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Constituents of Pollen. V.[1] Constituents of *Betula platyphylla* var. *japonica*

Taichi Ohmoto, Tamotsu Nikaido, and Masa Ikuse

Faculty of Pharmacy, Toho University[2]

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A new triterpene 3-epiocotillol II was isolated along with sinapic acid, apige hydroxyhopanone, ocotillol II, and betulafolientriol oxide I from pollen grains of silver birch (*Betula platyphylla* Sukatchev var. *japonica* Hara) and the structure was clarified from chemical and spectral data. Presence of formic acid and other organic acids in the pollen grains were confirmed and analyzed quantitatively.

Keywords——*Betula platyphylla* Sukatchev var. *japonica* Hara; Betulaceae; pollen grains; 3-epiocotillol; formic acid; hydrocarbon; sinapic acid; hydroxyhopanone; ocotillol II; betulafolientriol oxide I

Silver birch, *Betula platyphylla* Sukatchev var. *japonica* Hara, belonging to Betulaceae family is a monoecious, deciduous tree growing native in the highlands of northern regions of Japan, from central Honshu to Hokkaido. The bark has been used as a medicine for skin diseases, and gout, for diuresis, *etc.* since olden days. As chemical constituents of the bark, presence of some phenolics such as paeonol and many triterpenes such as betulin, betulinic acid, and others were reported by Kosuge, *et al.*,[3] and others. As the constituents of the leaves, presence of hydroxyhopanone, betulafolientriol oxide I, and others were reported by Nagai, *et al.*,[4] and the presence of betulafolientetraol A and B *etc.* was reported by Ikekawa, *et al.*[5] There are only few reports on the constituents of pollen grains, male single cell and, therefore oil-soluble constituents of white birch pollen grains were mainly examined in the present study.

After extracting the constituents of pollen grains with ether, the extracts were fractionated into acidic, phenolic, and neutral compounds as demonstrated in Chart 1, and each fraction was studied for isolation and identification.

Since the presence of lower fatty acids was presumed from the special stimulative odor in the acidic fraction, the fraction was methylated by the conventional method. The presence of some organic acids such as formic acid and other acids were confirmed by gas-liquid chromatography (GLC). From the consideration that formic acid may be one of substances inducing pollen disease by the physical stimulation, quantitative analysis of organic acid in pollen grains of white birch and also some other species for comparison was triedand the result obtained is shown in Table I. Presence of a large amount of formic acid in the pollen grains of *Ambrosia elatior* L. and *Humulus scandens* Merrill aroused interest. In this connection, the correlation between the increase in patients with acute conjunctivitis occurring frequentry during spring and flying of pollen grains in air was examined by Shimizu, *et al.*,[6] but further examination will be necessary.

1) Part IV: T. Ohmoto, T. Nikaido, T. Nozaki, and M. Ikuse, *Yakugaku Zasshi*, **97**, 176 (1977).
2) Location: *Miyama-cho, Funabashi, Chiba.*
3) T. Kosuge, Y. Torigoe, and T. Yamamoto, Abstr. Papers, 95th Annu. Meet. Pharm. Soc. Jap., 1975, p. 194.
4) M. Nagai, N. Tanaka, S. Ichikawa, and O. Tanaka, *Tetrahedron Lett.*, **1968**, 4239.
5) N. Ikekawa, A. Ohta, M. Seki, and A. Takahashi, *Phytochemistry*, **11**, 3037 (1972).
6) Y. Shimizu and Y. Nakayama, *Nippon-no-Ganka*, **49**, 578 (1976).

As another organic acid, a phenolic acid of mp 191—192° (Crystal I) showing positive ferric chloride reaction was obtained, and was identified as sinapic acid. From the sodium hydroxyde soluble fraction, yellow granular crystal II of mp over 300° was obtained. It showed a green color with ferric chloride and positive reaction for flavones, and its UV and IR spectra were corresponding to those of apigenin. Therefore, crystal II was methylated with diazomethane to gave yellow needles of mp 173—174°, and the methyl ether were identified with an authentic sample of 7,4′-dimethoxy apigenin. Accordingly, crystal II was proved to be apigenin. This is the first isolation of apigenin from pollen grains.

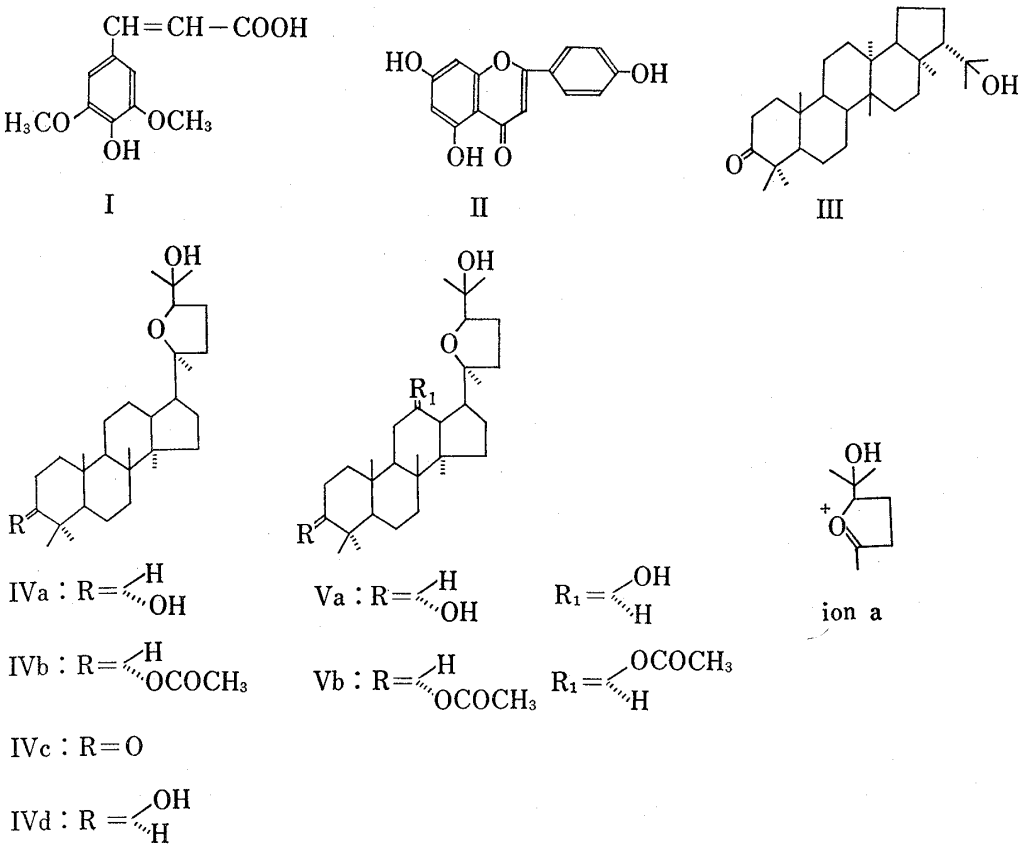

Chart 1

TABLE I. Amount of Organic Acids in Pollen Grains[a] (%)

Species	Organic acids								
	For.	AcOH	Pro.	Pyr.	Lac.	Mal.	Suc.	Cit.	α-Ket.
Ginkgo biloba L.	0.009	0.008	—	0.037	0.013	0.016	0.047	0.244	—
Cryptomeria japonica D. DON	0.002	0.003	—	—	0.001	0.013	0.051	0.159	0.004
Typha latifolia L.	0.006	0.059	—	0.027	0.013	0.023	trace	0.184	—
Alnus hirsuta TURCZ.	0.011	0.019	—	—	0.024	0.038	0.041	—	—
Alnus sieboldiana MATSUM.	0.007	0.017	—	—	0.006	0.300	0.017	—	—
Betula platyphylla SUKATCHEV var. *japonica* HARA	0.009	0.670	0.019	0.032	0.005	0.004	0.004	0.826	—
Quercus acutissima CARRUTH.	0.015	0.057	—	0.053	0.035	—	0.092	—	—
Quercus serrata THUNB.	0.009	0.024	—	—	0.004	0.020	0.015	—	—
Humulus scandens MERRILL	0.025	0.078	—	0.012	—	0.550	0.051	0.380	—
Camellia japonica L.	0.001	—	—	—	—	0.017	0.004	—	—
Ambrosia elatior L.	0.028	0.100	—	0.032	—	0.082	0.060	0.300	—

For: Formic acid, AcOH: Acetic acid, Pro: Propionic acid, Pyr: Pyruvic acid, Lac: Lactic acid, Mal: Malic acid, Suc: Succinic acid, Cit: Citric acid, α-Ket: α-Ketoglutaric acid.
a) Collected April, 1975.

The neutral fraction was treated by column chromatography, and a was-like substance was obtained from the fraction eluted with hexane. This substance was recognized as saturated hydrocarbons of C_{17} to C_{35} mainly containing C_{25} and C_{27} from comparison with authentic samples by GLC. Hydrocarbons hitherto found in pollen grains are listed in Table II, and it is interesting that the hydrocarbons show characteristics of a plant family.

Table II. Main Hydrocarbons in Pollen Grains of Plants of Betulaceae, Fagaceae and Compositae Families

Species	Families	Main hydrocarbons
Alnus japonica Steud.[a]	Betulaceae	$C_{25}H_{52}$, $C_{27}H_{56}$
Alnus sieboldiana Matsum.[b]	Betulaceae	$C_{25}H_{52}$, $C_{27}H_{56}$
Betula platyphylla Skatchev var. *japonica* Hara	Betulaceae	$C_{25}H_{52}$, $C_{27}H_{56}$
Quercus acutissima Carruth.[c]	Fagaceae	$C_{27}H_{56}$, $C_{29}H_{60}$
Ambrosia elatior L.[d]	Compositae	$C_{29}H_{60}$, $C_{31}H_{64}$

a) T. Ohmoto, T. Nikaido, and M. Ikuse, *Yakugaku Zasshi*, **94**, 367 (1974).
b) T. Ohmoto, T. Nikaido, T. Nozaki, and M. Ikuse, *Yakugaku Zasshi*, **97**, 176 (1977).
c) T. Ohmoto, T. Nikaido, and M. Ikuse, *Shoyakugaku Zasshi*, **26**, 36 (1972).
d) T. Ohmoto, T. Nikaido, and M. Ikuse, *Yakugaku Zasshi*, **94**, 362 (1974).

From the chromatography after elution of hydrocarbons, colorless plate crystals (III), mp 254—256°, were obtained from the fraction eluted with benzene–chloroform (1: 1). Substance III was identified as hydroxyhopanone by admixture with an authentic sample and the comparison with it on IR, PMR, and MS spectra.

Colorless plate crystals (IVa) of mp 167—169° with positive Libermann–Burchard reaction were obtained from the fraction eluted with chloroform. The presence of hydroxyl and ether groups were presumed from its IR spectrum. Its PMR spectrum exhibited the presense of 8 methyl protons at δ 0.84—1.21, methine proton (>CHOH) of hydroxyl group on C-3 position at δ 3.38 and another methine proton at δ 3.71 was comfirmed. Since the MS spectrum of IVa showed, besides M^+-15 at *m/e* 445, a fragment peak of ion a at *m/e* 143 in betulafolientriol oxide I was observed as the base peak, the side chain was considered to have the same structure as betulafolientriol oxide I. Acetylation of IVa by the usual method gave granular crystals (IVb) of mp 137°. The presence of hydroxyl and acetyl groups in IVb from IR spectrum and formation of monoacetate from PMR spectrum were clarified. Oxidation of IVa with chromic acid gave colorless needle crystals (IVc) of mp 164—166°, which had hydroxyl and carbonyl groups from the IR spectrum. From the above findings,

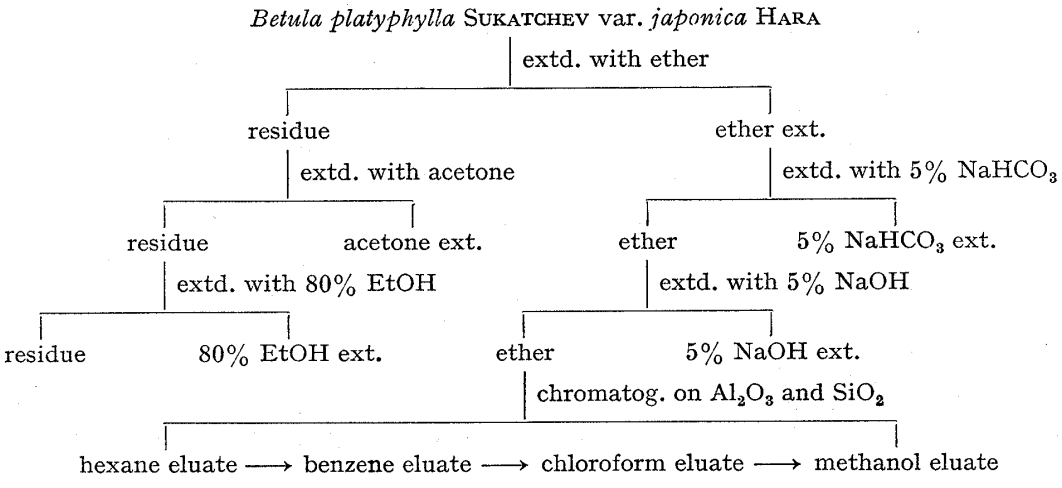

Chart 2

it is clear that one two hydroxyl groups is a tertiary hydroxyl group and this corresponds to the formation of ion a in IVa in MS spectrum. When IVc was reduced with $NaBH_4$, IVd was obtained as crystals of mp 196—198°. IVa was considered to be the 3-epimer of ocotillol II[7] from complete correspondence of its melting point to those of ocotillol II. This can also be understood from the fact that the multiplet of 3α-H shows W 1/2=17 Hz in the PMR spectrum of ocotillol II while 3β-H triplet of IVa shows a coupling constant of 6 Hz. Therefore IVa was recognized as a new compound and it was designated as 3-epiocotillol II. Colorless needle crystals (Va) of mp 237—239° with a positive Liebermann–Burchard reaction was obtained from the fraction eluted with chloroform–methanol (99:1). Acetylation of Va by the usual method gave crystals (Vb) of mp 178—180°, and its PMR spectrum showed the formation of a diacetate Vb. Oxidation of Va with chromic acid gave Vc of mp 165—167°, whose melting point and IR spectrum were corresponding to those of betulafolientoriol oxide I obtained from the leaves of white birch. Va was identified as betulafolientriol oxide I from admixture with an authentic sample and comparison of IR spectrum.

By repeated purification of the fraction eluted with chloroform–methanol (49:1), IVd was obtained as needles of mp 196—198°. IVd was identified with ocotillol II, derived from 3-epiocotillol II (IVa), by admixture and comparison of IR spectra.

It is interesting, from the pattern of development and aspect of biosynthesis in plants, that components analogous to those in the leaves and bark are present in the stage of pollen grains, a single cell of reproduction.

Experimental

Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. UV, IR, PMR, and mass spectra were taken on a Hitachi Model 139, Hitachi Model EPI-G3, JEOL Model JNM-4H-100, and JEOL Model JMS-01-SG-2 spectrometer, respectively. GLC was carried out on Hitachi Model 063 gas-liquid chromatograph using a stainless column (3 mm×1 m) packed with 2% SE-30 on Chromosorb-W (60—80 mesh) with N_2 carrier gas at the flow-rate of 30 ml/min. Carboxylic acid analysis was carried out on Seishin-Seiyaku Model S-603 using a stainless column (3 mm×1 m) packed with anion-exchange resin. Column chromatography was made on Al_2O_3 (Wako, 300 mesh) and silica gel (Wako, C-200). TLC was carried out on Wako gel B-5, and spots were detected with 10% H_2SO_4 (Triterpenoids) and 5% $FeCl_3$ (phenolic derivatives), using S-1 [triterpenoids, hexane–benzene (1:1)], and S-2 [phenolic derivatives, $CHCl_3$–AcOEt (10:1)] as the developing solvents.

Extraction and Fractionation of Components——Pollen grains (2040 g) collected in May, 1974, at Kuriyama, Shioya district, Tochigi Prefecture, were extracted with ether in a Soxhlet apparatus. The extract (190.0 g) was dissolved with ether, and the ether solution was shaken with 5% $NaHCO_3$ and 5% NaOH solution respectively.

Formic, Acetic, and Other Organic Acids——Components in the 5% $NaHCO_3$ extract (3.11 g) were determined with a carboxylic acid analyser.

Quantitative Analysis of Organic Acids——Fresh pollen grains (1.0 g) were ground in a homogenizer and extracted with H_2O. H_2O extract was submitted to carboxylic acid analyser (Table I).

Isolation of Sinapic Acid (I)——5% $NaHCO_3$ extract was added with dil. HCl and precipitated crystals were recrystallized from acetone, yielding a crystalline compound (0.22 g) which showed one spot on TLC (S-2). I: Colorless needles, mp 191—192°; $FeCl_3$ test, red. *Anal.* Calcd. for $C_{11}H_{12}O_5$: C, 58.93; H, 5.53. Found: C, 58.89; H, 5.38. MS *m/e*: 224 (M^+), 209 (M^+-CH_3). IR ν_{max}^{KBr} cm^{-1}: 3385 (OH), 1690 (>C=O), 1614, 1520 (benzene ring). PMR (CD_3COCD_3) δ: 3.91 (6H, s, $-OCH_3\times2$), 6.38 (1H, d, J=16 Hz, -CH=CH-). 7.00 (2H, s, C_2–H and C_6–H), 7.58 (1H, d, J=16 Hz, -CH=CH-).

Isolation of Apigenin (IIa)——5% NaOH extract was added with dil. HCl and precipitated crystals were recrystallized from 80% EtOH, yielding a crystalline compound (0.41 g) which showed one spot on TLC (S-2). IIa: Yellow granular crystals, mp>300°: $FeCl_3$ test, dark green: Mg+HCl test rose. *Anal.* Calcd. for $C_{15}H_{10}O_6$: C, 62.94; H, 3.52. Found: C, 62.91; H, 3.62. UV λ_{max}^{EtOH} nm (log ε): 270 (4.11), 337 (4.14). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1646, 1604, 1580, 1546, 1492 (chromone ring).

Methylation of Apigenin (IIa)——To a solution of IIa (80 mg) in MeOH an ether solution of CH_2N_2 was added and the mixture was allowed to stand at room temperature for 5 hr. After usual work up, the crude product obtained was recrystallized from $CHCl_3$ to 81 mg of a dimethyl ether (IIb) as pale yellow needles,

7) E.W. Warnhoff and C.M.M. Halls, *Canad. J. Chem.*, **43**, 3311 (1965).

mp 173—174°. *Anal.* Calcd. for $C_{17}H_{14}O_5$: C, 68.47; H, 4.70. Found: C, 68.19; H, 4.76. MS *m/e* 298 (M^+). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1668, 1605, 1510 (chromone ring). PMR ($CDCl_3$) δ: 3.92 (6H, s, $-OCH_3\times2$), 6.38 (1H, d, J=2.5 Hz, C_6–H), 6.49 (1H, d, J=2.5 Hz, C_8–H), 6.58 (1H, s, C_3–H), 7.03 (2H, d, J=9 Hz, $C_{3'}$–H, $C_{5'}$–H), 7.85 (2H, d, J=9 Hz, $C_{2'}$–H, $C_{6'}$–H), 12.91 (1H, s, C_5–OH). Mixed melting point on admixture with an authentic samples of 7,4′-dimethoxyapigenin showed no depression, and IR and PMR spectra, and TLC of the two samples were entirely identical.

Separation of Hydrocarbons and Triterpenoids——Neutral portion (42.50 g) was chromatographed on alumina (500 g) and silica gel (100 g), and the columns were eluted successively with hexane, benzene, $CHCl_3$, and MeOH.

Hydrocarbons——The fraction (5.48 g) eluted with hexane was compared with retention time on GLC and were identical with authentic samples of the following hydrocarbons: $C_{17}H_{36}$, $C_{18}H_{38}$, $C_{19}H_{40}$, $C_{20}H_{42}$, $C_{21}H_{44}$, $C_{22}H_{46}$, $C_{23}H_{48}$, $C_{24}H_{50}$, $C_{25}H_{52}$, $C_{26}H_{54}$, $C_{27}H_{56}$, $C_{28}H_{58}$, $C_{29}H_{60}$, $C_{30}H_{62}$, $C_{31}H_{64}$.

Isolation of Hydroxyhopanone (III)——The fraction (3.61 g) eluted with benzene–$CHCl_3$ (1: 1) was repeatedly chromatographed on silica gel (40 g) and eluted with benzene–$CHCl_3$ (2: 1), and the eluted substance was recrystallized from CH_2Cl_2, yielding a crystalline compound (III) (143 mg) which showed one spot on TLC (S-1). III: Colorless plates, mp 254—256°, $[\alpha]_D^{17}$ +64.2° (c=1.15, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{50}O_2$: C, 81.44; H, 11.31. Found: C, 81.46; H, 11.63. MS *m/e*: 442 (M^+), 207, 189, 149. IR ν_{max}^{KBr} cm^{-1}: 3460 (OH), 1710, 1690 (>C=O). Mixed melting point on admixture with an authentic sample of hydroxyhopanone was entirely identical.

Isolation of 3-Epiocotillol II (IVa)——The fraction (4.10 g) eluted with $CHCl_3$ was repeatedly chromatographed on silica gel (60 g) and eluted with $CHCl_3$, and the eluted substance was recrystallized from acetone, yielding a crystalline compound (IVa) (2.70 g) which showed one spot on TLC. IVa: Colorless plates, mp 167—169°; $[\alpha]_D^{13}$ +19.46 (c=2.24, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{52}O_3$: C, 78.26; H, 11.30; Found: C, 78.31; H, 11.62. MS *m/e*: 445 (M^+-CH_3), 401 ($M^+-C_3H_7O$), 383 ($M^+-C_3H_7O-H_2O$), 143 ($M^+-C_{22}H_{37}O$, base peak). IR (KBr) cm^{-1}: 3380, 2955, 2855, 1475, 1455, 1390, 1378, 1370, 1161, 1132, 1088, 1069, 1046, 994. PMR ($CDCl_3$) δ: 0.84—1.21 (3H×8), 3.38 (1H, t, J=6 Hz, C_3–H), 3.71 (1H, m, $W_{1/2}$=13 Hz, C_{24}–H).

Acetylation of 3-Epiocotillol II (IVa)——A solution of 0.49 g of IVa, 5 ml of pyridine, and 1 ml of Ac_2O was allowed to stand for 18 hr at room temperature, and poured into ice-water. A white powder (0.48 g) that appeared was collected and crystallized from MeOH to give 3-epiocotillol II acetate (IVb) as colorless granules, mp 137°, $[\alpha]_D^{23}$ −3.37° (c=4.07, $CHCl_3$). *Anal.* Calcd. for $C_{32}H_{54}O_4$: C, 76.49; H, 10.76. Found: 76.37; H, 10.89. MS *m/e*: 443 ($M^+-OCOCH_3$), 383 ($M^+-OCOCH_3-C_3H_7O-H_2O$), 143 ($M^+-C_{24}H_{39}O_2$, base peak). IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1725, 1245 ($OCOCH_3$). PMR ($CDCl_3$) δ: 0.85—1.22 (3H×8), 2.10 (3H, s, $OCOCH_3$), 3.70 (1H, m, $W_{1/2}$=14 Hz, C_{24}–H), 4.68 (1H, t, J=6.5 Hz).

Oxidation of 3-Epiocotillol II (IVa)——A solution of 0.40 g of IVa, 5 ml of pyridine, and 1.1 g of CrO_3 was allowed to stand over night at room temperature, and poured into 80% MeOH. A white powder (0.38 g) that appeared was collected and recrystallized from benzene–hexane to give ocotilone II (IVc) as colorless needles, mp 164—166°; $[\alpha]_D^{14}$ +60.49° (c=2.06, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{50}O_3$: C, 78.60; H, 10.92. Found. C, 78.66; H, 11.08. MS *m/e*: 443 (M^+-CH_3), 399 ($M^+-C_3H_7O$), 381 ($M^+-C_3H_7O-H_2O$), 143 ($M^+-C_{22}H_{35}O$). IR ν_{max}^{KBr} cm^{-1}: 3480 (OH), 1697 (>C=O). PMR ($CDCl_3$) δ: 0.91—1.23 (3H×8), 2.44 (2H, m, $W_{1/2}$=17 Hz, C_2–H), 3.72 (1H, m, $W_{1/2}$=14 Hz, C_{24}–H).

Reduction of Ocotillone II (IVc)——A solution of 114 mg of IVc, 10 ml of iso-PrOH, and 390 mg of $NaBH_4$ was allowed to stand over night at room temperature, added with AcOH, and poured into ice-water. After ether extraction of the aqueous solution, ether layer was washed with 5% $NaHCO_3$ and water, and dried over Na_2SO_4, and evaporated. The residue (98 mg) was recrystallized from acetone to give ocotillol II (IVd) as colorless needles, mp 196°; $[\alpha]_D^{19}$ +38.91° (c=1.22, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{52}O_3$: C, 78.26; H, 11.30. Found: C, 77.79; H, 11.21. MS *m/e*: 445 (M^+-CH_3), 401 ($M^+-C_3H_7O$), 383 ($M^+-C_3H_7O-H_2O$), 143 ($M^+-C_{22}H_{40}O$, base peak). IR ν_{max}^{KBr} cm^{-1}: 3480 (OH), 1122, 1089, 1050, 1037 (–O–). PMR ($CDCl_3$) δ: 0.78—1.25 (3H×8), 3.22 (1H, m, $W_{1/2}$=17 Hz, C_3–H), 3.71 (1H, m, $W_{1/2}$=14 Hz, C_{24}–H).

Acidic Oxidation of 3-Epiocotillol II (IVa)——A solution of 133 mg of IVa, 6 ml of 85% AcOH, and 90 mg of CrO_3 was allowed to stand over night at room temperature, poured into H_2O, and extracted with benzene. Benzene layer was washed with 5% $NaHCO_3$ and H_2O, dried over Na_2SO_4, and evaporated. The residue (121 mg) was recrystallized from EtOH to give trisnorketolactone (IVe) as colorless plates, mp 184—186°, $[\alpha]_D^{16}$ +70.77° (c=1.04, $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{42}O_3$: C, 78.26; H, 10.15. Found: C, 78.09; H, 10.21. MS *m/e*: 414 (M^+), 315 ($M^+-C_5H_7O_2$), 99 ($M^+-C_{22}H_{35}O$, base peak). IR ν_{max}^{KBr} cm^{-1}: 1760 (lactone), 1709 (>C=O). PMR ($CDCl_3$) δ: 0.90—1.38 (3H×6).

Isolation of Betulafolientriol Oxide I (Va)——The fraction (16.20 g) eluted with $CHCl_3$–MeOH (99: 1) was repeatedly chromatographed on silica gel (180 g) with $CHCl_3$–MeOH (49: 1), and the eluted substance was recrystallized from hexane–acetone, yielding a crystalline compound (132 mg) which showed one spot on TLC (S-1). Va: Colorless needles, mp 237—239°, $[\alpha]_D^{17}$ −16.9° (c=2.43, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{52}O_4$: C, 75.63; H, 10.92. Found: C, 75.79; H, 11.24. MS *m/e*: 461 (M^+-CH_3), 400 ($M^+-C_3H_7O-H_2O$), 382 ($M^+-C_3H_7O-2H_2O$), 143 ($M^+-C_{22}H_{37}O_2$, base peak). IR ν_{max}^{KBr} cm^{-1}: 3475, 3360 (OH), 1170, 1160, 1127, 1090, 1080, 1060, 1035 (tetrahydrofuran ring). PMR ($CDCl_3$) δ: 0.81—1.26 (3H×8), 3.37 (1H, t, J=8 Hz, C_3–H), 3.58 (1H, m, $W_{1/2}$=16 Hz, C_{12}–H), 3.85 (1H, m, $W_{1/2}$=15 Hz, C_{24}–H). Mixed melting point on admixture

with an authentic sample of betulafolientriol oxide I showed no depression, and IR spectrum and TLC of the two samples were entirely identical.

Acetylation of Betulafolientriol Oxide I (Va)——A solution of 70 mg of Va, 2 ml of pyridine, and 0.3 ml of Ac_2O was allowed to stand 15 hr at room temperature, and poured into ice-water. A white powder (62 mg) that appeared was collected and recrystallized from acetone–petroleum ether to give betulafolientriol oxide I acetate (Vb) as colorless plates, mp 178—180°; $[\alpha]_D^{14}$ −20.76° (c=2.56, $CHCl_3$). *Anal.* Calcd. for $C_{34}H_{56}O_6$: C, 72.86; H, 10.00. Found: C, 72.71; H, 10.12. MS m/e: 501 ($M^+-OCOCH_3$), 442 ($M^+-OCOCH_3\times2$), 143 ($M^+-C_{26}H_{41}O_4$). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1740, 1250 ($OCOCH_3$). PMR ($CDCl_3$) δ: 0.83—1.20 (3H×8), 2.02 (3H, s, $OCOCH_3$), 2.08 (3H, s, $OCOCH_3$), 3.67 (1H, m, $W_{1/2}$=14 Hz, C_{24}–H), 4.62 (1H, t, J=6 Hz, C_3–H), 4.85 (1H, m, $W_{1/2}$=15 Hz, C_{12}–H).

Oxidation of Betulafolientriol Oxide I (Va)——A solution of 62 mg of Va, 2 ml of pyridine, and 180 mg of CrO_3 was allowed to stand over night at room temperature, and poured into 50% MeOH. A white powder (49 mg) that appeared was collected and recrystallized from hexane to give a diketone (Vc) as colorless needles, mp 165—167°. *Anal.* Calcd. for $C_{30}H_{48}O_4$: C, 76.26; H, 10.17. Found: C, 75.64; H, 10.08. IR ν_{max}^{KBr} cm^{-1}: 3470 (OH), 1712 (>C=O).

Isolation of Ocotillol II (IVd)——The fraction (3.30 g) eluted with $CHCl_3$–MeOH (49:1) was repeatedly chromatographed on silica gel (60 g) with $CHCl_3$–MeOH (40:1), and the eluted substance was recrystallized from MeOH, yielding a crystalline compound (IVd) (47 mg) which showed one spot on TLC (S-1). IVd: Colorless needles, mp 196—198°; $[\alpha]_D^{22}$ +39.63° (c=1.04, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3485 (OH), 1123, 1079, 1046, 1039 (tetrahydrofuran ring). Mixed melting point on admixture with an authentic sample of ocotillol II showed no depression, and IR, PMR, and MS spectra, and TLC of the two samples were entirely identical.

Acetylation of Ocotillol II (IVd)——A solution of 13 mg of IVd, 1 ml of pyridine, and 0.2 ml of Ac_2O was allowed to stand over night at room temperature, and poured into ice-water. A white powder (9 mg) that appeared was collected and recrystallized from benzene to give ocotillol II acetate (IVf) as colorless prisms, mp 254—256°, $[\alpha]_D^{21}$ +36.67° (c=0.97, $CHCl_3$). MS m/e: 443 ($M^+-OCOCH_3$), 383 ($M^+-OCOCH_3-H_2O$), 143 ($M^+-C_{24}H_{39}O_2$, base peak). PMR ($CDCl_3$) δ: 0.83—1.20 (3H×8), 2.03 (3H, s, $OCOCH_3$), 3.70 (1H, m, $W_{1/2}$=13 Hz, C_{24}–H), 4.50 (1H, m, $W_{1/2}$=16 Hz, C_3–H).

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