Thermolysis of Taxinine and Taxinine H. Allylic Rearrangement of the Ester Moiety in the C-Ring

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Thermolysis of taxinine and taxinine H, esters of 2 α ,9 α ,10 β -triacetoxo-8 β ,12,15 α ,15 β -tetramethyl-4-methylene-13-oxotricyclo[9.3.1.0^{3,8}]pentadec-11-en-5 α -ol, resulted in the smooth formation of the corresponding 4-en-4-ylmethanol esters and the 4(20),5-diene by virtue of migration and elimination of the 5-O-ester moiety, thus providing useful methods for chemical modifications of the C-ring in taxinine derivatives.

Key words taxinine; taxinine H; thermolysis; allylic rearrangement; (2 α ,9 α ,10 β -triacetoxo-8 β ,12,15 α ,15 β -tetramethyl-13-oxotricyclo[9.3.1.0^{3,8}]pentadeca-4,11-dien-4-yl)methanol; 2 α ,9 α ,10 β -triacetoxo-8 β ,12,15 α ,15 β -tetramethyl-4-methylene-13-oxotricyclo[9.3.1.0^{3,8}]pentadeca-5,11-diene

Recently, we have demonstrated¹⁾ that regio-selective *O*-deacylations of the 2-, 5-, 9- and 10- ester moieties in taxinine (**1a**),²⁾ which is a taxane diterpenoid isolable from the needles of Japanese yew, *Taxus cuspidata*,³⁾ can be accomplished by treatment with barium hydroxide hydrate, diisobutylaluminum hydride (DIBAL-H) or Red-Al® under mild conditions, and that the *O*-deacylation of the biologically inactive taxoid **1a** at the 9- and 10-positions induces biological activity such as the differentiation of PC-12 tumor cells. These facts are of interest in connection with the development of bioactive taxoids⁴⁾ and with the valuable utilization of natural products.

During the course of our studies on chemical modifications of taxinine derivatives, two types of thermal reaction of the 5-*O*-ester moieties in the taxoid **1a** and its analog, taxinine H⁵⁾ (**1b**), were observed. In this paper, we describe the present observations in detail.

When the taxoid **1a** [4(20)-en-5-*O*-cinnamate] was heated at the temperature of its melting point (265°C) in a degassed sealed tube without any solvent, it was smoothly consumed (3% after 3 min; 37% after 10 min) and converted into the 4(5)-en-20-*O*-cinnamate **2a**, which had a slightly higher polarity compared with that of the starting 5-*O*-cinnamate **1a**. The structure of **2a** was assigned on the basis of its microanalytical and spectral data, *e.g.*, retention of the molecular peak (*m/z* 606) in the mass spectrum and a drastic downfield shift of the signal originating from the C-5 carbon (δ 78.2 ppm for the taxoid **1a**, δ 131.3 ppm for the product **2a**) with a marked upfield shift of the signal originating from the C-20 *exo*-methylene carbon (δ 117.2 ppm for the taxoid **1a**; δ 69.0 ppm for the product **2a**) in the ¹³C-NMR spectrum, and this structure was supported by two-dimensional NMR data including ¹H-¹H correlation spectroscopy (COSY), heteronuclear multiple bond correlation (HMBC) and nuclear Overhauser and exchange spectroscopy (NOESY) spectra. It should be noted that the product **2a** possesses a unique ring-system which resembles quite closely that of the initial cyclic product⁶⁾ in the biosynthesis of paclitaxel (Taxol®) which is one of the most promising chemotherapeutic drugs for the treatment of a variety of advanced solid cancers.⁷⁾

In the thermal reaction of the taxoid **1a** (for 10 min)

described above, the formation of trace amounts of a more polar product **3** and cinnamic acid were observed. The product **3** was assigned to be the 4(20),5-diene **3** on the basis of its microanalytical and spectral data, *e.g.*, disappearance of characteristic signals for the cinnamoyl group and the appearance of two signals at δ 5.94 (1H, brd, *J* = 8 Hz) and δ 5.54 (1H, m) originating from the C₅,C₆-vinyl protons were observed in the ¹H-NMR spectrum. Prolongation of the reaction time or the use of more drastic conditions in the thermolysis of the taxoid **1a** resulted in the decomposition of the product **2a** and increased formation of the diene **3** and cinnamic acid.

Analogous results were obtained in the thermolysis of taxinine H (**1b**) at 230°C, though the corresponding 20-*O*-acetate **2b** could not be isolated easily in a pure state even after repeated column chromatography of the reaction mixture, because its chromatographic behavior was close to that of the starting taxoid **1b**.

The 20-*O*-cinnamate **2a** was unstable under thermal conditions. Treatment of **2a** at 200°C without solvent caused 6% consumption of the starting **2a** after 10 min to give the 5-*O*-cinnamate **1a**, and the employment of more drastic condition (at 265°C for 10 min) allowed the formation of the 5-*O*-cinnamate **1a** and the diene **3** in 34% and 14% yields, together with a small amount of cinnamic acid. The major product was identical to the taxoid **1a** bearing the 5 α -*O* stereochemistry. In this reaction, no evidence supporting the formation of the 5 β -isomer of the taxoid **1a** was obtained. Thus, the interconversion between the 5 α -*O*-cinnamate **1a** and the 20-*O*-cinnamate **2a** was observed under the conditions employed. In sharp contrast to these facts, taxinine A⁸⁾ (**1c**) bearing a hydroxyl group at the C-5 position was very stable under these thermal conditions. This fact clearly indicates that the thermal

Chart 1

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Fig. 1

migration of a substituent in taxinines is characteristic of the derivatives bearing the allylic ester moiety at the C-5 position.

Taking the above facts and chemical reactivities of the allylic esters⁹⁾ into consideration, the interconversion between the taxinine derivatives **1** and the 20-*O*-esters **2** can be reasonably explained by virtue of allylic rearrangement of the ester moiety under the thermal conditions as shown in Fig. 1. The exclusive formation of the 5 α -*O*-ester **1** in the thermolysis of the 20-*O*-ester **2** seems to be due to the steric hindrance on the β -face of this unusually complex ring-system with a unique cage-conformation,¹⁰⁾ especially by the 2 α -*O*-acetyl and C-8 β methyl groups. The employment of more drastic conditions allowed the elimination of the appropriate acids from the taxoids **1** and the 20-*O*-ester **2** leading to the 4(20),5-diene **3**.

It has been documented that treatment with ethanolic potassium hydroxide¹¹⁾ or photolysis¹²⁾ of the taxoid **1a** resulted in contraction of its B-ring leading to anhydro-taxininol in the former case or taxuspine C in the latter case. The two types of thermal reaction of the C-5 ester moiety described above provide useful methods for the chemical modifications of the C-ring in taxinine derivatives.

Experimental

Melting points (uncorrected) were determined on a Yanagimoto melting point apparatus. Elemental analysis was performed by the microanalytical laboratory of our university. Mass spectra and high-resolution mass spectra were obtained by electron ionization (EI) or fast atom bombardment (FAB) method on a JEOL JMS-SX 102A. ¹H- and ¹³C-NMR spectra were recorded on a JEOL EX-400 (400 MHz) spectrometer and are reported in ppm downfield from tetramethylsilane. Thin-layer chromatographic analyses and preparative TLC were carried out on pre-coated Silica gel 60 F₂₅₄ plates (Merck, Art 5715). Column chromatographic separation was performed with Merck silica gel 60 (70–230 mesh). All thermal reactions were carried out in a degassed sealed tube with Microdistillation and Sublimation apparatus (Miyamoto Riken Ltd.).

Thermolysis of Taxinine Derivatives (1) **1**) Taxinine (**1a**, 100 mg, 0.165 mmol) was heated at 265 °C without solvent for 10 min. The resulting mixture was subjected to silica gel column chromatography eluting with CH₂Cl₂–AcOEt (50/1) to separate 3-phenylacrylic acid (2 α ,9 α ,10 β -triacetoxo-8 β ,12,15 α ,15 β -tetramethyl-13-oxotricyclo[9.3.1.0^{3,8}]pentadeca-4,11-dien-4-yl)methyl ester (**2a**) (37 mg, 37%), the starting material **1a** (60 mg, 60%), and trace amounts of acetic acid (9 α ,10 β -diacetoxo-8 β ,12,15 α ,15 β -tetramethyl-4-methylene-13-oxotricyclo[9.3.1.0^{3,8}]pentadeca-5,11-dien-2 α -yl) ester **3** and cinnamic acid.

2a: Colorless amorphous powder, mp 166–167 °C (from AcOEt), [α]_D²⁵ = +79.43° (CHCl₃, *c* = 0.35). EI-MS *m/z* (rel. int. %): 606 (*M*⁺, 3), 564 (4), 547 (3), 458 (8), 416 (7), 296 (43), 131 (100). IR (KBr) cm^{–1}: 1744 (C=O), 1711 (C=O), 1676 (C=O), 1639, 1372, 1234. UV $\lambda_{\max}$ (EtOH) nm (ϵ): 277 (24300). ¹H-NMR (CDCl₃) δ : 7.64 (1H, d, *J* = 16,

H-23), 7.62 (2H, m, Ph-H), 7.38 (3H, m, Ph-H), 6.46 (1H, d, *J* = 16, H-22), 6.03 (1H, d, *J* = 11, H-9), 5.91 (1H, d, *J* = 11, H-10), 5.86 (1H, m, H-5), 5.69 (1H, br d, *J* = 2, H-2), 4.92 (1H, d, *J* = 12, H-20a), 4.82 (1H, d, *J* = 12, H-20b), 3.30 (1H, brs, H-3), 2.88 (1H, brs, H-14a), 2.86 (1H, br d, *J* = 5, H-14b), 2.19 (1H, d, *J* = 5, H-1), 2.09 (1H, m, H-6), 2.08 (3H, brs, H-18), 2.07, 2.05 and 2.03 (each 3H, each s, OAc), 1.82 (3H, brs, H-16), 1.17 (3H, brs, H-17), 0.97 (3H, brs, H-19). ¹³C-NMR (CDCl₃) δ : 199.7 (C-13), 170.0 (OAc), 169.6 (OAc), 169.1 (OAc), 166.7 (C-21), 150.4 (C-11), 145.1 (C-23), 138.2 (C-12), 134.5 (Ar), 133.8 (C-4), 131.3 (C-5), 130.3 (Ar), 128.9 (Ar), 128.3 (Ar), 118.1 (C-22), 75.5 (C-9), 72.7 (C-10), 71.5 (C-2), 69.0 (C-20), 47.3 (C-1), 43.0 (C-3), 41.7 (C-8), 38.9 (C-15), 37.5 (C-17), 36.1 (C-14), 28.3 (C-7), 25.1 (C-16), 22.4 (C-6), 21.5 (OAc), 20.9 (OAc), 20.7 (OAc), 17.7 (C-19), 13.6 (C-18). HR FAB-MS *m/z*: 607.2923 [*M*+H]⁺ (Calcd for C₃₅H₄₃O₉: 607.2907 [*M*+H]⁺). The ¹H- and ¹³C-NMR spectra were assigned on the basis of several types of two-dimensional NMR data including ¹H–¹H COSY, ¹³C–¹H COSY, HMBC and NOESY spectra. Detailed analyses of the ¹H–¹H and ¹³C–¹H COSY spectra revealed connectivities of C-1 to C-14, C-2 to C-3, C-5 to C-6, and C-9 to C-10. There are HMBC correlations of H-1 β to C-13, H-2 β to C-8, H-9 β to C-8, C-11 and C-19, H-10 α to C-9, C-11, C-12 and C-15. Also, the NOESY spectrum showed cross peak correlations for H-1 β /H-2 β , H-14 β and H₃-17, H-2 β /H-9 β , H₃-16 and H₃-19, H-3 α /H-14 α , H-5/H-20b, H-9 β /H₃-16 and H₃-19, H-14 α /H-3 α and H-20a.

3: Colorless amorphous powder, mp 161–162 °C (from AcOEt), [α]_D²⁵ = +118.2 (CHCl₃, *c* = 1.00). FAB-MS *m/z* (rel. int. %): 459 (*M*⁺ + H, 3), 400 (0.5), 289 (16), 136 (58). IR (KBr) cm^{–1}: 1746 (C=O), 1681 (C=O), 1373, 1237, 1027. UV $\lambda_{\max}$ (EtOH) nm (ϵ): 264 (4700), 231 (13700). ¹H-NMR (CDCl₃) δ : 6.00 (1H, d, *J* = 10, H-10), 5.96 (1H, d, *J* = 10, H-9), 5.94 (1H, br d, *J* = 8, H-5), 5.68 (1H, br d, *J* = 3, H-2), 5.59 (1H, brs, H-20a), 5.54 (1H, m, H-6), 4.99 (1H, brs, H-20b), 2.88 (1H, dd, *J* = 7, 20, H-14 β), 2.82 (1H, brs, H-3), 2.47 (1H, d, *J* = 20, H-14 α), 2.18 (2H, br d, *J* = 4, H-7), 2.12 (1H, d, *J* = 7, H-1), 2.07, 2.04 and 2.03 (each 3H, each s, OAc), 2.01 (3H, brs, H-18), 1.75 (3H, brs, H-16), 1.12 (3H, brs, H-17), 0.86 (3H, br s, H-19). ¹³C-NMR (CDCl₃) δ : 199.4 (C-13), 169.7 (OAc), 169.6 (OAc), 168.9 (OAc), 150.0 (C-11), 140.9 (C-4), 137.8 (C-12), 132.9 (C-5), 123.3 (C-6), 117.9 (C-20), 75.9 (C-10), 73.0 (C-9), 71.0 (C-2), 47.8 (C-1), 44.1 (C-3), 41.2 (C-8), 38.6 (C-15), 36.7 (C-14), 36.5 (C-17), 33.0 (C-7), 25.4 (C-16), 21.2 (OAc), 20.8 (OAc), 20.6 (OAc), 18.6 (C-19), 13.8 (C-18). Anal. Calcd for C₂₆H₃₄O₇: C, 68.10; H, 7.47. Found: C, 67.90; H, 7.51. The ¹H- and ¹³C-NMR spectra were assigned on the basis of several types of two-dimensional NMR data including ¹H–¹H COSY, ¹³C–¹H COSY, HMBC and NOESY spectra. Detailed analyses of the ¹H–¹H and ¹³C–¹H COSY spectra revealed connectivities of C-1 to C-14, C-2 to C-3, C-5 to C-6, C-6 to C-7 and C-9 to C-10. In its HMBC spectrum, H-1 β showed long-range ¹H–¹³C correlations with C-2, C-3, C-11, C-13, C-14 and C-15, H-2 β with C-4, C-8, C-14 and C-15, H-6 with C-4 and C-8, H-7 with C-5, C-6 and C-8, H-9 β with C-7, H-10 α with C-8 and C-9, H-14 α with C-1, C-2, C-13 and C-15, and H-20b with C-5. Also, NOESY correlations of H-2 β /H-9 β and H₃-16, H-5/H-6, H-9 β /H₃-16 and H₃-16/H₃-17 were observed.

2) Prolonged reaction time in this thermolysis allowed the formation of the 20-*O*-cinnamate **2a** and the 4(20),5-diene **3** in 36% and 11% yields after 15 min, and in 27% and 22% yields after 30 min, respectively, with recovery of the taxoid **1a** and formation of the pertinent cinnamic acid. Analogous results were obtained in the thermolysis of the taxoid **1a** at 275 °C for 8 min (the yields of the products: 32% for the 20-*O*-cinnamate **2a**, 19% for the diene **3**, with the 49% recovery of the taxoid **1a**). No formation of detectable amounts of other products in these reactions was shown by TLC and ¹H-NMR spectral analyses.

3) Under analogous conditions (at 230 °C for 10 min), taxinine H (**1b**) (26 mg, 0.05 mmol) gave acetic acid (9 α ,10 β -diacetoxo-4-acetoxymethyl-8 β ,12,15 α ,15 β -tetramethyl-13-oxotricyclo[9.3.1.0^{3,8}]pentadeca-4,11-dien-2 α -yl) ester (**2b**) (5.2 mg, 20%) and the diene **3** (9.2 mg, 40%), with recovery of the taxoid **1b**. No formation of detectable amounts of other products except cinnamic acid was observed. The product **2b** was isolated in a pure state by repeated preparative TLC eluted by CHCl₃–AcOEt (10/1).

2b: Amorphous powder, [α]_D²⁵ = +44.3 (CHCl₃, *c* = 0.6). FAB-MS *m/z* (rel. int. %): 519 (*M*⁺ + H, 10), 459 (35), 417 (5), 399 (5), 357 (13), 339 (7), 307 (27), 297 (25). IR (KBr) cm^{–1}: 1741 (C=O), 1372, 1230, 1024. UV $\lambda_{\max}$ (EtOH) nm (ϵ): 268 (1960). ¹H-NMR (CDCl₃) δ : 6.02 (1H, d, *J* = 11, H-9), 5.90 (1H, d, *J* = 11, H-10), 5.79 (1H, brs, H-5), 5.67 (1H, dd, *J* = 2.0, 2.4, H-2), 4.79 (1H, d, *J* = 13, H-20a), 4.58 (1H, d, *J* = 13,

H-20b), 3.25 (1H, br s, H-3), 2.82 (1H, dd, $J=7$, 20, H-14b), 2.68 (1H, d, $J=20$, H-14a), 2.05 (1H, dd, $J=2.4$, 7, H-1), 2.07, 2.05, 2.03, 2.02 and 2.01 (each 3H, each s, H-18 and four OAc), 1.81 (3H, br s, H-16), 1.16 (3H, br s, H-17), 0.95 (3H, br s, H-19). ^{13}C -NMR (CDCl_3) δ : 199.5 (C-13), 170.9 (OAc), 170.4 (OAc), 170.0 (OAc), 169.7 (C-21), 150.4 (C-11), 138.3 (C-12), 133.7 (C-4), 131.1 (C-5), 75.5 (C-9), 72.7 (C-10), 71.5 (C-2), 69.0 (C-20), 47.1 (C-1), 43.0 (C-3), 41.6 (C-8), 39.0 (C-15), 37.5 (C-17), 36.0 (C-14), 28.3 (C-7), 25.1 (C-16), 22.4 (C-6), 21.5 (OAc), 21.1 (OAc), 20.9 (OAc), 20.7 (OAc), 17.8 (C-19), 13.5 (C-18). HR FAB-MS m/z : 519.2601 $[\text{M}+\text{H}]^+$ (Calcd for $\text{C}_{28}\text{H}_{39}\text{O}_9$: 519.2594 $[\text{M}+\text{H}]^+$).

Thermolysis of the 20-*O*-Cinnamate (2a) The compound **2a** (5.0 mg, 0.008 mmol) was heated without solvent at 200 °C for 10 min. TLC densitometric analysis of the mixture showed the formation of the taxoid **1a** (6%) with recovery of a large amount of the unreacted **2a**. Thermolysis of the compound **2a** (10.0 mg, 0.016 mmol) at 265 °C gave the taxoid **1a** (3.4 mg, 34%) and the diene **3** (1.1 mg, 14%), with recovery of the starting **2a** and formation of the pertinent cinnamic acid. The ^1H -NMR spectrum of the reaction mixture showed no formation of detectable amounts of other products. Prolongation of the heating time (for 30 min) in this reaction caused the degradation of the products which was evidenced by the presence of complex mixtures by TLC and NMR spectroscopic analyses.

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