TAXOL AND ITS RELATED TAXOIDS FROM THE NEEDLES OF TAXUS SUMATRANA

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Through bioassay-guided separation of the chemical constituents of the needles of Taxus sumatrana, taxol (1), cephalomannine (2), and a new taxoid 19-hydroxy-13-oxobaccatin III (8) have been isolated together with 7-epi-10-deacetyltaxol (3), 7-epi-10-deacetylccephalomannine (4), baccatin III (5), 19-hydroxybaccatin III (6), and 10-deacetyl-13-oxobaccatin III (7). The chemical structure of 8 has been elucidated on the bases of its chemical and physicochemical properties.

**KEY WORDS** Indonesian medicinal plant; Taxus sumatrana; taxol; cancer chemotherapeutic agent

Over the last two decades, taxol (1)1 has attracted much attention from scientists and is currently considered to be one of the most exciting leads in cancer chemotherapy.2 The clinical development of taxol was undertaken intensively, and the drug was brought to market for the treatment of ovarian cancer.3 However, the large-scale clinical usage of taxol has been hampered by its limited supply. Although the total and semi-syntheses of taxol have been reported recently,4,5 the supply of the drug is still dependent on natural resources, currently the bark of the Pacific yew, Taxus brevifolia Nutt. Typical yields of taxol from large-scale collections are below 0.01 %.6 In other words, to obtain one kilogram of taxol, 10,000 kilograms of bark are required, which are equal to the sacrifice of about 3000 trees.7

As a part of our search for biologically active compounds from Indonesian medicinal plants,8 we have investigated the chemical constituents of the needles of the Sumatran yew, Taxus sumatrana (Miquel), which was collected in Sumatra island. In this paper, we describe the isolation and chemical characterization of taxol (1) and its related taxoids including a new compound 19-hydroxy-13-oxobaccatin III (8).

The CH₂Cl₂ - MeOH (1:1) extract of the needles was partitioned into an ethyl acetate - water (1:1) mixture. Through the guidance of bioassay of cytotoxicity against KB cells, the ethyl acetate-soluble portion was subjected to silica gel and Sephadex LH-20 column chromatography and subsequently HPLC to provide taxol (1, 0.006 % from the air-dried needles), cephalomannine (2, 0.005 %),9 7-epi-10-deacetyltaxol (3, 0.008 %),10 and 7-epi-10-deacetylccephalomannine (4, 0.003 %),10 together with baccatin III (5, 0.02 %),10 19-hydroxybaccatin III (6, 0.05 %),10 10-deacetyl-13-oxobaccatin III (7, 0.02 %),11 and the new taxoid 19-hydroxy-13-oxobaccatin III (8, 0.02 %).

Taxol (1) was obtained as needles of mp 203-204 °C (from MeOH) (lit. mp 213-216 °C1), mp 198-203 °C9). The high-resolution FAB-MS spectrum of 1 substantiated the molecular formula as C₄₇H₅₁NO₁₄, and the physicochemical properties of 1, including the optical rotation,12 were identical with those reported previously.1
The other taxol-related compounds, such as cephalomannine (2), 7-epi-10-deacetyltaxol (3), 7-epi-10-deacetylcephalomannine (4), baccatin III (5), 19-hydroxybaccatin III (6), and 10-deacetyl-13-oxobaccatin III (7) were also provided in yields comparable to those of other Taxus species. The physicochemical properties of each were identical with those of reported compounds. Among them, 10-deacetyl-13-oxobaccatin III (7) was first isolated from natural sources.

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\text{taxol (1)}
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<th>R&lt;sup&gt;2&lt;/sup&gt;</th>
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<td>H&lt;sub&gt;3&lt;/sub&gt;C&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Ac</td>
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\[
\text{cephalomannine (2)}
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\text{7-epi-10-deacetyltaxol (3)}
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\text{7-epi-10-deacetylcephalomannine (4)}
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A new taxoid, 19-hydroxy-13-oxobaccatin III (8), was obtained as needles of mp 144-146 °C (from MeOH). The FAB-MS of 8 showed a quasi-molecular (M+Na)<sup>+</sup> ion peak at m/z 623, which was defined as C<sub>31</sub>H<sub>36</sub>O<sub>12</sub>Na by high-resolution FAB-MS analysis. The IR (KBr) spectrum of 8 showed absorption bands assignable to hydroxyl (3514 cm<sup>-1</sup>), acetoxy (1722 cm<sup>-1</sup>), and enone (1676 cm<sup>-1</sup>) groups, and UV absorption maxima were observed at 229 nm (ε = 15500) and 274 nm (ε = 4700).

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\text{baccatin III (5)}
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19-hydroxybaccatin III (6)

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\text{10-deacetyl-13-oxobaccatin III (7)}
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\[
\text{19-hydroxy-13-oxobaccatin III (8)}
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The <sup>1</sup>H-NMR spectrum of 8 was very similar to that of 19-hydroxybaccatin III (6), except that the 13-H signal was missing and the signals due to methylene protons at C-14 were observed at δ 2.65 and δ 2.93 (both 1H, d, J=20 Hz) in 8, while 13-H methine and 14-H<sub>2</sub> methylene proton signals in 6 were observed at δ 4.85 (1H, m) and δ 2.60 (2H, m), respectively. This evidence has led us to presume that 8 is a 13-oxo derivative of 6. This presumption was also supported by other physicochemical properties. Furthermore, treatment of 6 with activated MnO<sub>2</sub> in CH<sub>2</sub>Cl<sub>2</sub> provided the enone 8. Consequently, the chemical structure of 19-hydroxy-13-oxobaccatin III has been determined to be 8.
We are currently continuing further chemical and biological investigations of the chemical constituents of the needles of Taxus sumatrana. The details will be reported in due course.

ACKNOWLEDGEMENT

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REFERENCES AND NOTES

12) I: [α]D - 43° (c = 0.41, MeOH, 24 °C) (lit. [α]D - 49° (MeOH, 20 °C)1), [α]D - 42° (c = 0.37, MeOH, 23 °C)2). 

FAB-MS m/z : 854 (M+H)-. IR ν max (KBr) cm-1 : 3433, 2930, 1722, 1653, 1602, 1244. UV λ max (MeOH) nm (ε) : 227 (25500), 265 (1800). 1H-NMR (500 MHz, CDC13) δ : 1.14 (3H, s, 16-Me), 1.24 (3H, s, 17-Me), 1.68 (3H, s, 19-Me), 1.76 (1H, s, 1-0H), 1.79 (3H, s, 18-Me), 1.88 (1H, m, 6β-H), 2.24 (3H, s, 10-OAc), 2.29 (1H, m, 14β-H), 2.35 (1H, m, 14α-H), 2.38 (3H, s, 4-OAc), 2.45 (1H, d, J=4.5 Hz, 7-OH), 2.55 (1H, m, 6α-H), 3.35 (1H, d, J=5 Hz, 2'-OH), 3.80 (1H, d, J=7 Hz, 3'-H), 4.20 (1H, d, J=8 Hz, 20β-H), 4.30 (1H, d, J=8 Hz, 20α-H), 4.40 (1H, m, 7-H), 4.79 (1H, br s, 2'-H), 4.94 (1H, d, J=8 Hz, 5'-H), 5.67 (1H, d, J=7 Hz, 2-H), 5.79 (1H, dd, J=8.5, 2.6 Hz, 3'-H), 6.23 (1H, t, J=8.5 Hz, 13-H), 6.27 (1H, s, 10-H), 6.97 (1H, d, J=8.5 Hz, 3'-NH).
14) 7: needles of mp 168-169 °C (from MeOH). 

FAB-MS m/z : 543 (M+H)+. IR ν max (KBr) cm-1 : 3449, 2926, 1726, 1670, 1201, 1271, 1424. UV ν max (EtOH) nm (ε) : 229 (14100), 274 (4200). 1H-NMR (270 MHz, CDC13) δ : 2.65 (1H, d, J=20 Hz, 14-Ha), 2.95 (1H, d, J=20Hz, 14-Hb), 4.00 (1H, d, J=7 Hz, 3-H), 4.14 (1H, d, J=8 Hz, 20β-H), 4.28 (1H, m, 7-H), 4.34 (1H, d, J=8 Hz, 20α-H), 4.94 (1H, br d, J=8 Hz, 5'-H), 5.41 (1H, s, 10-H), 5.68 (1H, d, J=7 Hz, 2-H). 13C-NMR (67.8 MHz, CDC13) δc : 9.2 (C-19), 13.5 (C-18), 17.6 (C-16), 21.6 (4-OCCOH3), 32.8 (C-17), 37.0 (C-6), 42.5 (C-14), 43.3 (C-15), 45.9 (C-3), 58.3 (C-8), 71.8 (C-7), 72.8 (C-10), 75.9 (C-2), 76.2 (C-20), 78.5 (C-1), 80.3 (C-4), 83.8 (C-5), 128.7 (2-OCCOHs (quaternary), and 2-OCCOHs (meta)), 129.9 (2-OCCOHs (ortho)), 134.0 (2-OCCOHs (para)), 139.2 (C-12), 156.3 (C-11), 166.7 (2-OCCOHs), 170.1 (4-OCCOHs), 198.0 (C-13), 209.1 (C-9).
15) 8: 1H-NMR (270 MHz, CDC13) δ : 2.65 (1H, d, J=20 Hz, 14-Ha), 2.93 (1H, d, J=20 Hz, 14-Hb), 3.92 (1H, d, J=7 Hz, 3-H), 4.24 (1H, d, J=8 Hz, 20β-H), 4.41 (1H, d, J=8 Hz, 20α-H), 4.45 (1H, m, 7-H), 4.71 (2H, ABq, J=12.5 Hz, 19-Hz), 5.00 (1H, br d, J=8 Hz, 5-H), 6.44 (1H, d, J=7 Hz, 2-H), 6.53 (1H, s, 10-H). 13C-NMR (67.8 MHz, CDC13) δc : 13.9 (C-18), 18.6 (C-16), 20.8, 21.7 (4-OCCOH3, 10-OCCOH3), 33.1 (C-17), 36.3 (C-6), 41.9 (C-14), 43.6 (C-15), 45.8 (C-3), 60.0 (C-19), 61.9 (C-8), 72.3 (C-7), 73.5 (C-2), 76.0 (C-20), 76.5 (C-10), 79.0 (C-1), 80.3 (C-4), 84.2 (C-5), 128.7 (2-OCCOHs (meta)), 128.8 (2-OCCOHs (quaternary)), 130.1 (2-OCCOHs (ortho)), 133.9 (2-OCCOHs (para)), 141.5 (C-12), 152.0 (C-11), 167.1 (2-OCCOHs), 170.2 (4-OCCOHs and 10-OCCOHs), 198.0 (C-13), 203.4 (C-9).

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