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Specific oxidation of C-14 oxygenated 4(20),11-taxadienes by microbial transformation

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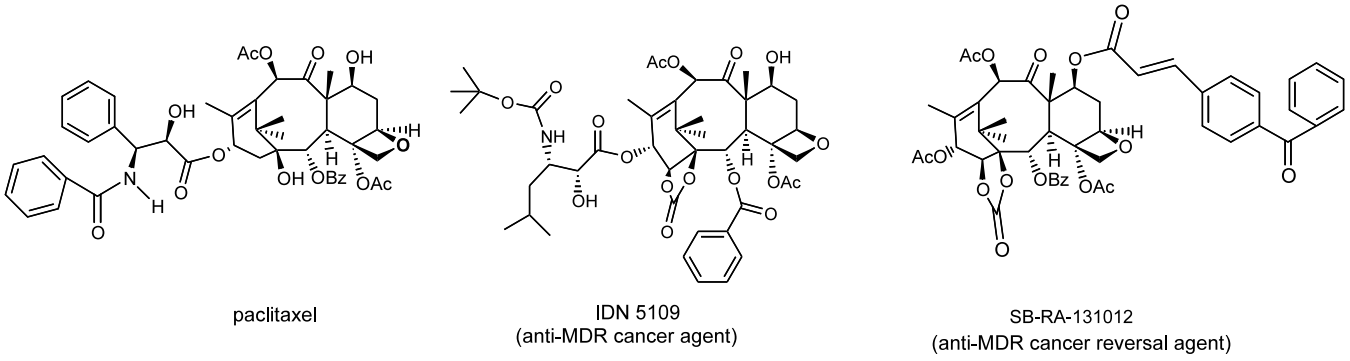
Abstract—Three C-14 oxygenated taxanes, 2α,5α,10β,14β-tetraacetoxytaxa-4(20),11-diene (**1**), 2α,5α,10β-triacetoxy-14β-(2-methylbutyryloxy)taxa-4(20),11-diene (**2**), and yunanaxane (**3**), major products of callus cultures of *Taxus* spp., were regio- and stereoselectively hydroxylated at the 7β position by a fungus, *Absidia coerulea* IFO 4011. Intriguingly, when **1** was co-administered with β-cyclodextrin and incubated with the fungus cell cultures, three other compounds 5α,9α,10β,13α-tetraacetoxytaxa-4(20),11-dien-14β-ol (**7**), 5α,9α,10β,13α-tetraacetoxytaxa-4(20),11-dien-1β-ol (**8**) and 5α,9α,10β,13α-tetraacetoxy-11(15→1) abeotaxa-4(20),11-dien-15-ol (**9**) were obtained.

Taxuyunnanine C [2α,5α,10β,14β-tetraacetoxytaxa-4(20),11-diene, **1**], and its analogues, 2α,5α,10β-triacetoxy-14β-(2-methylbutyryloxy)taxa-4(20),11-diene (**2**), yunnanxane [2α,5α,10β-triacetoxy-14β-(3-hydroxy-2-methylbutyryloxy)taxa-4(20),11-diene, **3**], are the three major C-14 oxygenated taxanes produced by the cell cultures of *Taxus* spp. in high yields (ca. 5–6% of the dry weight).[1–3] Their high content in the cultures and their taxane-skeleton endow them with valuable potential for the semi-synthesis of paclitaxel (Taxol®), one of the most effective anticancer agents, and other structurally related bioactive agents, such as anti-MDR (multi-drug resistance) cancer agents or anti-MDR cancer reversal agents (Scheme 1).[4–6] Unfortunately, taxanes **1**–**3**, have fewer functional groups bearing on the skeleton in comparison with paclitaxel and other bioactive taxoids in Scheme 1. The regio- and stereoselective introduction of oxygen functional groups at their C-1, C-7, C-9 and C-13 positions seems very difficult through traditional chemical methods. In this context, bioconversion by using microorganisms or plant cell suspension cultures is a potential alternative, and some interesting progress has been achieved.[7–9] Furthermore, the enzymatic systems of micro-organisms or plant cell cultures may be useful tools to mimic some steps of taxoid biosynthesis and can provide some useful help



Scheme 1.

Keywords: taxane; oxidation; microbial transformation; *Absidia coerulea* IFO 4011.
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for the study of taxoid biosynthesis, especially for extensive oxidation of the taxane skeleton. This short communication describes the specific oxidation and rearrangement of these taxanes by the fungus, *Absidia coerulea* IFO 4011.

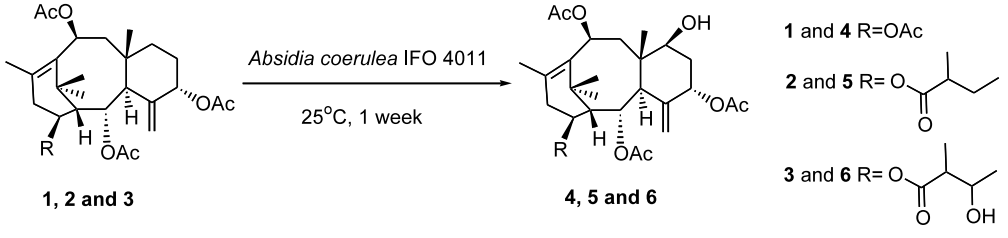
First, to 2-day-old cell cultures of a fungus, *A. coerulea* IFO 4011 (obtained from Institute for Fermentation, Osaka, Japan), **1** was added, and after another week of incubation, 7β hydroxyl product **4** was obtained in 5% yield (Scheme 2). The structure of **4** was determined on the basis of the ^{1}H NMR, ^{1}H–^{1}H COSY, ^{13}C NMR, DEPT, HMQC, HMBC, NOE, HREIMS and IR spectra.[10] The HREIMS spectrum of **4** showed an elemental composition of $C_{28}H_{40}O_9$ (found $[M]^+$ m/z 520.2666, calcd 520.2672), suggesting that a hydroxyl group may be introduced. The presence of an OH group in **4** was supported by the IR absorption at 3614 cm^{-1}. ^{1}H and ^{13}C NMR of **4** also showed the existence of a new oxymethine proton signal at δ 3.90 (1H, dd, $J=5.1$, 11.7 Hz) and the connected carbon signal at δ 68.92 (*d*). HMBC correlation of this carbon with H-19, H-9, H-6 and H-5 strongly suggested that the hydroxyl group was introduced at the C-7 position. The stereochemistry of 7-OH was determined to be β-configuration by the NOE difference spectrum; the integration values of H-3, H-6α, H-10 and H-18 were enhanced in 17, 5, 17 and 9%, respectively, when H-7 was irradiated. Therefore, the structure of **4** was determined as 2α,5α,10β,14β-tetraacetoxytaxa-4(20),11-dien-7β-ol.

To confirm the specific hydroxylation ability of the fungus and to gain insight into the influence of the different substrates on the biotransformation process, two other related compounds, **2** and **3**, were also used as exogenous substrates and incubated with the cell cultures. As expected, 7β hydroxyl products (**5**, **6**) were obtained under the same incubation conditions in 10 and 15% yields (Scheme 2), respectively. Their structures were also determined by spectral methods.[11,12] It is interesting that the longer the alkyl chain of the acyloxyl groups at C-14 became, the higher the yield of 7-hydroxylated products became.

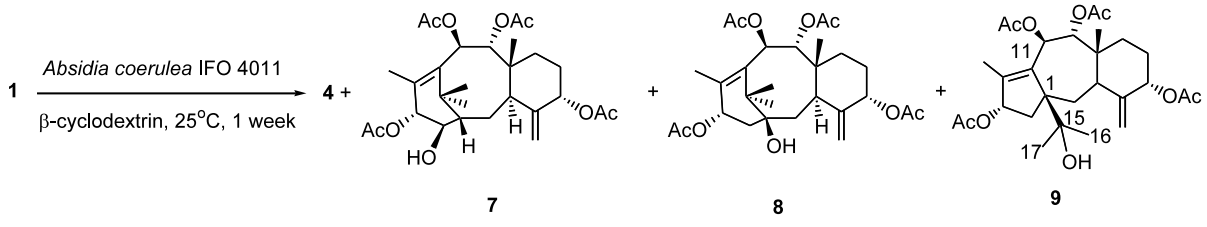
In an attempt to enhance the yield of **4**, β-cyclodextrin, which has been used commonly and successfully in the biotransformation to increase yield,[13] was co-administered to the cell cultures of the fungus with **1**. The yield of the desired product **4** was ca. 5% and was not increased as expected; however, very intriguingly, three other products were produced in addition to **4**. Their structures were determined as 5α,9α,10β,13α-tetraacetoxytaxa-4(20),11-dien-14β-ol (**7**),[14,15] 5α,9α,10β,13α-tetraacetoxytaxa-4(20),11-dien-1β-ol (**8**)[16] and 5α,9α,10β,13α-tetraacetoxy-11(15→1)abeotaxa-4(20),11-dien-15-ol (**9**)[17](Scheme 3). Compounds **7** and **8** were produced in about 5% yields individually, and **9** in a trace yield.

Product **7** is a known compound which was isolated from the bark of *T. mairei*.[15] There are substantial differences between the structures of the substrate **1** and product **7**, including disappearance of the 2α acetoxyl group, appearance of 9α and 13α acetoxyl groups, and 14β deactylation. Therefore, product **7** was produced from **1** through several steps (plausibly acetoxyl group intramolecular migration, or acetoxyl group reduction or acetoxylation, etc.).

The molecular formula of **8** was established to be $C_{28}H_{40}O_9$ by combined analyses of the HREIMS, ^{1}H and ^{13}C NMR spectral data. The ^{1}H NMR spectrum of **8** was similar to that of **7** except the signals of H-1, H-13 and H-14. In the ^{1}H NMR spectra of **8**, the methine proton of H-1 in **7** (δ 1.78, dd, $J=2.5$, 4.7 Hz) disappeared, and new signals of a methylene proton of H-14 [δ 2.53 (1H, dd, $J=10.0$, 15.0 Hz), δ 1.61 (1H, m)] appeared. The ^{13}C NMR indicated that one hydroxyl group existed at the C-1 position of **8** [δ 76.09 (*s*)], the location of which was supported by DEPT, HMQC and HMBC experiments. So, the structure of **8** was identified as 5α,9α,10β,13α-tetraacetoxytaxa-



Scheme 2.



Scheme 3.

4(20),11-dien-1β-ol, plausibly produced from **7** via hydroxyl group transfer from C-14 to C-1.

HREIMS showed that **9** had the same molecular formula $C_{28}H_{40}O_9$ as that of **8**. The ^1H, ^{13}C NMR, DEPT, HMQC and HMBC spectra of **9** showed unusual chemical shifts for C-1 (δ 62.95, *s*), and C-15 (δ 75.55, *s*) compared with those of **8**. The C-11 and C-12 carbon signals showed cross-peaks with H-14 resonances in HMBC, indicating that both C-11 and C-12 were three bonds apart from H-14. These NMR data indicated that **9** should be 11(15→1)abeotaxane.[18] The 11(15→1)abeotaxane **9** was probably produced under the conditions of the biotransformation by the migration of the 11–15 bond to the C-1 position, because 11(15→1)abeotaxanes are observed in *Taxus* plants as natural products.[19]

The results suggested that the substrate goes, in the presence of β-cyclodextrin, into the organelles of the cells where there are many different enzymes catalyzing the unusual transformations. We observed a considerable difference in the reaction modes of these biotransformations in the presence and in the absence of β-cyclodextrin. Obviously, the difference in the reaction mode means that each step of taxoid biotransformation takes place in different compartments in the cells. This biotransformation by a fungus gave hypothetical biosynthetic intermediates of paclitaxel and its analogues **4**, **7** and **8** from C-14 oxygenated taxoids, in fair yield. Since the availability of taxanes bearing a functionalized group both at C-13 and C-14 such as **7** is very limited in yew trees, this kind of taxane may be the intermediates between C-14 functionalized taxanes and C-13 functionalized taxanes, even the intermediates of paclitaxel biosynthesis. The fact that the functional group at C-2 in **1** was removed after incubation with the fungus cell cultures suggested that the same reaction may take place in the *Taxus* plant.

In conclusion, here we have reported a useful 7β hydroxylation and 9α acetoxylation of C-14 oxygenated taxanes by employing fungus *A. coerulea* IFO 4011 cell cultures. In the latter case the reaction is accompanied by 2α de-acetoxylation and oxygen functional group transfer to the C-1 and (or) C-13 positions. To our best knowledge, these are the first examples of hydroxylation or acetoxylation of the C-7 or C-13 methylene of taxane derivatives by biocatalytic reactions. These biotransformations would provide not only valuable intermediates for the synthesis of paclitaxel or other bioactive taxoids, but also some helpful hints for the taxoid biosynthetic pathway in the *Taxus* plant.

Acknowledgements

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10. 2α,5α,10β,14β-Tetraacetoxytaxa-4(20),11-dien-7β-ol (**4**): white powder (mp 79–82°C); $[\alpha]_D^{20}$ +47.8° (*c* 0.140, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3614, 2972, 2880, 1732, 1646, 1436, 1374, 1240, 1102, 1020 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.92 (1H, d, J=2.1 Hz, H-1), 5.40 (1H, dd, J=2.2, 6.6 Hz, H-2), 2.76 (1H, d, J=6.1 Hz, H-3), 5.33 (1H, t, J=2.4 Hz, H-5), 2.12 (1H, m, H-6α), 1.69 (1H, m, H-6β), 3.90 (1H, dd, J=5.1, 11.7 Hz, H-7), 2.23 (1H, dd, J=5.1, 15.1 Hz, H-9α), 2.12 (1H, m, H-9β), 5.97 (1H, dd, J=5.1, 12.0 Hz, H-10), 2.79 (1H, dd, J=9.3, 19.3 Hz, H-13β), 2.42 (1H, dd, J=4.4, 19.0 Hz, H-13α), 4.95 (1H, dd, J=4.9, 9.3 Hz, H-14), 1.69 (3H, s, H-16), 1.14 (3H, s, H-17), 2.03 (3H, brs, H-18), 0.75 (3H, s, H-19), 5.29 (1H, s, H-20a), 4.93 (1H, s, H-20b), 2.17, 2.07, 2.05, 2.01 [3H each, s, OAc (CH_3)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 58.79 (C-1), 70.41 (C-2), 40.55 (C-3), 140.86 (C-4), 77.61 (C-5), 36.87 (C-6), 68.92 (C-7), 44.37 (C-8), 36.94 (C-9), 69.58 (C-10), 135.76 (C-11), 134.92 (C-12), 39.46 (C-13), 70.08 (C-14), 37.34 (C-15), 25.37 (C-16), 31.79 (C-17), 21.70 (C-18), 16.53 (C-19), 118.06 (C-20), 21.46×2, 21.41, 21.14 [OAc (CH_3)], 170.64, 169.99, 169.96, 169.54 [OAc (CO)]; HREIMS m/z calcd for $C_{28}H_{40}O_9$ 520.2672, found 520.2666.
11. 2α,5α,10β-Triacetoxy-14β-(2-methylbutyryloxy)taxa-4(20),11-dien-7β-ol (**5**): white powder (mp 78–80°C); $[\alpha]_D^{20}$ +28.5° (*c* 0.220, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3615, 2976, 2940, 2884, 1732, 1646, 1462, 1374, 1250, 1020 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.91 (1H, d, J=2.0 Hz, H-1), 5.40 (1H, dd, J=2.2, 6.6 Hz, H-2), 2.78 (1H, d, J=6.6 Hz, H-3), 5.34 (1H, t, J=3.4 Hz, H-5), 2.11 (1H, m, H-6α), 1.66 (1H, m, H-6β), 3.90 (1H, dd, J=5.1, 12.2 Hz, H-7), 2.22 (1H, dd, J=5.3, 15.3 Hz, H-9α), 2.13 (1H, m, H-9β), 5.97 (1H, dd, J=5.4, 12.0 Hz, H-10), 2.83 (1H, dd, J=9.3, 19.3 Hz, H-13β), 2.37 (1H, dd, J=5.4, 19.3 Hz, H-13α), 4.95 (1H, dd, J=4.9, 9.0 Hz, H-14), 1.69 (3H, s, H-16), 1.15 (3H, s, H-17), 2.04 (3H, brs, H-18),

0.75 (3H, s, H-19), 5.29 (1H, s, H-20a), 4.89 (1H, s, H-20b), 2.33 (1H, tq, $J=7.1$, 7.0 Hz, H-2′), 1.62 (1H, ddq, $J=7.0$, 7.0, 7.3 Hz, H-3′a), 1.46 (1H, ddq, $J=7.0$, 7.0, 7.3 Hz, H-3′b), 0.89 (3H, t, $J=7.3$ Hz, H-4′), 1.12 (3H, d, $J=7.1$ Hz, H-5′), 2.18 [3H, s, 2-OAc (CH_3)], 2.03 [3H, s, 5-OAc (CH_3)], 2.07 [3H, s, 10-OAc (CH_3)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 59.10 (C-1), 70.07 (C-2), 40.56 (C-3), 140.85 (C-4), 77.51 (C-5), 36.88 (C-6), 68.89 (C-7), 44.33 (C-8), 36.94 (C-9), 69.60 (C-10), 135.71 (C-11), 135.10 (C-12), 39.65 (C-13), 69.97 (C-14), 37.28 (C-15), 25.35 (C-16), 31.73 (C-17), 21.45 (C-18), 16.57 (C-19), 117.92 (C-20), 175.66 (C-1′), 41.06 (C-2′), 26.74 (C-3′), 11.61 (C-4′), 16.57 (C-5′), 21.77 [2-OAc (CH_3)], 21.12 [5-OAc (CH_3)], 21.34 [10-OAc (CH_3)], 169.59 [2-OAc (CO)], 169.87 [5-OAc (CO)], 170.66 [10-OAc (CO)]; HRMS [+ESI, in MeOH/H_2O (1:1), 3% AcOH, NaCl] m/z calcd for $[C_{31}H_{46}O_9+Na]^+$ 585.30397, found 585.30729; HRMS [-ESI, in MeOH/H_2O (1:1)], calcd for $[C_{31}H_{46}O_9-H]^+$ 561.31419, found 561.31083.

12. 2α,5α,10β-Triacetoxy-14β-(3-hydroxy-2-methylbutyryloxy)taxa-4(20), 11-dien-7β-ol (**6**): white powder (mp 89–91°C); $[\alpha]_D^{20}$ +31.4° (c 0.60, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3624, 2992, 2936, 1732, 1646, 1606, 1458, 1374, 1318, 1102, 1020 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.92 (1H, d, $J=2.0$ Hz, H-1), 5.40 (1H, dd, $J=2.2$, 6.6 Hz, H-2), 2.76 (1H, d, $J=6.3$ Hz, H-3), 5.34 (1H, t, $J=3.2$ Hz, H-5), 2.09 (1H, m, H-6α), 1.69 (1H, m, H-6β), 3.90 (1H, dd, $J=5.1$, 11.7 Hz, H-7), 2.23 (1H, dd, $J=5.1$, 15.1 Hz, H-9α), 2.14 (1H, m, H-9β), 5.97 (1H, dd, $J=5.1$, 12.0 Hz, H-10), 2.83 (1H, dd, $J=9.0$, 19.0 Hz, H-13β), 2.38 (1H, dd, $J=4.7$, 19.0 Hz, H-13α), 4.99 (1H, dd, $J=4.7$, 9.0 Hz, H-14), 1.69 (3H, s, H-16), 1.14 (3H, s, H-17), 2.04 (3H, brs, H-18), 0.75 (3H, s, H-19), 5.30 (1H, s, H-20a), 4.90 (1H, s, H-20b), 2.40 (1H, dq, $J=7.0$, 7.3 Hz, H-2′), 3.87 (1H, dq, $J=7.0$, 6.3 Hz, H-3′), 1.21 (3H, d, $J=6.4$ Hz, H-4′), 1.16 (3H, d, $J=7.3$ Hz, H-5′), 2.18, 2.07, 2.03 [3H each, s, OAc (CH_3)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 58.97 (C-1), 69.99 (C-2), 40.58 (C-3), 140.82 (C-4), 77.57 (C-5), 36.94 (C-6), 69.51 (C-7), 44.35 (C-8), 36.89 (C-9), 69.53 (C-10), 135.83 (C-11), 134.81 (C-12), 39.48 (C-13), 70.56 (C-14), 37.30 (C-15), 25.34 (C-16), 31.72 (C-17), 21.38 (C-18), 16.56 (C-19), 118.02 (C-20), 174.78 (C-1′), 47.00 (C-2′), 68.92 (C-3′), 20.87 (C-4′), 14.02 (C-5′), 21.76, 21.44, 21.13 [OAc (CH_3)], 170.60, 169.84, 169.55 [OAc (CO)]; HREIMS m/z calcd for $C_{31}H_{46}O_{10}$ 578.3091, found 578.3087.
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14. 5α,9α,10β,13α-Tetraacetoxytaxa-4(20),11-dien-14β-ol (**7**): white powder (mp 205–208°C; lit.[15] mp 208–209°C); {$[\alpha]_D^{20}$ +80.7° (c 1.733, $CHCl_3$); lit.[15] $[\alpha]_D^{24}$ +76.4° (c 0.480, $CHCl_3$)}; IR ν_{max} ($CHCl_3$): 3620, 2968, 1732, 1648, 1440, 1374, 1134, 1020 cm^{-1}; HREIMS m/z calcd for $C_{28}H_{40}O_9$ 520.2672, found 520.2664; the NMR data of **7** are in good agreement with those reported in lit. 15.
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16. 5α,9α,10β,13α-Tetraacetoxytaxa-4(20),11-dien-1β-ol (**8**): white powder (mp 80–82°C); $[\alpha]_D^{20}$ +88.9° (c 1.007, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3620, 3024, 2968, 1734, 1648, 1440, 1374, 1136, 1018 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.89 (1H, dd, $J=8.5$, 14.2 Hz, H-2a), 1.75 (1H, dd, $J=5.9$, 14.2 Hz, H-2b), 2.94 (1H, d, $J=5.9$ Hz, H-3), 5.37 (1H, t, $J=2.7$ Hz, H-5), 1.85 (1H, m, H-6a), 1.73 (1H, m, H-6b), 1.80 (2H, m, H-7), 5.87 (1H, d, $J=10.8$ Hz, H-9), 6.07 (1H, d, $J=10.8$ Hz, H-10), 6.03 (1H, t, $J=8.0$ Hz, H-13), 2.53 (1H, dd, $J=10.0$, 15.0 Hz, H-14a), 1.61 (1H, m, H-14b), 1.62 (3H, s, H-16), 1.19 (3H, s, H-17), 2.10 (3H, d, $J=1.0$ Hz, H-18), 0.76 (3H, s, H-19), 5.24 (1H, d, $J=1.2$ Hz, H-20a), 4.91 (1H, d, $J=1.2$ Hz, H-20b), 2.08 [3H, s, 5-OAc (CH_3)], 2.02 [3H, s, 9-OAc (CH_3)], 2.05 [3H, s, 10-OAc (CH_3)], 2.16 [3H, s, 13-OAc (CH_3)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 76.09 (C-1), 38.10 (C-2), 41.49 (C-3), 147.80 (C-4), 76.22 (C-5), 27.23 (C-6, C-7), 43.18 (C-8), 76.76 (C-9), 72.03 (C-10), 134.49 (C-11), 138.93 (C-12), 70.96 (C-13), 41.43 (C-14), 43.58 (C-15), 27.12 (C-16), 21.95 (C-17), 14.84 (C-18), 17.67 (C-19), 114.27 (C-20), 20.99 [5-OAc (CH_3)], 21.36 [9-OAc (CH_3)], 21.78 [10-OAc (CH_3)], 20.72 [13-OAc (CH_3)], 169.82 [5-OAc (CO)], 170.34 [9-OAc (CO)], 169.82 [10-OAc (CO)], 170.20 [13-OAc (CO)]; HREIMS m/z calcd for $C_{28}H_{40}O_9$ 520.2672, found 520.2671.
17. 5α,9α,10β,13α-Tetraacetoxy-11(15→1)abeotaxa-4(20),11-dien-15-ol (**9**): white powder (mp 200–202°C); $[\alpha]_D^{20}$ −20° (c 0.08, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3620, 3020, 2964, 1732, 1644, 1370, 1120, 1018 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.17 (1H, dd, $J=8.3$, 14.4 Hz, H-2a), 1.38 (1H, d, $J=14.2$ Hz, H-2b), 2.70 (1H, d, $J=8.5$ Hz, H-3), 5.30 (1H, t, $J=2.7$ Hz, H-5), 1.88 (1H, m, H-6a), 1.78 (1H, m, H-6b), 1.72 (2H, m, H-7), 5.78 (1H, d, $J=10.2$ Hz, H-9), 6.15 (1H, d, $J=10.2$ Hz, H-10), 5.52 (1H, t, $J=7.6$ Hz, H-13), 2.48 (1H, dd, $J=7.3$, 13.9 Hz, H-14a), 1.21 (1H, m, H-14b), 1.31 (3H, s, H-16), 1.14 (3H, s, H-17), 1.83 (3H, d, $J=1.0$ Hz, H-18), 0.77 (3H, s, H-19), 5.17 (1H, s, H-20a), 4.76 (1H, s, H-20b), 2.03, 2.01, 2.00, 1.99 [3H each, s, OAc (CH_3)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 62.95 (C-1), 29.26 (C-2), 40.29 (C-3), 147.04 (C-4), 75.07 (C-5), 27.37 (C-6), 27.79 (C-7), 41.91 (C-8), 77.86 (C-9), 69.57 (C-10), 137.74 (C-11), 145.15 (C-12), 79.43 (C-13), 44.33 (C-14), 75.55 (C-15), 24.73 (C-16), 27.09 (C-17), 11.52 (C-18), 16.85 (C-19), 112.28 (C-20), 21.16, 21.00, 20.88, 20.77 [OAc (CH_3)], 170.54, 170.10, 169.66, 168.66 [OAc(CO)]; HREIMS m/z calcd for $C_{28}H_{40}O_9$ 520.2672, found 520.2684.
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19. (a) Kiyota, H.; Shi, Q.; Oritani, T.; Li, L. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 35–40 and references cited therein; (b) Baloglu, E.; Kingston, G. I. *J. Nat. Prod.* **1999**, *62*, 1448–1472.