

## TWO TAXANE DITERPENES FROM *TAXUS MAIREI*

JING-YU LIAN, ZHI-DA MIN, MIZUO MIZUNO,\* TOSHIYUKI TANAKA\* and MUNEKAZU IINUMA\*

Department of Phytochemistry, China Pharmaceutical University, Nanjing, China, \*Department of Pharmacognosy, Gifu Pharmaceutical University, 6-1 Mitahora-higashi 5 chome, Gifu 502, Japan

(Received 2 March 1988)

**Key Word Index**—*Taxus mairei*, Taxaceae, diterpene, 1-hydroxy-4-deacetylbaaccatin IV, 1-acetoxy-5-deacetylbaaccatin I

**Abstract**—Two new baaccatin type diterpenes were isolated from the stem bark of *Taxus mairei*. Their structures were established as 1-dehydroxy-4-deacetylbaaccatin IV and 1-acetoxy-5-deacetylbaaccatin I on the basis of spectroscopic analysis.

### INTRODUCTION

In the previous paper [1, 2], we have reported three new non-taxane type diterpenes from the bark of *Taxus mairei*, taxamairins A, B and C. As the continuation of our chemical investigation on *T. mairei*, we now report the structures of two new baaccatin type diterpenes: 1-dehydroxy-4-deacetylbaaccatin IV (1) and 1-acetoxy-5-deacetylbaaccatin I (2). The present paper deals with the isolation, structure elucidation and identification of the compounds.

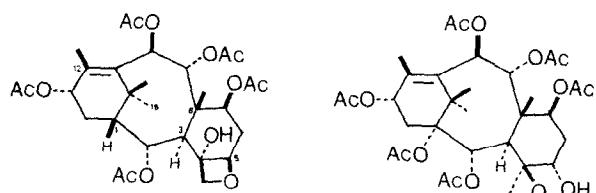
### RESULTS AND DISCUSSION

An ethanolic extract of the bark of *Taxus mairei* (Lemée et Lével.) S. Y. Hu growing in Fujing province, China was fractionated by column chromatography on silica gel or neutral aluminium oxide. Finally 1 and 2 were purified by recrystallization.

Compound 1,  $C_{30}H_{42}O_{12}$  ( $M^+ m/z$  594 266), showed a UV maximum at 220 nm which revealed the absence of the cinnamate group. It was shown in the IR spectrum an absorption at  $3461\text{ cm}^{-1}$  relating to a hydroxyl group. The  $^1\text{H}$  NMR spectrum exhibited the presence of tertiary methyl groups of taxane type;  $\delta 1.20$  ( $C_{17}$ ),  $1.56$  ( $C_{19}$ ),  $1.67$  ( $C_{16}$ ) and  $1.96$  ( $C_{18}$ ) ppm. In the range of 2.0–2.2 ppm, there were five methyl signals assignable to acetyl groups. The signals at  $4.20$  ( $1\text{H}, d, J=7.4\text{ Hz}$ ) and  $4.53$  ( $1\text{H}, d, J=7.2\text{ Hz}$ ) ppm appeared as an AX system indicating a methylene at C-20 of taxane belonging to baaccatin IV type [3]. The configuration of five acetyl groups could be assigned as follows: an AX system, which consisted of  $6.03$  and  $6.16$  ( $J=12.4\text{ Hz}$ ) ppm, was attributed to  $9\beta$ -H and  $10\alpha$ -H ( $\theta=180^\circ$ ). It was resulted in the acetyl groups being  $9\alpha$  and  $10\beta$ . On the other hand, three signals at  $2.46$  ( $1\text{H}, ddd, J_{14\beta, 14\alpha}=15.2, J_{14\beta, 13\beta}=7.2, J_{14\beta, 1}=1.4\text{ Hz}$ ),  $1.83$  ( $1\text{H}, ddd, J_{14\alpha, 14\beta}=15.2, J_{14\alpha, 13\beta}=9.0, J_{14\alpha, 1}=1.4\text{ Hz}$ ) and  $6.13$  ( $1\text{H}, dd, J_{13\beta, 14\alpha}=9.1, J_{13\beta, 14\beta}=7.2\text{ Hz}$ ) were assignable to the  $14\beta$ -H,  $14\alpha$ -H and  $13\beta$ -H, respectively. An acetyl group was shown to be at  $13\alpha$  and a proton to be at C- $1\beta$ . Furthermore, a characteristic signal of 3-H of taxane appeared at  $3.03$  ( $d, J=5.5\text{ Hz}$ ) ppm. Because the dihedral angle between  $1\beta$ -H and  $2\beta$ -H was nearly  $90^\circ$ , a peak for 2-H, which only coupled with

3-H, was observed in a doublet at  $5.65$  ppm. The result suggested an acetyl group at  $2\beta$ -C. The signal of  $5.52$  ( $1\text{H}, dd, J_{7\alpha, 6\beta}=11.0, J_{7\alpha, 6\alpha}=6.5\text{ Hz}$ ) ppm being attributed to the  $H-7\alpha$  that made clear an acetyl group at the  $7\beta$ -C. As a matter of course, a hydroxyl was assigned to be at  $4\alpha$ -C. Finally, the structure of 1-dehydroxy-4-acetylbaaccatin I could be proposed for 1.

Compound 2,  $C_{32}H_{44}O_4$  (FD and  $M^+ m/z$  652), a UV maximum at  $220\text{ nm}$ . It showed a IR absorption at  $3569\text{ cm}^{-1}$  which was related to a hydroxyl group. The  $^1\text{H}$  NMR spectrum of 2 revealed the presence of four tertiary methyl groups of taxane type,  $\delta 1.22$  ( $17\text{-Me}$ ),  $1.24$  ( $19\text{-Me}$ ),  $1.62$  ( $16\text{-Me}$ ) and  $2.24$  ( $18\text{-Me}$ ) ppm. There were signals of six acetyl groups in the range from  $2.0$  to  $2.4$  ppm. The signals of  $2.25$  ( $1\text{H}, d, J=5.0\text{ Hz}$ ) and  $3.63$  ( $1\text{H}, d, J=5.0\text{ Hz}$ ) ppm appeared as an AX system, indicative of a methylene group of the oxetan ring of baaccatin I type [4]. In the  $^1\text{H}$  NMR spectrum of 2, it was shown  $9\text{-C}$ ,  $10\text{-C}$  and  $13\text{-C}$  bearing an acetyl group, respectively. Two signals of  $2.12$  ( $1\text{H}, ddd, J_{6\beta, 6\alpha}=15.0, J_{6\beta, 7\alpha}=9.9, J_{6\beta, 5\beta}=4.4\text{ Hz}$ ) and  $1.76$  ( $1\text{H}, ddd, J_{6\alpha, 6\beta}=15.0, J_{6\alpha, 7\alpha}=4.1, J_{6\alpha, 5\beta}=3.0\text{ Hz}$ ) ppm were assignable for methylene at  $6\text{-C}$ , and a signal of  $5.52$  ( $1\text{H}, dd, J_{7\alpha, 6\beta}=9.9, J_{7\alpha, 6\alpha}=4.0\text{ Hz}$ ) ppm was also attributable to  $7\alpha$ -H, which showed an acetyl group to be at  $7\beta$ -C. On the other hand, two signals at  $2.52$  ( $1\text{H}, dd, J_{14\beta, 14\alpha}=15.0, J_{14\beta, 13\beta}=9.7\text{ Hz}, 14\beta\text{-H}$ ) and  $1.96$  ( $1\text{H}, dd, J_{14\alpha, 14\beta}=15.0, J_{14\alpha, 13\beta}=7.2\text{ Hz}$ ) ppm suggested two acetyl groups attaching at  $13\alpha$ -C and  $1\beta$ -C on the basis of the respective coupling constants. The final acetyl group was at the  $2\text{-C}$ ,



1

2

supported by their chemical shifts and coupling constant between  $3\alpha$ -H [3.18 ( $d$ ,  $J = 4.4$  Hz)] and  $2\beta$ -H [5.52 ( $J = 4.4$  Hz)]. Della and coworkers reported [5], if the  $5\alpha$ -C position was substituted with an acetyl group, the signal of  $5\beta$ -H appeared at 5.62 ppm. If the same position was substituted by a hydroxy group, the signal of  $5\beta$ -H appeared at 4.16 ppm. So, the signal of 4.15 ppm of **2** had to show the presence of a hydroxyl group at  $5\alpha$ -C. The structure of **2**, hence could be written as 1-acetoxy-5-deacetyl**baccatin I**.

## EXPERIMENTAL

*Plant material* Stem barks of *Taxus mairei* were collected in Fujian province, and a voucher was deposited in the Herbarium of China Pharmaceutical University.

*Separation and isolation of 1 and 2.* According to our previous papers [1, 2], the EtOAc fraction was chromatographed on a neutral alumina by CC, eluted by a portion of a mixed soln of EtOAc in cyclohexane (5:2 and 1:2). Compounds **1** and **2** were obtained after being recrystallized from MeOH, yielding 0.0017 and 0.005%, respectively.

**1-Dehydroxy-4-deacetyl**baccatin IV (1)**** Colourless crystals, mp 229–230° (EtOH).  $[\alpha]_D^{24} = 40$  (acetone;  $c$  0.5),  $M_r$  594.2665 ( $M^+$ , calcd. 594.2777),  $C_{30}H_{42}O_{12}$ , UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm (log  $\epsilon$ ). 219 (3.81). IR  $\nu_{\text{max}}$  (KBr) 3461, 1744, 1721, 1651, 1241, 1226, 1027  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (400 MHz,  $\text{CD}_3\text{OD}$ ).  $\delta$  1.20 (3H, s, 17-Me), 1.56 (3H, s, 19-Me), 1.67 (3H, s, 16-Me), 1.96 (3H, s, 18-Me), 1.83 (1H, *ddd*,  $J_{14\alpha, 14\beta} = 15.2$ ,  $J_{14\alpha, 14\beta} = 9.0$ ,  $J_{14\alpha, 1} = 1.4$  Hz, 14 $\alpha$ -H), 2.46 (1H, *ddd*,  $J_{14\beta, 14\alpha} = 15.2$ ,  $J_{14\beta, 13\beta} = 7.2$ ,  $J_{14\beta, 1} = 7.2$  Hz, 14 $\beta$ -H), 1.95 (3H, s, OAc), 2.1 (6H, s, 2  $\times$  OAc), 2.17 (3H, s, OAc), 2.20 (3H, s, OAc), 3.03 (1H, *d*,  $J_{3\alpha, 2\beta} = 5.5$  Hz, 3 $\alpha$ -H), 4.20 (1H, *d*,  $J = 7.2$  Hz, 20-H), 4.53 (1H, *d*, 20-H), 5.00 (1H, *d*,  $J = 10$  Hz, 5 $\alpha$ -H), 5.52 (1H, *dd*,  $J_{7\alpha, 6\beta} = 11.0$ ,  $J_{7\alpha, 6\alpha} = 6.5$  Hz, 7-H), 5.56 (1H, *d*,  $J_{2\beta, 3\alpha} = 5.5$  Hz, 2 $\beta$ -H), 6.03 (1H, *d*,  $J_{9\beta, 10\alpha} = 12.4$  Hz, 9 $\beta$ -H), 6.13

(1H, *dd*,  $J_{13\beta, 14\alpha} = 9.0$ ,  $J_{14\beta, 13\beta} = 7.2$  Hz, 13- $\beta$ -H), 6.16 (1H, *d*,  $J_{10\alpha, 9\beta} = 12.4$  Hz, 10 $\alpha$ -H). MS  $m/z$  (rel int): 594 ( $M^+$ , 6.0), 593 (39.2), 490 (31.2), 472 (39.4), 430 (39.4), 412 (9), 205 (25), 195 (93.7), 149 (100), 105 (62.5), 93 (39.7), 67 (28.1), 55 (40.6)

**1-Acetoxy-5-deacetyl**baccatin I (2)**** Colourless crystal, mp 240–241° (EtOH).  $M_r$  652,  $C_{32}H_{44}O_{14}$ . UV  $\lambda_{\text{max}}^{\text{MeOH}}$  220 nm. IR  $\nu_{\text{max}}$  (KBr) 3569, 1742, 1631, 1241  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (400 MHz,  $\text{CD}_3\text{OD}$ ).  $\delta$  1.22 (3H, s, 17-Me), 1.24 (3H, s, 19-Me), 1.62 (3H, s, 16-Me), 2.24 (3H, s, 18-Me), 1.89 (3H, s, OAc), 2.00 (3H, s, OAc), 2.18 (6H, s, 2  $\times$  OAc), 2.22 (3H, s, OAc), 2.24 (3H, s, OAc), 1.76 (1H, *ddd*,  $J_{6\alpha, 6\beta} = 15.0$ ,  $J_{6\alpha, 7\alpha} = 4.1$ ,  $J_{6\alpha, 5\beta} = 3.0$  Hz, 6 $\alpha$ -H), 2.12 (1H, *ddd*,  $J_{6\beta, 6\alpha} = 15.0$ ,  $J_{6\beta, 7\alpha} = 9.9$ ,  $J_{6\beta, 5\beta} = 4.4$  Hz, 6 $\beta$ -H), 2.52 (1H, *d*,  $J = 5.0$  Hz, 20-H), 3.63 (1H, *d*,  $J = 5.0$  Hz, 20-H), 1.96 (1H, *dd*,  $J_{14\alpha, 14\beta} = 15.0$ ,  $J_{14\alpha, 13\beta} = 7.2$  Hz, 14 $\alpha$ -H), 2.52 (1H, *dd*,  $J_{14\beta, 14\alpha} = 15.0$ ,  $J_{14\beta, 13\beta} = 9.7$  Hz, 14 $\beta$ -H), 3.18 (1H, *d*,  $J_{3\alpha, 2\beta} = 4.4$  Hz, 3 $\alpha$ -H), 4.16 (1H, *dd*,  $J_{5\beta, 6\beta} = 4.4$ ,  $J_{5\beta, 6\alpha} = 3.0$  Hz, 5 $\beta$ -H), 5.50 (1H, *d*,  $J_{2\beta, 3\alpha} = 4.4$  Hz, 2 $\beta$ -H), 5.52 (1H, *dd*,  $J_{7\alpha, 6\beta} = 9.9$  Hz,  $J_{7\alpha, 6\alpha} = 4.1$  Hz, 7 $\alpha$ -H), 6.09 (1H, *q*,  $J_{13\beta, 14\beta} = 9.9$ ,  $J_{13\beta, 14\beta} = 7.2$ , 13- $\beta$ -H), 6.10 (1H, *d*,  $J_{9\beta, 10\alpha} = 11.0$  Hz, 9 $\beta$ -H), 6.22 (1H, *d*,  $J_{10\alpha, 9\beta} = 11.0$  Hz, 10 $\alpha$ -H). FDMS  $m/z$  (rel int.) 653 ( $M + 1$ , 32), 652 ( $M$ , 60), 595 (7), 593 (100). EIMS  $m/z$  (rel int.) 594 (4), 533 (17), 490 (14), 430 (14.2), 352 (8), 310 (8), 195 (95), 253 (100), 93 (40)

## REFERENCES

1. Liang, J., Min, Z., Mizuno, M., Tanaka, T. and Iinuma M. (1987) *Chem. Pharm. Bull.* **35**, 1613.
2. Liang, J., Min, Z., Mizuno, M., Tanaka, T. and Iinuma, M. (1988) *Acta Chim. Sinica* **46**, 21.
3. Della, D. P., Marcano, C. de and Halsall, T. G. J. (1975) *Chem. Comm.* 365.
4. Senish, V., Blechert, S., Colin, M., Guenard, D., Picot, F., Potier, P. and Varenne, P. (1984) *J. Nat. Prod.* **47**, 131.
5. Della, D. P., Marcano, C. de and Halsall, T. G. (1969) *Chem. Comm.* 1284