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# Triterpenes of Japanese White Birch and the Configuration at C-24 of Ocotillol II and Its Related Compounds[1]

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A new triterpene named now betulafolientriol oxide I (IV), mp 237—240°, $[\alpha]_D^{22}$ +2.6° ($CHCl_3$), was isolated from the leaves of *Betula platyphylla* Sukatchev var. *japonica* Hara in addition to betulafolientriol (I), hydroxyhopanone (II), and dammar-24-ene-12$\beta$,20(*S*)-diol-3-one (III).

The isolation of III was the first example of its occurrence in nature. The chemical and spectrometric investigation of IV and its derivatives showed that IV is a derivative of I, having a modified side chain of a substituted tetrahydrofuran ring. The oxidation of betulafolientriol (I) with perbenzoic acid gave betulafolientriol oxide I (IV) along with its C-24-epimer (XIV). The structure of XIV including its all stereochemistries was clarified by the derivation of XIV from 25-bromo-20(*S*),24(*S*)-epoxy-dammarane-3$\alpha$,12$\beta$-diol (XV). Accordingly, the structure of betulafolientriol oxide I (IV) was established as 20(*S*),24(*R*)-epoxy-dammarane-3$\alpha$,12$\beta$,25-triol. The achievement of chemical conversion of IV into ocotillone II (XII) showed that the stereochemistry at C-24 of ocotillol II (VIII) and its derivatives is expressed in term of (*R*) configuration (the methyl at C-20/ the hydroxyisopropyl at C-24: *trans*).

A dammarane type triterpene, betulafolientriol (I), first obtained from the leaves of European white birch, *Betula alba* L. (Betulaceae) by Fischer and Seiler,[3] has been isolated also from Japanese white birch, *B. platyphylla* Sukatchev var. *japonica* Hara in our laboratory.[4] It has been concluded that the stereochemistry at C-20 of dammarenediol I and its related compounds is (*R*) configuration, and that of dammarenediol II and its homologues (*S*).[5,6] Betulafolientriol (I), one of the derivatives of dammarenediol II, has inevitably 20 (*S*) configuration.

Three minor triterpenes A, B and C in addition to I, have now been obtained from the unsaponifiable fraction of the ethereal extract of the leaves of Japanese white birch. The triterpene A, mp 245—250°, and the triterpene B, mp 196—199°, were found to be identical with hydroxyhopanone (II), and dammar-24-ene-12$\beta$,20(*S*)-diol-3-one (III) respectively by direct comparisons with authentic samples. The ketone (III) has already been prepared from I by Seiler, *et al.*,[3a] but this is the first example of its occurrence in nature. The triterpene B (III) is isomeric in its position of the double bond to alnusfoliendiolone[7] from *Alnus glutinosa* (Betulaceae), the double bond of which is located in C-25 (26).

1) Preliminary communication of this study: M. Nagai, N. Tanaka, S. Ichikawa and O. Tanaka, *Tetrahedron Letters*, **1968**, 4239.
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3) *a*) F.G. Fischer and N. Seiler, *Ann.*, **626**, 185 (1959); *b*) *Idem, ibid.*, **644**, 146 (1961).
4) S. Shibata, M. Nagai and O. Tanaka, *Shoyakugaku-Zasshi* (*Japan. J. Pharmacog.*), **18**, 27 (1964).
5) *a*) O. Tanaka, M. Nagai, T. Ohsawa, N. Tanaka, K. Kawai and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **20**, 1204 (1972) and the references there cited; *b*) O. Tanaka, N. Tanaka, T. Ohsawa, Y. Iitaka and S. Shibata, *Tetrahedron Letters*, **1968**, 4235.
6) J.F. Biellman, *Bull. Soc. Chim. France*, **1967**, 3459 (*Tetrahedron Letters*, **1966**, 4803).
7) F.G. Fischer and N. Seiler, *Ann.*, **644**, 162 (1961).

The triterpene C (IV), mp 237—240°, $C_{30}H_{52}O_4$, $[\alpha]_D^{22}$ +2.6° ($CHCl_3$), shows hydroxyl bands at 3420 (concentration independent) and 3620 $cm^{-1}$ in its infrared (IR) spectrum in $CHCl_3$. Its nuclear magnetic resonance (NMR) spectrum in $CDCl_3$ shows no vinylic proton signal, but exhibits eight tertiary methyl signals and signals due to protons on carbon atoms bearing oxygen functions at $\tau$ 6.15 (H, broad), 6.47 (H, doublet-triplet) and 6.61 (H, triplet), the latter two of which are similar to those of protons on C-12, and C-3 of betulafolientriol (I) respectively. On oxidation with chromic acid in pyridine, the triterpene C (IV) afforded a diketone (V), mp 176.5—178°, whose IR spectrum in $CCl_4$ still showed a hydroxyl band at 3581 $cm^{-1}$ (concentration independent) along with a carbonyl absorption at 1714 $cm^{-1}$. The NMR spectrum of V lacks the signals observed at $\tau$ 6.47 and 6.61 in the spectrum of the triterpene C (IV) (*vide supra*). Whereas, on oxidation with Jones reagent, IV yielded a compound, mp 233—236°, which was found to be identical with the trisnordiketolactone (VI), previously derived from I.[3] These evidences strongly suggest that the triterpene C (IV) would be a derivative of betulafolientriol (I), having a hindered and intramolecularly hydrogen-bonded hydroxyl on a modified side chain of I.

Referring the assignment of the mass spectrum (MS) of gratiogenin (VII),[8] the base peak at *m/e* 143 of the mass spectrum of the triterpene C (IV) would be attributed to the ion *a*, indicating the presence of the side chain of the same type as that of VII. The triterpene C is a new compound, and is named now betulafolientriol oxide I (IV).

Warnhoff and Halls isolated a triterpene named ocotillol II (VIII) from *Fouquieria splendens* Engelm and assigned its structure as VIII, whose configuration at C-24 has been left unsolved.[6,9] They reported the formation of the trisnorketolactone (IX) from VIII on oxidation with chromic acid and the transformation of 3-O-acetyldammarenediol II (X) to 3-acetate (XI) of VIII on oxidation with monoperphthalic acid. Afterwards, Bisset, *et al.* isolated ocotillone II (XII) and its C-20 epimer from the resin of *Dipterocarpus hispidus* Thw.[10] Hirose, *et al.* also isolated XII and its C-20 epimer from the wood of Kapur (*Dryobalanopus sp.*).[11] It is evident that ocotillone II (XII) has the same configuration at C-24 as that of ocotillol II (VIII), since XII gave VIII on reduction with $NaBH_4$.[10,11] Biellman found that oxidation of X with *p*-nitro-perbenzoic acid gave the C-24 epimer (XIII) of XI in addition to XI.[6]

Betulafolientriol (I) was subjected to oxidation with perbenzoic acid and the reaction mixture was separated chromatographically on alumina to give two crystalline compounds, tentatively named oxide I and oxide II, the former of which was proved to be identical with betulafolientriol oxide I (IV). On the basis of these results, the structure of betulafolientriol oxide I (IV) can be formulated as IV, except for the stereochemistry at C-24. The other product, oxide II (XIV), mp 145—150°, $C_{30}H_{52}O_4$, $[\alpha]_D^9$ +13.1° ($CHCl_3$), was assumed to be the C-24 epimer of IV, because of similarities of its spectral data to those of IV.

In our preceding paper,[5b,12] it has been reported that on the action of N-bromosuccinimide, betulafolientriol (I) yielded a bromo-compound (XV), whose structure was rigorously determined by X-ray crystallographic analysis. The substitution reaction of the bromine atom at C-25 of XV with a hydroxyl group by treatment with silver oxide in boiling aqueous ethanol, furnished the oxide II (XIV), establishing the structure of XIV including all its stereochemi-

8) R. Tschesche, G. Biernoth and G. Snatzke, *Ann.*, **674**, 196 (1964).
9) E.W. Warnhoff and C.M.M. Halls, *Canad. J. Chem.*, **43**, 3311 (1965).
10) N.G. Bisset, M.A. Diaz, C. Ehret, G. Ourisson, M. Palmade, F. Patil, P. Pesnelle and J. Streith, *Phytochem.*, **5**, 865 (1966).
11) Y. Hirose, T. Yanagawa, Y. Sayama, T. Igarashi and T. Nakatsuka, *Mokuzai Gakkaishi* (*J. Japan. Wood Research Soc.*), **14**, 36 (1968); Y. Hirose, T. Yanagawa and T. Nakatsuka, *ibid.*, **14**, 59 (1968). Ocotillol II has also been isolated from *Neolloydia texensis* (X.A. Dominguez, R. Carrero, R. Gonzalez, P. Rojas and R. Kecham, *Chem. Ind.*, **1967**, 2147).
12) T. Ohsawa, N. Tanaka, O. Tanaka and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **20**, 1890 (1971).

stries as shown in the Chart 1. Accordingly, the stereochemistry at C-24 of betulafolientriol oxide I (IV), the C-24 epimer of XIV, should be expressed in term of (*R*) configuration (the methyl group at C-20/the hydroxyisopropyl group at C-24: *trans*).

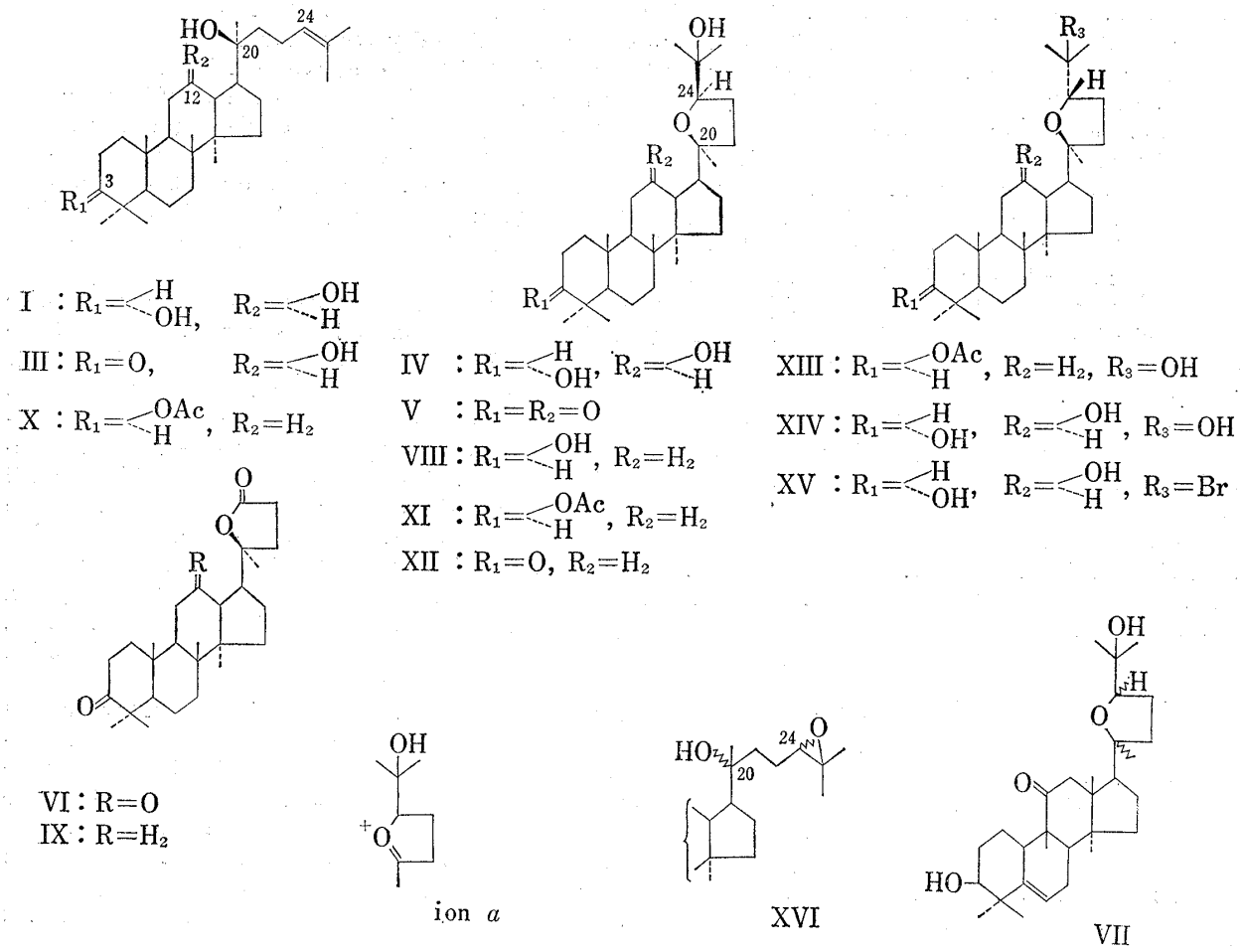


Chart 1

The transformation of betulafolientriol oxide I (IV=20(*S*), 24(*R*)-epoxy-dammarane-3$\alpha$,12$\beta$,25-triol) to ocotillone II was accomplished as follows. On acetylation with acetic anhydride in pyridine, IV gave 3-O-acetyl derivative, which was oxidized with chromic acid in pyridine, yielding a keto-acetate. Wolff-Kischner reduction of the keto-acetate and subsequent oxidation of the resulted diol with chromic acid afforded a ketone, mp 164—167°, which was proved to be identical with ocotillone II (XII) by mixed melting point and comparisons of the infrared (IR) spectrum and the thin-layer chromatogram (TLC) with those of the authentic sample. This chemical correlation led unequivocally to establish (*R*) configuration at C-24 of ocotillol II (VIII) as well as of ocotillone II (XII), and inevitably (*S*) configuration at C-24 of XIII.

It should be noted that the dammarane type triterpenes having the substituted tetrahydrofuran ring, such as IV, VIII, XII and C-20 epimer of XII (ocotillone I) might be the artifacts formed from epoxides such as XVI through nucleophilic attack of the oxygen atom of the hydroxyl at C-20 to C-24 of the epoxides during the process of the extraction or the separation of them, since the epoxides of this type have been shown to be unstable and to be converted very easily to the tetrahydrofuran derivatives.[6,9]

## Experimental[13]

**Isolation of Hydroxyhopanone (II), and Dammar-24-ene-12β,20(*S*)-diol-3-one (III)**——The fresh leaves (about 25 kg) of Japanese white birch collected in Nobeyama in June were treated in the manner described in Shoyakugaku-Zasshi,[3,4] affording 47 g of crude crystals of betulafolientriol (I). On successive recrystallizations from MeOH and EtOAc, the crude crystals yielded pure betulafolientriol (I). The combined mother liquor was concentrated to dryness, and the residue was chromatographed on alumina (Brockman) with benzene and $CHCl_3$ as the solvents. The eluate was concentrated to dryness and again chromatographed on alumina (Brockman). The fraction eluted with 10—40% $CHCl_3$ in benzene gave a small amount of colorless plates, mp 245—250°, on repeated recrystallizations from MeOH–$CH_2Cl_2$ and from EtOH, which was found to be identical with authentic sample of hydroxyhopanone (II) by direct comparisons (mixed mp and IR). The eluate with a mixture of ether–$CHCl_3$–benzene (1 : 8 : 11) afforded an oily residue, which, on standing in ether, gave crystalline material. On repeated recrystallization from acetone, the crystals were purified to a small amount of colorless plates, mp 196—199°, IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 3300 (OH), 1690 (>C=O), 812 (trisubstituted double bond), which was found to be identical by mixed mp, IR and TLC with 3-ketone (III) derived from betulafolientriol (I) after Seiler, *et al.*

**Isolation of Beutlafolientriol Oxide I (IV)**——Air-dried leaves (1.2 kg) of Japanese white birch were twice extracted with MeOH (6 liters) at room temperature. To the extract was added ether (0.9 liter) and the ethereal solution was washed with water, dried over anhydrous $Na_2SO_4$, and concentrated to a dark green viscous residue. The residue was treated in a similar way described by Seiler, *et al.*[3,4] The precipitates (5 g) containing betulafolientriol (I) were obtained from a part soluble in 80% aq. MeOH out of the unsaponifiable fraction dissolved in petroleum ether, and were chromatographed on silica gel (200 g). Some fractions eluted with $CHCl_3$ were collected and evaporated to dryness. The residue was dissolved in a small volume of ether, affording the precipitates (150 mg), which were purified by recrystallization from acetone to colorless prisms of betulafolientriol oxide I (IV), mp 237—240°, $[\alpha]_D^{22}$ +2.6° ($c$=1.2, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{52}O_4$: C, 75.58; H, 11.00. Found: C, 75.51; H, 10.85. IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3620 (free OH), 3420 (bonded OH). NMR (100 MHz, $CDCl_3$) $\tau$: 6.15 (H, m), 6.47 (H, d-t, $J$=5.3, 10.4 Hz respectively), 6.61 (H, t, $J$=2.2 Hz), 8.74 (3H×2, s each), 8.91 (3H, s), 9.01 (3H, s), 9.07 (3H×2, s each), 9.13 (3H, s), 9.15 (3H, s). Mass Spectrum $m/e$: 461 ($M^+$−15), 143 (base peak). The presence of IV in the unsaponifiable fraction of the extract of the fresh leaves was shown by TLC.

**Oxidation of Betulafolientriol Oxide I (IV)**——(i) A mixture of $CrO_3$ (2.0 g) and pyridine (9 ml) was added to a solution of the oxide (IV) (0.7 g) in pyridine (9 ml) and the reaction mixture was allowed to stand overnight at room temperature with magnetical stirring. A small volume of MeOH was added to it to decompose an excess of $CrO_3$. After filtration, the precipitates were washed with pyridine. The combined filtrate was concentrated *in vacuo* to afford a crystalline residue, which was recrystallyzed from *n*-hexane, giving the ketone (V) as colorless needles, mp 176.5—178°, $[\alpha]_D^{23}$ +77.2° ($c$=1.1, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 76.38; H, 10.06. IR $\nu_{max}^{CCl_4}$ $cm^{-1}$: 3581 (OH), 1714 (>C=O). NMR (60 MHz, $CDCl_3$) $\tau$: 6.30 (H, m), 8.76 (3H, s), 8.79 (3H, s), 8.90 (3H×3, s each), 8.93 (3H, s), 8.97 (3H, s), 9.22 (3H, s).

(ii) The oxide (IV) (50 mg) in acetone (4 ml) was oxidized with an excess of Jones reagent for 17 hr in a usual way. After addition of dil. $Na_2SO_3$ to decompose the excess of the reagent, the mixture was extracted with ether. The ethereal solution was washed with water, dried over anhydrous $Na_2SO_4$, and concentrated to dryness. The crystalline residue was recrystallized from MeOH, affording colorless needles, mp 233—236°, the identity of which with the trisnordiketolactone (VI) previously derived from betulafolientriol (I) was achieved by mixed mp and direct comparisons of their IR and TLC.

**Oxidation of Betulafolientriol (I) with Perbenzoic Acid**——To a solution of betulafolientriol (I) (1.05 g) in $CHCl_3$ (20 ml) was added a perbenzoic acid solution in $CHCl_3$ (10 ml, 68 mg of the peracid in 1 ml of the solution) in small portions under ice cooling. After 17 hr, the reaction mixture was poured into ice–dil. $Na_2CO_3$ mixture. The organic phase separated was washed with water and dried over anhydrous $Na_2SO_4$. After evaporation of the solvent, the oily product obtained was chromatographed on alumina (35 g, Wöelm, neutral, grade V). From the fractions eluted with benzene, a substance (0.4 g) was obtained as colorless prisms, mp 236—239°, on recrystallization from acetone, which was proved to be identical with betulafolientriol oxide I (IV) by mixed mp and comparisons of their IR and TLC. On evaporation of the solvent of the chromatographic fractions eluted with 10—20% ether in benzene, a viscous residue (0.4 g) was obtained, which, on crystallization from a small volume of acetone, gave C-24 epimer (oxide II) (XIV) of IV as colorless massive crystals, mp 145—150°, $[\alpha]_D^9$ +13.1° ($c$=1, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{52}O_4$: C, 75.58; H, 11.00. Found: C, 75.62; H, 11.03. IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3580, 3320 (OH). NMR (60 MHz, $CDCl_3$) $\tau$: 6.08 (H, m), ~6.3 (H, br), 6.59 (H, t, $J$=2.2 Hz), 8.74 (3H, s), 8.77 (3H, s), 8.89 (3H, s), 8.93 (3H, s), 9.06 (3H×2, s each), 9.09 (3H, s), 9.15 (3H, s).

13) All melting points were determined on a Kofler's block and uncorrected. Silica gel G (Merck) was used for the absorbant of the TLC.

**Oxidation of C-24 Epimer (Oxide II) (XIV) of Betulafolientriol Oxide I (IV)**——The epimer (XIV) (50 mg) in acetone (5 ml) was oxidized with Jones reagent in a similar way described above for the oxidation of IV. The product obtained, mp 234—236°, was proved to be identical with authentic sample of the trisnordiketolactone (VI) by mixed mp and comparisons of their IR and TLC.

**Derivation of C-24 Epimer (Oxide II) (XIV) of Betulafolientriol Oxide I (IV) from the Bromo-compound (XV)**——A mixture of the bromide (XV) (100 mg), $Ag_2O$ (5 mg), and 70% aq. EtOH (35 ml) was heated under reflux for 8 hr. After filtration, the filtrate was concentrated *in vacuo* to a small volume, to which water was added. The product was extracted with ether, and the ethereal solution was washed with water, dried over anhydrous $Na_2SO_4$. After evaporation of the solvent, the residue was chromatographed on silica gel with benzene–ether as the solvent. Referring the TLC, fractions containing the oxide II (XIV) were collected and concentrated *in vacuo*. On crystallization from acetone, the residue afforded the oxide II (XIV) as colorless massive crystals, mp 145—150°, which was proved to be identical with authentic sample of the oxide II (XIV) by mixed mp and comparisons of their TLC and IR ($CHCl_3$).

**Derivation of Ocotillone II (XII) from Betulafolientriol Oxide I (IV)**——A mixture of betulafolientriol oxide I (IV) (150 mg), $Ac_2O$ (1.5 ml) and pyridine (2 ml) was allowed to stand overnight at room temperature, and then poured into ice–water. The product was extracted with ether. The ethereal solution was washed with dil. HCl and then with water, and dried over anhydrous $Na_2SO_4$. Evaporation of the solvent left an oily residue. The residue (150 mg) was dissolved in pyridine (2 ml) and dropwise added to a mixture of $CrO_3$ (400 mg) and pyridine (6 ml). After 17 hr, the reaction mixture was poured into ice–water, and the product was taken up in ether. The ethereal solution was washed with water, dil. HCl and water successively. After being dried over anhydrous $Na_2SO_4$, the solution was concentrated to dryness. The residue was chromatographed on silica gel (5 g). Fractions eluted with 70—100% $CHCl_3$ in benzene were concentrated to dryness, affording a residue (70 mg), which consisted of 12-keto-3-acetyl derivative of IV. The residue was mixed with KOH (90 mg), anhydrous hydrazine (0.15 ml), anhydrous diethylene glycol (1.5 ml), anhydrous EtOH (1.5 ml) and boiling stones, and heated with a reflux cooler for 1 hr 10 min on an oil bath preheated at 120°. A downward cooler was replaced for the reflux cooler, and the temperature of the oil bath was gradually raised from 120° to 230°. The temperature was maintained at 230° for further 5 hr. After cooling, the reaction mixture was poured into ice–water, and the product was extracted with ether. The ethereal solution was washed repeatedly with water and dried over anhydrous $Na_2SO_4$. On evaporation of the solvent, a crystalline residue (60 mg), mp 165—170° was obtained, which was, without further investigation, subjected to oxidation with $CrO_3$ (200 mg) and pyridine (4 ml) for 17 hr at room temperature. After filtration, the filtrate was poured into ice–water and the product was taken up into ether. The ethereal solution was washed with water, dil. HCl and water successively and dried over anhydrous $Na_2SO_4$. On removal of the solvent a crystalline residue was obtained, and recrystallized from MeOH as colorless needles, mp 164—167°, which was proved to be identical with ocotillone II (XII) by mixed mp and comparisons of the IR (KBr and $CHCl_3$) and the TLC with those of the authentic sample, mp 164—170°.

**Addendum**——Very recently, Ikekawa, *et al.** have reported the isolation of two new dammarane type triterpenes from the leaves of Japanese white birch (* N. Ikekawa, A. Ohta, M. Seki, A. Takahashi, *Phytochemistry*, **11**, 3037 (1972)).

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