



## *Abeo-taxanes from Taxus mairei*

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### Abstract

One new taxane (baccatin III 13-cinnamate), two new 11(15 → 1)*abeo*-taxanes (taxumains A and B) and 15 known taxanes were isolated from the twigs of *Taxus mairei*. The structures of new compounds were determined by spectroscopic methods. © 1998 Published by Elsevier Science Ltd. All rights reserved.

**Keywords:** *Taxus mairei*; Taxanes; *Taxus*; Twigs; Taxanes; 11(15 → 1)*abeo*-taxanes

### 1. Introduction

The chemical constituents of the plants of the *Taxus* genus have been extensively investigated (Appendino, 1995; Kingston, Molinero & Rimoldi, 1993; Chen, 1990), partly due to the discovery of the antitumor agent, taxol (Wani et al., 1971). *T. mairei* is the only species belonging to the genus *Taxus* found in Taiwan. A few taxane derivatives have been isolated from the heartwood of this plant (Yeh, Wang & Chen, 1988; Min, Jiang & Liang, 1989). In a previous paper (Yang, Fang & Cheng, 1996), we have reported 16 taxane diterpenes having C4(20)-exocyclic double bonds isolated from the twigs. As the continuation of our chemical investigation on *T. mairei*, we report herein 18 additional taxanes, including two new 11(15 → 1)*abeo*-taxanes.

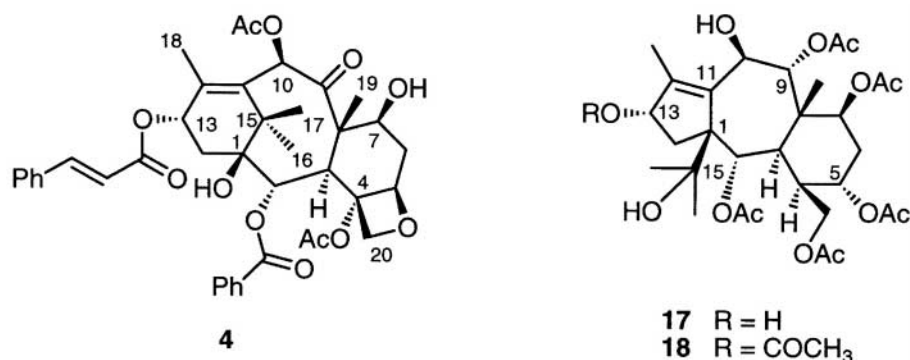
### 2. Results and discussion

A concentrated acetone extract of the twigs of *T. mairei* was taken in ethyl acetate, the soluble part was concentrated and chromatographed to give 18 taxanes. By analysis of their physical and spectral properties (mp,  $[\alpha]$  NMR, IR, MS,  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra), 15 known compounds were identified as baccatin III **1** (Henilh et al., 1984), 13-dehydro-10-deacetyl baccatin

III **2** (Barboni et al., 1994), 13-dehydrobaccatin III **3** (Wani et al., 1971), taxol **5** (Wani et al., 1971), taxol C **6** (Kitagawa et al., 1995), taxol 7-( $\beta$ -triacytylxyloside) **7** (Henilh et al., 1984), 1-deoxybaccatin IV **8** (Della Casa de Marcano & Halsall, 1975), baccatin IV **9** (Della Casa de Marcano & Halsall, 1975), 1-deoxybaccatin VI **10** (Min, Jiang & Liang, 1989), baccatin VI **11** (Min, Jiang & Liang, 1989), 2 $\alpha$ -benzoyloxy-4 $\alpha$ ,20-dihydroxy-5 $\alpha$ ,7 $\beta$ ,9 $\alpha$ ,10 $\beta$ ,13 $\alpha$ -pentaacetoxytax-11-ene **12** (Liang & Kingston, 1993), 2 $\alpha$ -benzoyloxy-4 $\alpha$ ,5 $\alpha$ -dihydroxy-7 $\beta$ ,9 $\alpha$ ,10 $\beta$ ,13 $\alpha$ ,20-pentaacetoxytax-11-ene **13** (Liang & Kingston, 1993), 4 $\beta$ ,20-epoxy-2 $\alpha$ ,5 $\alpha$ ,10 $\beta$ ,13 $\alpha$ -tetraacetox-1 $\beta$ ,7 $\beta$ ,9 $\alpha$ -trihydroxytax-11-ene **14** (Shen, Tai & Chen, 1996), 1 $\alpha$ ,7 $\beta$ -dihydroxy-4 $\beta$ ,20-epoxy-2 $\alpha$ ,5 $\alpha$ ,9 $\alpha$ ,10 $\beta$ ,13 $\alpha$ -pentaacetoxytax-11-ene **15** (Chu et al., 1993), and 1 $\beta$ -hydroxybaccatin I **16** (Yeh, Wang & Chen, 1988).

The new taxane **4** was determined to be baccatin III 13-cinnamate by analysis of its  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra. The assignments of individual protons and carbons were made by the assistance of  $^1\text{H}$ - $^1\text{H}$  COSY, HMQC and HMBC spectra. The carbon resonance at  $\delta_{\text{C}}$  166.2 was attributable to the cinnamoyl group, of which *E*-configuration was inferred from a large coupling constant of 16 Hz between the two olefinic protons at  $\delta_{\text{H}}$  7.83 and 6.51. The HMBC spectrum showed the correlation of cinnamoyl carbonyl (at  $\delta_{\text{C}}$  166.2) with the C-13 proton (at  $\delta_{\text{H}}$  6.18), and the correlation of benzoyl carbonyl (at  $\delta_{\text{C}}$  166.9) with the C-2 proton (at  $\delta_{\text{H}}$  5.66). The characteristic resonances of oxetane occurred at  $\delta_{\text{H}}$  4.15 and 4.31 (AB type,  $J = 8.4$  Hz).

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The 10 $\alpha$ -H occurring at  $\delta$  6.32 (s) was consistent with the structure having C-10 acetoxy and C-9 carbonyl groups. The H-3 occurred at a relatively low field of  $\delta$  3.85, presumably due to the deshielding effect of the carbonyl group at C-9.

Compound **17** (taxumain A) and **18** (taxumain B) are 11(15  $\rightarrow$  1)*abeo*-taxanes. The molecular formula of **17** was deduced to be C<sub>30</sub>H<sub>44</sub>O<sub>13</sub> as its molecular ion occurred at  $m/z$  612.2722 in the HR mass spectrum. From the NMR spectral analysis, no oxetane group was present in **17**; instead, a carbon resonance at  $\delta$  62.7 and the proton resonances at  $\delta$  3.75 (*d*,  $J$  = 11.6 Hz) and 4.19 (*dd*,  $J$  = 11.6, 8.4 Hz) indicated the presence of a CH<sub>2</sub>OAc moiety. The assignment of H-3 at  $\delta$  2.73 (*dd*,  $J$  = 8.0, 4.4 Hz) was supported by its correlation with H-4 (at  $\delta$  2.12) in the <sup>1</sup>H–<sup>1</sup>H COSY spectrum. Similarly, H-2 at  $\delta$  5.69 (*d*,  $J$  = 8.0 Hz) was assigned. The presence of a hydroxy substituent on C-15 was indicated by the carbon resonance at  $\delta$  76.3. The HMBC spectrum also showed the correlations of H-2 with C-1 (at  $\delta$  67.6), C-4 (at  $\delta$  41.5) and C-15, in agreement with the structure of 11(15  $\rightarrow$  1)*abeo*-taxane **17**. The large coupling constant  $J_{9,10}$  = 10.4 Hz was consistent with the *trans* relationship of H-9 (at  $\delta$  5.53) and H-10 (at  $\delta$  4.54). The NOESY experiment showed correlation of H-2 (at  $\delta$  5.69) with H-9 and H-19 (at  $\delta$  0.98), supporting the assigned stereochemistry.

Compound **18** had <sup>1</sup>H and <sup>13</sup>C NMR spectral characteristics similar to those of **17**, except that the H-13 geminal to an acetoxy group appeared at a lower field of  $\delta$  5.57. The exact mass of molecular ion at  $m/z$  654.2813 led to a molecular formula C<sub>32</sub>H<sub>46</sub>O<sub>14</sub>. Thus compound **18** was determined to be the 13-acetyl derivative of **17**. By the assistance of HMQC and HMBC spectra, individual protons and carbons were assigned. The signals of H-9 and H-10 occurred at  $\delta$  5.59 (*d*,  $J$  = 10.2 Hz) and 4.55 (*d*,  $J$  = 10.2 Hz), respectively. The correlation of C-13 (at  $\delta_C$  78.7) with one of H-14 (at  $\delta_H$  1.67) was shown in the HMBC spectrum. In agreement with this assign-

ment, H-13 appeared at a low field of  $\delta$  5.57 (*br t*) due to the inductive effect of an acetoxy substituent at C-13.

In summary, the twigs of *T. mairei* contain various taxane diterpenes. The known *abeo*-taxanoids usually have either 4-oxy substituents or 4(20)-double bonds (Kingston, Samaranayake & Ivey, 1990; Zhang et al., 1994; Fuji et al., 1995). Taxumain A and taxumain B are, however, the first reported *abeo*-taxanes lacking any oxygenated functionality at C-4.

### 3. Experimental

#### 3.1. General

Yanagimoto (or MP-500D) micro melting point apparatus; Jasco Dip-180 digital polarimeter, Finnigan TSQ-46c mass spectrometer; <sup>1</sup>H NMR: 300 or 400 MHz; <sup>13</sup>C NMR: 75 or 100 MHz; Waters M-45 high-pressure liquid chromatograph with Hibar Lichrosorb Si 60 column (10  $\mu$ m or 7  $\mu$ m, 25  $\times$  1 cm i.d.) were used.

#### 3.2. Plant material

The twigs (1.2 kg) of *T. mairei* were collected in the remote mountains at an elevation of ca. 2100 m (Tong-Shi, Taichung county). A voucher specimen is deposited in the Herbarium of National Taiwan University. The air-dried material was exhaustively extracted with acetone (7 L  $\times$  3). The acetone extract was concentrated to give 100 g of residue, which was diluted with water and extracted with EtOAc (1 L  $\times$  3). The combined EtOAc extracts were concentrated to give an oil (75 g), which was absorbed with 110 g of SiO<sub>2</sub> and then chromatographed on a column packed with 650 g of SiO<sub>2</sub> by elution with gradients of hexane, EtOAc and acetone. The portion obtained from the elution of EtOAc/hexane (70–90%) was further subjected to flash chromatography and HPLC

with elution of EtOAc/CH<sub>2</sub>Cl<sub>2</sub> (50–60%) or acetone/hexane (20–40%). Compounds **8** (20 mg), **10** (22 mg), **9** (25 mg), **11** (17 mg), **16** (30 mg), **15** (8 mg), **3** (12 mg), **4** (8 mg), **1** (25 mg), **13** (7 mg), **12** (5 mg), **2** (20 mg), **14** (16 mg), **18** (14 mg), **17** (22 mg), **6** (6 mg), **5** (10 mg) and **7** (7 mg) were obtained in the ascending order of polarity.

### 3.3. Baccatin III (**1**)

Needles, mp 238–240°, [ $\alpha$ ]<sub>D</sub><sup>29</sup> –50 (CHCl<sub>3</sub>; *c* 1.0) { [8], mp 236–238°, [ $\alpha$ ]<sub>D</sub> –54 (CHCl<sub>3</sub>; *c* 1.2)}.

### 3.4. 13-Dehydro-10-deacetylbaaccatin III (**2**)

Needles, mp 167–170°, [ $\alpha$ ]<sub>D</sub><sup>23</sup> –12.4 (CHCl<sub>3</sub>; *c* 1.9) { [9] mp 168–169°}.

### 3.5. 13-Dehydrobaccatin III (**3**)

Needles, mp 211–213°, [ $\alpha$ ]<sub>D</sub><sup>29</sup> –32.4 (CHCl<sub>3</sub>; *c* 1.2) { [4], mp 210–212°, [ $\alpha$ ]<sub>D</sub> –40 (CHCl<sub>3</sub>; *c* 1.8)}.

### 3.6. Baccatin III 13-cinnamate (**4**)

Gum, [ $\alpha$ ]<sub>D</sub><sup>25</sup> –75.7 (CHCl<sub>3</sub>; *c* 1.0). IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>–1</sup>: 3428, 1712, 1596, 1509, 1176. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  1.11 (3 H, *s*, H-17), 1.22 (3 H, *s*, H-16), 1.66 (3 H, *s*, H-19), 2.00 (3 H, *s*, H-18), 2.22 (1 H, *dd*, 8.4, 15.2 Hz, H-14), 2.23 (3 H, *s*, AcO), 2.26 (3 H, *s*, AcO), 2.41 (1 H, *dd*, 8.4, 15.2 Hz, H-14), 2.55 (2 H, *m*, H-6), 3.85 (1 H, *d*, 6.8 Hz, H-3), 4.31 (1 H, *d*, 8.4 Hz, H-20), 4.15 (1 H, *d*, 8.4 Hz, H-20), 4.45 (1 H, *m*, H-7), 4.96 (1 H, *d*, 8.1 Hz, H-5), 5.66 (1 H, *d*, 6.8 Hz, H-2), 6.18 (1 H, *t*, 8.4 Hz, H-13), 6.32 (1 H, *s*, H-10), 6.51 (1 H, *d*, 16.0 Hz), 7.43 (3 H, *m*), 7.46 (2 H, *m*), 7.56 (2 H, *m*), 7.57 (1 H, *m*), 7.83 (1 H, *d*, 16.0 Hz), 8.04 (2 H, *d*, 7.2 Hz). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  9.4 (C-19), 15.5 (C-18), 20.9 (AcO), 21.3 (C-16), 22.6 (AcO), 26.8 (C-17), 35.6 (C-14), 36.2 (C-6), 43.0 (C-15), 45.8 (C-3), 58.7 (C-8), 70.0 (C-13), 72.3 (C-7), 74.8 (C-2), 75.7 (C-10), 76.4 (C-20), 79.2 (C-1), 81.0 (C-4), 84.3 (C-5), 116.7 (cinn.), 128.2, 128.6, 129.0, 129.1, 130.0, 130.9 (2 C, C-11 and BzO), 133.7, 133.8, 143.1 (C-12), 146.7 (cinn.), 166.2 (cinn.), 166.9 (BzO), 169.8 (AcO), 171.3 (AcO), 203.8 (C-9). FAB-MS (NBA) *m/z* (rel. int.): 657 [M-OAc]<sup>+</sup> (5), 639 (7), 579 (3), 509 (4), 327 (10), 289 (20), 154 (70), 131 (100). HR-MS for C<sub>38</sub>H<sub>41</sub>O<sub>10</sub> [M-OAc]<sup>+</sup> requires: 657.2700. Found: 657.2704.

### 3.7. Taxol (**5**)

Needles, mp 195–200°, [ $\alpha$ ]<sub>D</sub><sup>29</sup> –30 (CHCl<sub>3</sub>; *c* 0.9) { [4], mp 198–203°, [ $\alpha$ ]<sub>D</sub> –42 (CHCl<sub>3</sub>; *c* 1.0)}.

### 3.8. Taxol C (**6**)

Needles, mp 200–204°, [ $\alpha$ ]<sub>D</sub><sup>29</sup> –50 (CHCl<sub>3</sub>; *c* 0.5) { [10], mp 202–204°, [ $\alpha$ ]<sub>D</sub> –62 (CHCl<sub>3</sub>; *c* 0.8)}.

### 3.9. Taxol 7-( $\beta$ -triacetylxyloside) (**7**)

Gum, [ $\alpha$ ]<sub>D</sub><sup>29</sup> –10 (CHCl<sub>3</sub>; *c* 0.8) { [8], [ $\alpha$ ]<sub>D</sub> –23 (CHCl<sub>3</sub>; *c* 1.0)}.

### 3.10. 1-Deoxybaccatin IV (**8**)

Needles, mp 259–262°, [ $\alpha$ ]<sub>D</sub><sup>29</sup> +88 (CHCl<sub>3</sub>; *c* 1.2) { [11], mp 259–260°, [ $\alpha$ ]<sub>D</sub> +99 (CHCl<sub>3</sub>; *c* 1.5)}.

### 3.11. Baccatin IV (**9**)

Needles, mp 253–255°, [ $\alpha$ ]<sub>D</sub><sup>29</sup> +15 (CHCl<sub>3</sub>; *c* 1.6) { [11], mp 254–255°, [ $\alpha$ ]<sub>D</sub> +19 (CHCl<sub>3</sub>; *c* 1.0)}.

### 3.12. 1-Deoxybaccatin VI (**10**)

Needles, mp 219–222°, [ $\alpha$ ]<sub>D</sub><sup>29</sup> –18 (CHCl<sub>3</sub>; *c* 1.2) { [6], mp 220–221°, [ $\alpha$ ]<sub>D</sub> –21.2 (CHCl<sub>3</sub>; *c* 1.0)}.

### 3.13. Baccatin VI (**11**)

Needles, mp 238–241°, [ $\alpha$ ]<sub>D</sub><sup>29</sup> –15 (CHCl<sub>3</sub>; *c* 1.6) { [6], mp 239–241°, [ $\alpha$ ]<sub>D</sub> –9 (CHCl<sub>3</sub>; *c* 1.9)}.

### 3.14. 2 $\alpha$ -Benzoyloxy-4 $\alpha$ ,20-dihydroxy-5 $\alpha$ ,7 $\beta$ ,9 $\alpha$ ,10 $\beta$ ,13 $\alpha$ -pentaacetoxytax-11-ene (**12**)

Needles, mp 112–115°, [ $\alpha$ ]<sub>D</sub><sup>29</sup> –18 (CHCl<sub>3</sub>; *c* 0.5) { [12], mp 114–115°, [ $\alpha$ ]<sub>D</sub> –30 (CHCl<sub>3</sub>; *c* 0.1)}.

### 3.15. 2 $\alpha$ -Benzoyloxy-4 $\alpha$ ,5 $\alpha$ -dihydroxy-7 $\beta$ ,9 $\alpha$ ,10 $\beta$ ,13 $\alpha$ ,20-pentaacetoxytax-11-ene (**13**)

Needles, mp 120–124°, [ $\alpha$ ]<sub>D</sub><sup>29</sup> –20 (CHCl<sub>3</sub>; *c* 0.4) { [12], mp 122–123°, [ $\alpha$ ]<sub>D</sub> –37 (CHCl<sub>3</sub>; *c* 0.1)}.

### 3.16. 4 $\beta$ ,20-Epoxy-2 $\alpha$ ,5 $\alpha$ ,10 $\beta$ ,13 $\alpha$ -tetraacetox-1 $\beta$ ,7 $\beta$ ,9 $\alpha$ -trihydroxytax-11-ene (**14**)

Amorphous powder, [ $\alpha$ ]<sub>D</sub><sup>29</sup> +43 (CHCl<sub>3</sub>; *c* 0.8) { [13], [ $\alpha$ ]<sub>D</sub> +46 (CHCl<sub>3</sub>; *c* 1.5)}.

### 3.17. 1 $\alpha$ ,7 $\beta$ -Dihydroxy-4 $\beta$ ,20-epoxy-2 $\alpha$ ,5 $\alpha$ ,9 $\alpha$ ,10 $\beta$ ,13 $\alpha$ -pentaacetoxytax-11-ene (**15**)

Amorphous powder, [ $\alpha$ ]<sub>D</sub><sup>29</sup> +120 (CHCl<sub>3</sub>; *c* 0.6) { [14], [ $\alpha$ ]<sub>D</sub> +138.7 (CHCl<sub>3</sub>; *c* 1.2)}.

### 3.18. 1 $\beta$ -Hydroxybaccatin I (16)

Needles, mp 260–262°,  $[\alpha]_D^{29} + 65$  (CHCl<sub>3</sub>; *c* 1.4) { [5], mp 260–261°,  $[\alpha]_D + 71.8$  (CHCl<sub>3</sub>; *c* 1.0)}.

### 3.19. Taxumain A (17)

Needles, mp 284–286°C,  $[\alpha]_D^{23} -11.1$  (CHCl<sub>3</sub>; *c* 1.5). IR  $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$ : 3414, 1736, 1028. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  0.98 (3 H, *s*, H-19), 1.02 (3 H, *s*, H-17), 1.20 (3 H, *s*, H-16), 1.69 (1 H, *dd*, 7.2, 14.4 Hz, H-14), 1.82 (2 H, *m*, H-6), 1.84 (3 H, *s*, H-18), 2.02 (3 H, *s*, AcO), 2.03 (3 H, *s*, AcO), 2.05 (3 H, *s*, AcO), 2.06 (3 H, *s*, AcO), 2.08 (3 H, *s*, AcO), 2.10 (1 H, *m*, H-14), 2.73 (1 H, *dd*, 4.4, 8.0 Hz, H-3), 2.12 (1 H, *m*, H-4), 3.75 (1 H, *d*, 11.6 Hz, H-20), 4.19 (1 H, *dd*, 8.4, 11.6 Hz, H-20), 4.95 (1 H, *br s*, H-5), 4.54 (1 H, *d*, 10.4 Hz, H-10), 4.57 (1 H, *br t*, H-13), 5.22 (1 H, *dd*, 5.2, 11.2 Hz, H-7), 5.53 (1 H, *d*, 10.4 Hz, H-9), 5.69 (1 H, *d*, 8.0 Hz, H-2). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  11.0 (C-18), 14.1 (C-19), 20.9, 21.1, 21.2, 21.3, 21.4, 25.7 (C-16), 27.7 (C-17), 29.1 (C-6), 40.5 (C-14), 40.6 (C-3), 41.5 (C-4), 43.1 (C-8), 62.7 (C-20), 66.7 (C-10), 67.6 (C-1), 68.0 (C-2), 68.8 (C-7), 70.4 (C-5), 76.3 (C-15), 77.0 (C-13), 79.0 (C-9), 138.2 (C-11), 145.7 (C-12), 168.8, 170.7, 170.8, 171.1, 171.2. FAB-MS (NBA) *m/z* (rel. int.): 635 [M + Na]<sup>+</sup> (8), 594 (12), 577 (18), 535 (10), 417 (30), 307 (20), 154 (100). HR-MS for C<sub>30</sub>H<sub>44</sub>O<sub>13</sub> requires: 612.2782. Found: 612.2722.

### 3.20. Taxumain B (18)

Gum,  $[\alpha]_D^{25} -15.2$  (CHCl<sub>3</sub>; *c* 0.9). IR  $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$ : 3414, 1732, 1025. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  1.01 (3 H, *s*, H-19), 1.10 (3 H, *s*, H-17), 1.22 (3 H, *s*, H-16), 1.67 (1 H, *dd*, 7.5, 14.4 Hz, H-14), 1.85 (2 H, *m*, H-6), 1.87 (3 H, *s*, H-18), 2.01 (3 H, *s*, AcO), 2.02 (3 H, *s*, AcO), 2.03 (3 H, *s*, AcO), 2.04 (3 H, *s*, AcO), 2.08 (3 H, *s*, AcO), 2.09 (3 H, *s*, AcO), 2.11 (1 H, *m*, H-14), 2.13 (1 H, *m*, H-4), 2.66 (1 H, *dd*, *J* = 4.2, 8.1 Hz, H-3), 3.77 (1 H, *d*, *J* = 12.0 Hz, H-20), 4.19 (1 H, *dd*, 8.4, *J* = 12.0 Hz, H-20), 4.55 (1 H, *d*, *J* = 10.2 Hz, H-10), 4.97 (1 H, *br s*, H-5), 5.23 (1 H, *dd*, *J* = 5.7, 10.8 Hz, H-7), 5.59 (1 H, *d*, *J* = 10.2 Hz, H-9), 5.57 (1

H, *br t*, *J* = 7.5 Hz, H-13), 5.71 (1 H, *d*, *J* = 8.1 Hz, H-2). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):  $\delta$  11.0 (C-18), 14.2 (C-19), 20.8, 21.0, 21.1, 21.2, 21.3, 21.4, 25.7 (C-16), 27.8 (C-17), 29.0 (C-6), 38.1 (C-14), 40.6 (C-3), 41.5 (C-4), 43.0 (C-8), 62.7 (C-20), 66.4 (C-10), 67.7 (C-1), 67.9 (C-7), 68.7 (C-2), 70.5 (C-5), 76.4 (C-15), 78.7 (C-13), 79.7 (C-9), 140.5 (C-11), 142.1 (C-12), 168.5, 168.7, 170.6, 170.9, 171.1 (2 C). FAB-MS (NBA) *m/z* (rel. int.): 677 [M + Na]<sup>+</sup> (10), 637 (20), 577 (30), 517 (22), 475 (23), 417 (25), 136 (100). HR-MS for C<sub>32</sub>H<sub>46</sub>O<sub>14</sub> requires: 654.2888. Found: 654.2813.

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