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-deacetylcephalomannine (**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 CH_2Cl_2 - 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-deacetylcephalomannine (**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 °C[1)]; mp 198-203 °C[9)]). The high-resolution FAB-MS spectrum of **1** substantiated the molecular formula as $C_{47}H_{51}NO_{14}$, and the physicochemical properties of **1**, including the optical rotation,[12)] were identical with those reported previously.[1)]

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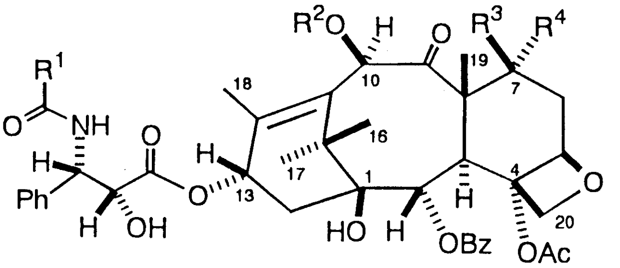
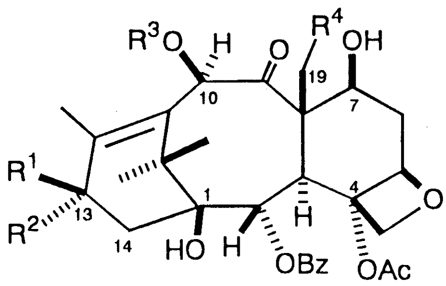
The other taxol-related compounds, such as cephalomannine (**2**),[9] 7-*epi*-10-deacetyltaxol (**3**),[10] 7-*epi*-10-deacetylcephalomannine (**4**),[10] baccatin III (**5**),[10] 19-hydroxybaccatin III (**6**),[10] and 10-deacetyl-13-oxobaccatin III (**7**),[11] were also provided in yields comparable to those of other *Taxus* species.[9, 10, 13] The physicochemical properties of each were identical with those of reported compounds. Among them, 10-deacetyl-13-oxobaccatin III (**7**)[14] was first isolated from natural sources.



	R^1	R^2	R^3	R^4
taxol (**1**)	Ph	Ac	OH	H
cephalomannine (**2**)	H_3C–CH=C(CH_3)–	Ac	OH	H
7-*epi*-10-deacetyltaxol (**3**)	Ph	H	H	OH
7-*epi*-10-deacetylcephalomannine (**4**)	H_3C–CH=C(CH_3)–	H	H	OH

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 $(M+Na)^+$ ion peak at *m/z* 623, which was defined as $C_{31}H_{36}O_{12}Na$ by high-resolution FAB-MS analysis. The IR (KBr) spectrum of **8** showed absorption bands assignable to hydroxyl (3514 cm^{-1}), acetoxyl (1722 cm^{-1}), and enone (1676 cm^{-1}) groups, and UV absorption maxima were observed at 229 nm (ε = 15500) and 274 nm (ε = 4700).

	R^1	R^2	R^3	R^4
baccatin III (**5**)	H	OH	Ac	H
19-hydroxybaccatin III (**6**)	H	OH	Ac	OH
10-deacetyl-13-oxobaccatin III (**7**)	O		H	H
19-hydroxy-13-oxobaccatin III (**8**)	O		Ac	OH



The ^{1}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 δ2.65 and δ2.93 (both 1H, d, *J*=20 Hz) in **8**, while 13-H methine and 14-H_2 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.[15] Furthermore, treatment of **6** with activated MnO_2 in CH_2Cl_2 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.

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12) **1**: $[\alpha]_D$ − 43 ° (*c* = 0.41, MeOH, 24 °C) (lit. $[\alpha]_D$ − 49 ° (MeOH, 20 °C)[1], $[\alpha]_D$ − 42 ° (*c* = 0.37, MeOH, 23 °C)[9]). 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). ^{1}H-NMR (500 MHz, $CDCl_3$) δ : 1.14 (3H, s, 16-Me), 1.24 (3H, s, 17-Me), 1.68 (3H, s, 19-Me), 1.76 (1H, s, 1-OH), 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), 3.53 (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).
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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, 1602, 1271, 1242. UV λ_{max} (EtOH) nm (ε) : 229 (14100), 274 (4200). ^{1}H-NMR (270 MHz, $CDCl_3$) δ : 2.65 (1H, d, *J*=20 Hz, 14-Ha), 2.95 (1H, d, *J*=20 Hz, 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). ^{13}C-NMR (67.8 MHz, $CDCl_3$) δc : 9.2 (C-19), 13.5 (C-18), 17.6 (C-16), 21.6 (4-OCO$\underline{C}H_3$), 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-OCO$\underline{C}_6H_5$ (quaternary), and 2-OCO$\underline{C}_6H_5$ (meta)), 129.9 (2-OCO$\underline{C}_6H_5$ (ortho)), 134.0 (2-OCO$\underline{C}_6H_5$ (para)), 139.2 (C-12), 156.3 (C-11), 166.7 (2-O$\underline{C}$OC_6H_5), 170.1 (4-O$\underline{C}$OCH$_3$), 198.0 (C-13), 209.1 (C-9).
15) **8**: ^{1}H-NMR (270 MHz, $CDCl_3$) 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-H_2), 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). ^{13}C-NMR (67.8 MHz, $CDCl_3$) δc : 13.9 (C-18), 18.6 (C-16), 20.8, 21.7 (4-OCO$\underline{C}H_3$, 10-OCO$\underline{C}H_3$), 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-OCO$\underline{C}_6H_5$(meta)), 128.8 (2-OCO$\underline{C}_6H_5$(quaternary)), 130.1 (2-OCO$\underline{C}_6H_5$(ortho)), 133.9 (2-OCO$\underline{C}_6H_5$(para)), 141.5 (C-12), 152.0 (C-11), 167.1 (2-O$\underline{C}$OC_6H_5), 170.2 (4-O$\underline{C}$OCH$_3$ and 10-O$\underline{C}$OCH$_3$), 198.0 (C-13), 203.4 (C-9).

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