 MHz), Nicolet NT-360 (360 MHz) or JEOL GX-400 (400 MHz) spectrometer with tetramethylsilane as an internal standard. Column chromatography was performed with Kieselgel 60 (70—230 mesh, E. Merck) and analytical thin-layer chromatography (TLC) with precoated Kieselgel 60 $F_{254}$ plates (0.25 mm thickness, E. Merck). Compounds **1**—**4** were described previously.[1,2]

**Cholesta-7,14-diene-3β,5α,6α-triol (6) and Its 3,6-Diacetate (7)**——7α,8α-Epoxycholestane-3β,5α,6α-triol 3,6-diacetate, mp 182—184 °C (methanol), $^1$H-NMR ($CDCl_3$) $\delta$: 0.78 (3H, s, 18-Me), 1.11 (3H, s, 19-Me), 2.00 (3H, s, 3-OAc), 2.20 (3H, s, 6-OAc), 3.58 (1H, br s, 7-H), 5.1 (1H, m, 3-H), 5.18 (1H, m, 6-H), MS *m/z*: 456 ($M^+ -$ AcOH), 440, 422, was obtained by permanganate oxidation of 7-dehydrocholesterol without destroying the formed manganese oxide, followed by acetylation according to the method of Anastasia *et al.*[7] A mixture of the epoxide (210 mg, 0.42 mmol) and 70% $HClO_4$ (0.2 ml) in tetrahydrofuran (THF) (10 ml)-$H_2O$ (1.0 ml) was heated at *ca.* 50 °C with stirring until the starting material disappeared (*ca.* 2 h). Extractive (ether) work-up gave a mixture of four major components [designated as A (*Rf* 0.56), B (*Rf* 0.50), C (*Rf* 0.33), and D (*Rf* 0.22) in increasing order of polarity] as determined by TLC (developed with hexane–ethyl acetate = 2 : 1). Each component was separated by preparative TLC.

Compound A (25 ml), mp 180—182 °C (methanol) (lit.[8] 179—180 °C), was characterized as the required diacetate **7** based on the spectroscopic data including $^1$H-NMR and ultraviolet (UV).[8]

Compound B (25 mg), mp 192—194 °C (methanol) (lit.[8] 191—192 °C) was identified as cholesta-7,9(11)-diene-3β,5α,6α-triol 3,6-diacetate, since the $^1$H-NMR and UV data were identical with the reported data.[8] Compound C (50 mg), mp 164—167 °C (methanol) was assigned as cholesta-7,9(11)-diene-3β,5α, 6α-triol 3-acetate based on $^1$H-NMR ($CDCl_3$) $\delta$: 0.52 (3H, s, 18-Me), 1.12 (3H, s, 19-Me), 2.01 (3H, s, 3-OAc), 4.0 (1H, m, 6-H), 5.0 (1H, m, 3-H), 5.16 (1H, m, 7-H), 5.7 (1H, m, 11-H) and UV $\lambda_{max}^{ethanol}$ nm (log ε): 237 (4.2), 243 (4.3), 252 (4.0). This assignment was confirmed by the fact that acetylation of compound C afforded compound B. Compound D (25 mg) did not show UV absorption and exhibited two acetyl resonances in the $^1$H-NMR spectrum. However, this compound was not further investigated.

Saponification of **7** with 5% KOH–methanol gave the triol **6**, mp 182—184 °C (methanol) (lit.[8] 182—184 °C). The UV spectrum of **6** was essentially identical with that reported previously.[8]

**Cholest-7-ene-3β,5α,6α-triol (8) and Its 3,6-Diacetate (9)**——These two compounds were prepared according to the method of Anastasia *et al.*[8] The crude product obtained from another run of permanganate oxidation of 7-dehydrocholesterol (2.0 g) without destroying the formed manganese oxide, followed by acetylation, was carefully chromatographed on silica gel (180 g). Following the elution of the main product, 7α,8α-epoxycholestane-3β,5α,6α-triol 3,6-diacetate, the diacetate **9** (230 mg) was obtained (eluted with hexane–ether = 2 : 1), mp 187—189 °C (ethanol) (lit.[8] 188—189 °C). Saponification of **9** with 5% KOH–methanol furnished the triol **8**, mp 229—231 °C (methanol) (lit.[8] 231—232 °C).

**Cholest-7-ene-3β,5α,6β-triol (10) and Its 3,6-Diacetate (11)**——*m*-Chloroperbenzoic acid (700 mg, 4.06 mmol) was added in several portions to a cooled (0 °C), stirred solution of 7-dehydrocholesterol acetate (1.28 g, 3.0 mmol) in dry ether (50 ml). After a total reaction time of 30 min, aq. sat. $NaHCO_3$ was added and the reaction mixture was extracted with ether. Chromatography (silica gel 25 g, eluted with hexane–ethyl acetate = 4 : 1) of the oily residue, obtained by usual work-up, afforded cholest-7-ene-3β,5α,6β-triol 3-acetate (350 mg, 27%). mp 227—230 °C (methanol). $^1$H-NMR δ: 0.60 (3H, s, 18-Me), 1.08 (3H, s, 19-Me), 2.02 (3H, s, OAc), 3.6 (1H, m, $W_{1/2}$ = 12 Hz, 6-H), 5.1 (1H, m, 3-H), 5.3 (1H, br d, $W_{1/2}$ = 6 Hz, 7-H). Saponification of the 3-acetate with 5% KOH–methanol afforded the triol **10**, mp 237—239 °C (ethyl acetate) (lit.[9] 240—242 °C). Acetylation of the 3-acetate with acetic anhydride–pyridine afforded the 3,6-diacetate **11**, mp 151—152 °C (methanol).

**Cholestane-3β,5α,6α-triol (12), Cholestane-3β,5α,6α-triol 3,6-Diacetate (13), Cholestane-3β,5α,6β-triol (14) and Cholestane-3β,5α,6β-triol 3,6-Diacetate (15)**——Compound **12**, mp 231—233 °C (methanol) (lit.[10] 232—234 °C) was prepared by $OsO_4$ treatment of cholesterol according to the literature.[10] Acetylation of **12** with acetic anhydride–pyridine afforded the diacetate **13**, mp 186—188 °C (methanol) (lit.[11] 184—185 °C). Compound **14**, mp 228—230 °C (methanol) (lit.[12] 234—235 °C), was prepared from cholesterol 5α,6α-oxide according to the literature.[12] Acetylation of **14** with acetic anhydride–pyridine afforded the diacetate **15**, mp 167—169 °C (methanol) (lit.[13] 167 °C).

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## References and Notes

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