# A Biodegradation Product of Betulin

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**A biodegradation product of betulin was isolated from decayed outer bark of *Betula platyphylla* SUKATCHEV var. *japonica* (MIQ.) HARA. The structure was elucidated as (17*R*,20*R*)-7$\beta$,20,23,29-tetrahydroxy-28-norlupane-3,16-dione by chemical and spectral means.**

**Keywords** *Betula platyphylla* var. *japonica*; betulin; biodegradation; 28-norlupane

Betulin (**2**) is a naturally occurring triterpene, abundantly available mainly from birch barks.[1] The outer bark of *Betula platyphylla* SUKATCHEV var. *japonica* (MIQ.) HARA (Japanese name, shirakanba) contains 10% betulin as a component of suberin wax.[2] Suberin is very resistant to biodegradation by fungi, but, over many years of decay, extracellular enzymes of fungi gradually degrade it.[3] In connection with this biodegradation process, our attention was focused on the fate of betulin in the bark of *B. platyphylla* var. *japonica*. In tracing this in decayed barks by thin layer chromatography, a new compound (**1**) was recognized to appear as the decay proceeded.[4] This paper describes the structure elucidation of this biodegradation product (**1**) of betulin.

Compound **1**, colorless needles, mp 268—273 °C, $[\alpha]_D$ *ca.* 0° ($c=1.0$, pyridine), was formulated as $C_{29}H_{46}O_6$ from the $[M+H]^+$ ion peak at $m/z$ 491 in the field desorption mass spectrum (FD-MS) and a signal count in the $^{13}$C-NMR spectrum ($5CH_3+10CH_2+7CH+7C$), indicating it to be a nor-triterpene. The $^1$H- and $^{13}$C-NMR spectra of **1** showed the presence of two ketone groups ($\delta_C$ 216.7, 216.8), two primary hydroxyl groups [$\delta_C$ 68.2, $\delta_H$ 3.70 (1H, d, $J=10.4$ Hz), 3.95 (1H, d, $J=10.4$ Hz); $\delta_C$ 68.9, $\delta_H$ 3.88 (1H, d, $J=11.0$ Hz), 3.92 (1H, d, $J=11.0$ Hz)], a secondary hydroxyl group [$\delta_C$ 73.9, $\delta_H$ 4.07 (1H, dd, $J=10.2$, 4.7 Hz)] and a tertiary hydroxyl group ($\delta_C$ 74.7).

Compound **1** gave a triacetate (**1a**) with acetic anhydride in pyridine and a mono acetonide (**1b**) with acetone containing a small amount of sulfuric acid. The latter derivative suggested the presence of a glycol system in the molecule. On Huang–Minlon reduction, **1** gave a compound (**1c**) in which one of the hydroxymethyl groups of removed, together with the two ketone groups. This means that the hydroxymethyl group is located at an $\alpha$-position to one of the ketone groups and undergoes retro-aldol condensation reaction under basic conditions.[5]

Taking into account the above evidence, the structure of **1** was elucidated by NMR spectroscopy. The $^1$H-signals were correlated with the $^{13}$C-signals by $^1$H–$^{13}$C shift correlation spectroscopy (COSY, Table I). Their sequence was determined to be as shown in Fig. 2 by $^1$H–$^1$H COSY and $^1$H–$^{13}$C long range COSY. Thus, the structure of **1** was deduced to be 28-norlupane having two ketones at C-3 and C-16 and four hydroxyl groups at C-7, C-20, C-23 (or C-24), and C-29.

The stereochemistry of **1** was confirmed by nuclear Overhauser effect correlation spectroscopy (NOESY). As shown in Fig. 3, the methyl group at C-4 showed a correlation with the methyl group at C-10, indicating a 1,3-diaxial arrangement. Therefore, the hydroxylmethyl group at C-4 was determined to be equatorial, that is, C-23.

The configuration of the secondary hydroxyl group at C-7 was assigned as $\beta$ considering the coupling pattern (dd, $J=10.2$, 4.7 Hz) of the proton signal at $\delta$ 4.07 (H-7) and its NOE correlation with the protons at C-5 and C-9 and methyl group at C-14 (Fig. 3).

The mode of fusion of rings *D* and *E* was determined as *cis* because the proton at C-17 showed a long-range coupling ($J=1.2$ Hz) with the $\alpha$-proton at C-15, ascribable to their W-arrangement (see Fig. 3).[6]

Finally, the configuration of C-20 was analyzed by circular dichroism (CD) spectroscopy. To avoid the complexity resulting from the ketone groups, we took the spectrum of compound **1c**, in which the glycol system showed negative maxima at 290 nm ($\Delta\varepsilon$ −0.81) and 630 nm ($\Delta\varepsilon$ −0.65) and a positive maximum at 320 nm ($\Delta\varepsilon$ +0.27) in the presence of nickel(II) acetylacetonate [$Ni(acac)_2$].[7] This means that the glycol system possesses

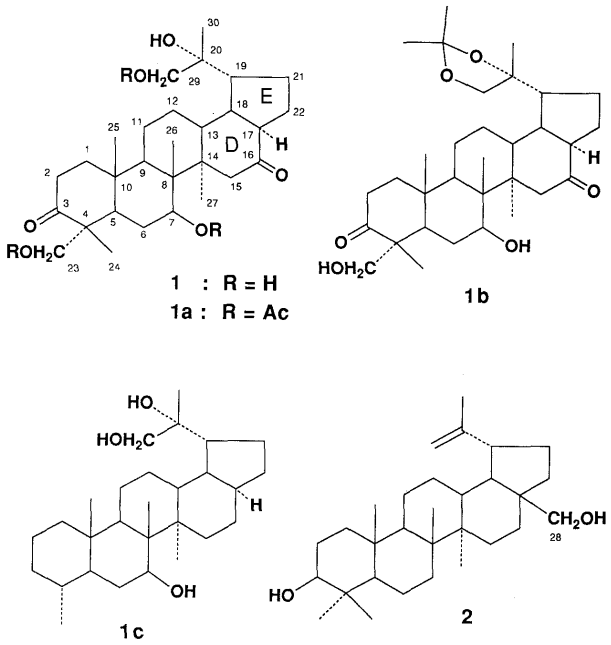


Fig. 1

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TABLE I. NMR Data for **1** in $C_5D_5N$

| Carbon | $^{13}C$ | $^{1}H$ |
|---|---|---|
| 1 | 38.7 | 1.53—1.60 (1H)[a] |
| | | 1.88—1.95 (1H)[a] |
| 2 | 36.6 | 2.57—2.64 (1H)[a] |
| | | 2.81 (1H, ddd, $J$=16.5, 9.5, 4.3 Hz) |
| 3 | 216.7 | |
| 4 | 52.3 | |
| 5 | 44.5 | 2.57—2.64 (1H)[a] |
| 6 | 31.2 | 1.85—1.95 (2H)[a] |
| 7 | 73.9 | 4.07 (1H, dd, $J$=10.2, 4.7 Hz) |
| 8 | 46.6 | |
| 9 | 50.4 | 1.39 (1H, td, $J$=12.8, 3.4 Hz) |
| 10 | 36.4 | |
| 11 | 22.2 | 1.30—1.40 (1H)[a] |
| | | 1.53—1.59 (1H)[a] |
| 12 | 27.9 | 1.09—1.16 (1H)[a] |
| | | 2.13—2.20 (1H)[a] |
| 13 | 40.5 | 1.86—1.95 (1H)[a] |
| 14 | 47.8 | |
| 15 | 48.1 | 2.97 (1H, d, $J$=14.0 Hz) |
| | | 3.60 (1H, dd, $J$=14.0, 1.2 Hz) |
| 16 | 216.8 | |
| 17 | 55.2 | 3.04 (1H, ddt, $J$=17.7, 8.2, 1.2 Hz) |
| 18 | 47.4 | 2.45 (1H, ddd, $J$=10.9, 7.6, 1.2 Hz) |
| 19 | 50.7 | 2.57—2.64 (1H)[a] |
| 20 | 74.7 | |
| 21 | 27.3 | 2.10—2.15 (1H)[a] |
| | | 2.20—2.25 (1H)[a] |
| 22 | 32.1 | 1.99—2.04 (2H)[a] |
| 23 | 68.2 | 3.70 (1H, d, $J$=10.4 Hz) |
| | | 3.95 (1H, d, $J$=10.4 Hz) |
| 24 | 17.4 | 1.09 (3H, s) |
| 25 | 16.7 | 0.95 (3H, s) |
| 26 | 10.1 | 1.33 (3H, s) |
| 27 | 14.7 | 1.04 (3H, s) |
| 28 | — | |
| 29 | 68.9 | 3.88 (1H, d, $J$=11.0 Hz) |
| | | 3.92 (1H, d, $J$=11.0 Hz) |
| 30 | 24.7 | 1.56 (1H, s) |

*a*) Coupling patterns were not confirmed because of the overlapping of signals.

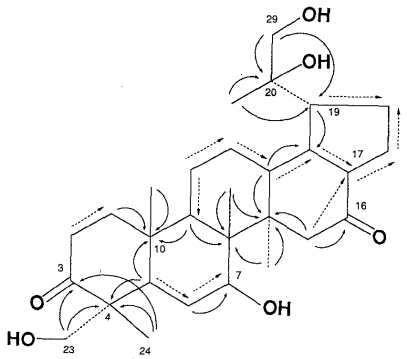


Fig. 2. $^{1}H$–$^{1}H$ COSY (·····) and Long-Range $^{1}H$–$^{13}C$ COSY ($^{1}H \curvearrowright {}^{13}C$) Connections for Compound **1**

(−)-chirality, that is, (20*R*) configuration, in the most stable conformation to form a complex with $Ni(acac)_2$ (see Fig. 4). Thus, the structure of **1** was established as (17*R*,20*R*)-7$\beta$,20,23,29-tetrahydroxy-28-norlupane-3,16-dione.

It is noteworthy that the ketonization at the $\beta$-position is an important step to eliminate an oxidized methyl group in this biodegradation process. The hydroxymethyl group

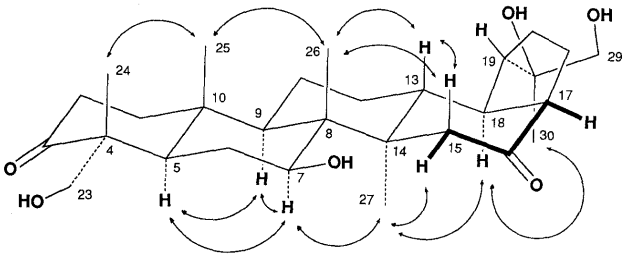


Fig. 3. NOESY Connections for Compound **1**

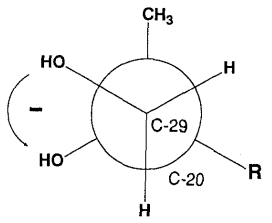


Fig. 4

at C-17 in betulin (**2**) must be eliminated by retro-aldol condensation reaction or by decarboxylation reaction after oxidation to the $\beta$-keto acid. The hydroxymethyl group at C-4 and also the methyl group at C-8 are considered to be eliminated in a similar manner to the above in the next step. Further investigation should clarify the next stage of biodegradation, which may be more complicated bacause of the participation of detritivores.[8]

**Experimental**

Melting points were determined with a Yanagimoto micromelting apparatus and are uncorrected. Optical rotations were taken with a JASCO DIP-360 automatic polarimeter. The $^{1}H$- and $^{13}C$-NMR spectra were measured with a JEOL GSX-500 spectormeter. Ultraviolet (UV) spectra were recorded on a Hitachi 323 spectrometer and infrared (IR) spectra on a Shimadzu IR-460 spectrometer. CD spectra were recorded on a JASCO J-600 spectrometer. MS were measured with Hitachi M-80A and JEOL SX-102 spectrometers.

**Isolation Procedure** Decayed outer bark (400 g) of *Betula platyphylla* SUKATCHEV var. *japonica* (MIQ.) HARA, collected at Iizunakogen, Nagano Prefecture, was extracted with 3 l of $CHCl_3$ overnight at room temperature. The residue was further extracted twice with 3 l of MeOH under reflux for 5 h. The MeOH extracts were combined and concentrated to a syrup under reduced pressure. The syrup was chromatographed on silica gel using EtOAc–MeOH and $CHCl_3$–EtOAc as eluents to yield compound **1** (270 mg).

**Compound 1** Colorless needles, mp 268—273 °C, $[\alpha]_D$ *ca.* 0° ($c$=1.0, pyridine). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3425, 2940, 1692, 1641, 1450, 1300, 1286, 1041, 1016, 679. FD-MS *m/z*: 491 $[M+H]^+$, 460, 429.

**Triacetate (1a)** A mixture of **1** (20 mg), pyridine (1 ml) and $Ac_2O$ (1 ml) was allowed to stand at room temperature overnight. The reaction mixture was poured into ice-water and extracted with ether. The extract was washed with 5% HCl solution, 5% $Na_2CO_3$ solution and water, then dried over anhydrous $Na_2SO_4$ and concentrated to yield **1a** (12 mg). Colorless amorphous solid. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3460, 2950, 1736, 1696, 1372, 1240, 1038. $^{1}H$-NMR ($CDCl_3$) $\delta$: 0.96 (3H, s), 1.01 (3H, s), 1.16 (3H, s), 1.23 (3H, s), 1.60 (3H, s), 2.06 (3H, s), 2.07 (3H, s), 2.12 (3H, s), 3.90 (1H, d, $J$=11.0 Hz), 3.94 (1H, d, $J$=11.0 Hz), 3.99 (1H, d, $J$=11.0 Hz), 4.06 (1H, d, $J$=11.0 Hz), 4.95 (1H, dd, $J$=9.9, 5.5 Hz). EI-MS *m/z*: 616 ($M^+$), 598, 556, 538, 496, 478, 436, 418. HR-MS *m/z*: 616.3618. Calcd for $C_{35}H_{52}O_9$: 616.3612.

**Monoacetonide (1b)** Compound **1** (10 mg) was dissolved in acetone (5 ml) containing one drop of concentrated $H_2SO_4$. The solution was allowed to stand at room temperature for 3 h. The resulting solution was poured into ice-water and extracted with EtOAc. The extract was washed with water, dried over anhydrous $Na_2SO_4$, and concentrated. The residue was chromatographed on silica gel using $CHCl_3$–MeOH to

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yield **1b** (8 mg). Colorless needles, mp 166—172 °C, $[\alpha]_D$ −1.4° ($c$=0.7, $CHCl_3$). $^1$H-NMR ($C_5D_5N$) $\delta$: 0.95 (3H, s), 1.05 (3H, s), 1.09 (3H, s), 1.29 (3H, s), 1.33 (3H, s), 1.45 (3H, s), 1.46 (3H, s), 2.91 (1H, d, $J$=14.1 Hz), 3.63 (1H, d, $J$=14.1 Hz), 3.70 (1H, d, $J$=10.3 Hz), 3.74 (1H, d, $J$=8.4 Hz), 3.84 (1H, d, $J$=8.4 Hz), 3.96 (1H, d, $J$=10.3 Hz), 4.09 (1H, dd, $J$=9.9, 4.4 Hz). EI-MS $m/z$: 515 $[M-CH_3]^+$, 500, 485, 456, 425, 407, 386, 368, 317, 301. HR-MS $m/z$: 515.3365 $[M-CH_3]^+$. Calcd for $C_{31}H_{47}O_6$: 515.3372.

**Huang-Minlon Reduction of 1** A mixture of **1** (22 mg), 100% hydrazine hydrate (0.2 ml), KOH (120 mg), diethylene glycol (2 ml) and EtOH (2 ml) was refluxed for 1.5 h. The water, EtOH and excess hydrazine were removed by distillation, and the temperature of the solution was allowed to rise to 200 °C, when refluxing was continued for 4 h. The cooled solution was diluted with water and extracted with ether. The extract was washed with water, dried over anhydrous $Na_2SO_4$ and concentrated. The residue was chromatographed on silica gel using $CHCl_3$ to yield compound **1c** (10 mg). Colorless amorphous solid. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3425, 2925, 2865, 1459, 1448, 1379, 1048. $^1$H-NMR ($C_5D_5N$) $\delta$: 0.80 (3H, d, $J$=6.6 Hz), 0.82 (3H, s), 1.18 (3H, s), 1.31 (3H, s), 1.58 (3H, s), 3.93 (2H, s), 4.00 (1H, dd, $J$=11.0, 4.4 Hz). EI-MS $m/z$: 432 ($M^+$), 401, 394, 358. CD (3.2 mg of **1c** in 5 ml of $5\times10^{-5}$ M $Ni(acac)_2/CCl_4$) $\Delta\varepsilon$: −0.65 (630), +0.27 (320), −0.81 (290).

**References and Notes**


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4) Compound **1** has not been identified in outer or inner barks which were not decayed. The possibility that compound **1** is a fungal metabolite may be ruled out, as no lupane-type triterpene has been reported from fungi and such fungal products as lanostane-type triterpenes or ergostane-type sterols were not isolated from the extract.
5) As the reaction occurs through an enol form, the C-4 methyl can adopt the less hindered configuration (4α). D. H. R. Barton, P. de Mayo, *J. Chem. Soc.*, **1954**, 887.
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