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Minor Diterpenes of *Aralia cordata* Thunb.: 17-Hydroxy-*ent*-kaur-15-en-19-oic Acid and Grandifloric Acid

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15α-Hydroxy-*ent*-kaur-16-en-19-oic acid (IX) (=grandifloric acid) and a new diterpene acid, 17-hydroxy-*ent*-kaur-15-en-19-oic acid (X) were isolated as the minor constituents from the roots of *Aralia cordata* Thunb. The final identification of IX and the structural elucidation of X were accomplished by preparation of IX and the methyl ester of X(XI) from methyl *ent*-kaurenoate (XII) by means of the double bond isomerization and the dye-sensitized photooxidation.

The isolation and the structural elucidation of the *ent*-pimarane type diterpenes, *ent*-pimara-8(14),15-dien-19-oic acid (I) (major), II, III, IV, V and VI of *Aralia cordata* Thunb. (Araliaceae, Japanese name "Udo") have been reported. *ent*-Kaurenoic acid (VII) (major) and its homologue (VIII) have also been isolated from the same plant.[2,3] The present paper deals with the further isolation of the two minor diterpenes from the roots of this plant.

On the chromatography of the acidic fraction of the ethereal extract, there was obtained an additional crystalline substance, mp 243—244°, $C_{20}H_{30}O_3$. In spite of its sharp melting point, this substance was revealed to be a mixed crystal of two isomeric compounds (1:1) by means of thin-layer chromatography (TLC) on $AgNO_3$-silica gel, gas-liquid chromatograpy (GLC), and nuclear magnetic resonance (NMR). The rechromatography on silica gel impregnated with $AgNO_3$ furnished the separation into two isomers IX and X, both of which showed the lower melting point, mp 228—229° (IX) and mp 193—194° (X) than that of the original crystals.

On the basis of infrared (IR), NMR, and mass spectra and its physical constant, the compound (IX) was suggested to be identical with grandifloric acid (15α-hydroxy-*ent*-kaur-16-en-19-oic acid) which has already been isolated from *Espeletia* spp.[4]

The other isomer (X), a new hydroxyditerpene acid, $C_{20}H_{30}O_3$ (mass spectrum, 318 (M^+)) showed IR bands (in Nujol) at 3420 (OH) and 1695 cm^{-1} (COOH) and NMR signals (in pyridine-d_5) at δ 1.18 (3H singlet, $-\overset{|}{C}-CH_3$), 1.36 (3H singlet, $-\overset{|}{C}-CH_3$), 4.47 (2H singlet, broadened by allylic coupling, $-HC=\underset{|}{C}-\underline{C}H_2-OH$) and 5.59 ppm (1H singlet, broadened by allylic coupling, $-\underline{H}C=\underset{|}{C}-CH_2-OH$). On treatment with CH_2N_2, X yielded a methyl ester (XI), mp 129—131°, with IR bands at 3610 (OH) and 1720 cm^{-1} (COOMe) (in $CHCl_3$) and NMR signals (in $CDCl_3$) at δ 0.83 (3H singlet, $-\overset{|}{C}-CH_3$), 1.18 (3H singlet, $-\overset{|}{C}-CH_3$), 3.62 (3H singlet, $COOCH_3$), 4.19 (2H singlet broadened by allylic coupling, $-CH=\underset{|}{C}-\underline{C}H_2-OH$), and 5.33 ppm (1H singlet broad-

1) Location: *a) Kasumi, Hiroshima-shi; b) Kitashinjuku, Shinjuku-ku, Tokyo.*
2) S. Shibata, S. Mihashi, and O. Tanaka, *Tetrahedron Letters*, **1967**, 5241; S. Mihashi, I. Yanagisawa, O. Tanaka, and S. Shibata, *ibid.*, **1969**, 1683.
3) O. Tanaka, S. Mihashi, I. Yanagisawa, T. Nikaido, and S. Shibata, *Tetrahedron*, **28**, 4523 (1972).
4) F. Piozzi, V. Spiro, S. Passannanti, M. Salvato, and R. Mondelli, *Gazz. Chim. Ital.*, **98**, 908 (1968) [*C.A.*, **70**, 29099 (1969)]; C.H. Brieskorn and E. Pöhlmann, *Tetrahedron Letters*, **1968**, 5661; *idem*, *Chem. Ber.*, **102**, 2621 (1969).

ened by allylic coupling, $-CH=\underset{|}{C}-CH_2-OH$). These evidences as well as the co-occurrence of *ent*-kaurenoic acid (VII) led to propose the structure, 17-hydroxy-*ent*-kaur-15-en-19-oic acid for X.

The above assumption was finally confirmed by the preparation of IX and XI from methyl *ent*-kaurenoate (XII). On treatment with iodine, XII afforded an equilibrated mixture of the double bond isomers, XII and methyl *ent*-isokaurenoate (XIII).[5] This mixture without further separation was subjected to the dye-sensitized photooxidation followed by the reduction of the resulted mixture of two hydroperoxides.[5,6] The products were chromatographed on silica gel impregnated with $AgNO_3$ to give two hydroxy-esters. One of them was proved to be identical with XI by direct comparison. The other hydroxy-ester was saponified to give a hydroxy acid which was revealed to be identical with IX in every respects.

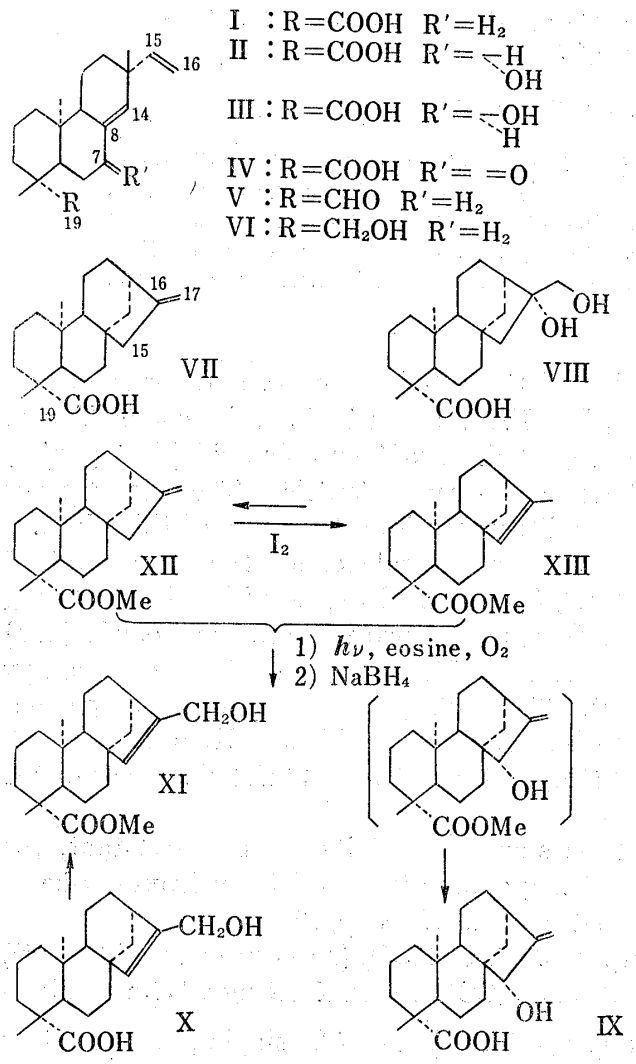

Chart 1

The diterpene acids I and VII have been isolated also from *A. racemosa* and *A. continentalis* in the similar yield to that from *A. cordata* (0.2—0.4%, slight excess of I).[7] The oriental plant drug "Dhuo" (Japanese name Dokkatsu) in the Japanese market has been known to be prepared from the roots of *A. cordata* usually containing I and VII as the major diterpenes in the same ratio as that of the original plant. However, we found recently that Dhuo sold in Hiroshima-shi in 1972—1973 gave *ent*-kaurenoic acid (VII) in an anomalous high yield (more than 1.2%) along with a relatively small amount of I. The hydroxy acid, IX and X were also isolated as the minor constituents and identified. The source of this unusual plant drug has been left to be identified.

Experimental

All melting points were determined on a micro hot-stage and uncorrected.

Conditions of GLC——1.5% SE-30 on Chromosorb W, glass column 2 m×2 mm, hydrogen flame ionization detector, N_2 1.5 kg/cm². For free acid: column temp. 230°, retention times IX 3.8 min, and X 4.3 min. For methyl ester: column temp. 210°, retention times methyl ester of IX 5.0 min, and XI 6.3 min.

5) The isomerization of the double bond of kaur-16-ene and the stereochemistry of the photooxidation of the kaur-15- and 16-ene type diterpenes: M.F. Barns and J. MacMillan, *J. Chem. Soc.* (*C*), **1967**, 361; R.A. Bell, R.E. Ireland, and L.N. Marder, *J. Org. Chem.*, **31**, 2536 (1966).

6) Just after our work was completed, there appeared the reported of the photooxidation of XII and XIII: A.K. Banerjee, A. Martin, and T. Nakano, *J. Org. Chem.*, **38**, 3807 (1973).

7) O. Tanaka, Y. Yasuda, K. Yamasaki, and S. Mihashi, *Yakugaku Zasshi*, **92**, 1058 (1972).

Extraction and Separation of Diterpenes——The roots (1 kg) of *A. cordata* collected in the suburbs of Sendai-shi were extracted with ether at room temperature. The concentrated ethereal extract was shaken with a) 5% $NaHCO_3$, b) 5% Na_2CO_3, c) 5% NaOH successively and finally washed with water. The major diterpenes, I and VII were obtained mainly from the water washing.

The fraction-b) was acidified and the precipitate was taken up in ether. The ethereal extract was concentrated to dryness and the residue was chromatographed on silica gel being eluted with $CHCl_3$. After I, VII, and fatty acid fractions, the oily substance was eluted, which was treated with a small amount of benzene or acetone to give crystalline material (170 mg). Further recrystallization of this material from MeOH–H_2O afforded colorless crystals,[8] mp 243—244°. *Anal.* Calcd. for $C_{20}H_{30}O_3$: C, 75.43; H, 9.50; molecular weight 318. Found: C, 75.43; H, 9.73. Mass Spectrum: 318 (M^+). Although the TLC on silica gel (solvent benzene: acetone (10: 1) or $CHCl_3$: MeOH (6: 1)) showed a single spot, the GLC and TLC on 3% $AgNO_3$–silica gel (solvent benzene: acetone (4: 1)) of this material exhibited two peaks and two spots, respectively. Its NMR spectrum (in pyridine-d_5) also indicated that this material was a mixted crystal of IX and X in a ratio of 1: 1.

The rechromatography of the material on silica gel impregnated 5% $AgNO_3$ (eluting solvent benzene: AcOEt (20: 1)) led to the separation into two isomers, IX and X. The first eluted compound was recrystallized from MeOH–H_2O or benzene–hexane to give colorless crystals, IX (grandifloric acid), mp 228—229°, $[\alpha]_D$ −130° (c=0.1 MeOH). Mass Spectrum: 318, (M^+) ($C_{20}H_{30}O_3$). IR ν_{max}^{Nujol} cm^{-1} 3410 (OH) and 1700 (COOH). NMR (in pyridine-d_5) δ ppm 1.18 (3H, singlet, $-\overset{|}{C}-CH_3$), 1.36 (3H, singlet, $-\overset{|}{C}-CH_3$), 4.12 (1H, singlet broadened by allylic coupling, $-\underline{H}COH-\overset{|}{C}=CH_2$), 5.16 and 5.44 (1H, each, broad singlets, $-\overset{|}{C}=CH_2$).

The other compound eluted after IX was recrystallized from MeOH–H_2O or benzene–hexane to give colorless crystals (X), mp 193—194°, $[\alpha]_D$ −60° (c=0.3, MeOH). Mass Spectrum: 318 (M^+), ($C_{20}H_{30}O_3$). On treatment with CH_2N_2 in ether under usual way, X yielded the methyl ester (XI), colorless crystals, from hexane, mp 129—131°, $[\alpha]_D$ −50° (c=0.3, MeOH). Mass Spectrum: 332 (M^+), ($C_{21}H_{32}O_3$).

Preparation of IX and XI from XII——Methyl *ent*-kaurenoate (XII) (100 mg) prepared from VII was dissolved in benzene (10 ml) and to this solution was added a small amount of iodine. The solution was refluxed for 9 hr and iodine was removed by washing with aqueous $NaHSO_3$. Evaporation of the solvent to dryness gave a equilibrated mixture of XII and methyl *ent*-isokaurenoate (XIII) (*ca.* 1: 2 by GLC).

To a solution of this mixture (100 mg) in pyridine (5 ml) was added eosine (1 mg) and the solution was irradiated by a tungsten filament lamp (300 W) for 120 hr under stream of oxygen with stirring. After dilution with ether and removal of eosine with active charcoal, the solvent was concentrated to dryness. The residue was reduced with $NaBH_4$ (50 mg) in aqueous MeOH at room temperature for 1 hr. After working up in the usual way, the crude reaction mixture was chromatographed on silica gel impregnated with 5% $AgNO_3$ using AcOEt: benzene (1: 20) as eluting solvent. The first eluted compound (30 mg) was dissolved in diethyleneglycol (25 ml) and to this solution was added KOH (800 mg). The mixture was heated under reflux for 1 hr and then acidified with aqueous AcOH. The resulted solution was extracted with ether repeatedly and the ethereal layer was washed with H_2O, dried, and concentrated to dryness. The residue was recrystallized from benzene–hexane affording colorless crystals (17 mg), mp 228—229°, $[\alpha]_D$ −128° (c= 0.5, MeOH), which was identical with IX by mixed melting point and comparison of IR, NMR, TLC, GLC, and optical rotation.

The second eluate of the above chromatography was recrystallized from hexane yielding colorless crystals, mp 127—129° (10 mg), $[\alpha]_D$ −53° (c=0.5, MeOH), which was proved to be identical with XI by mixed melting point and the comparison of IR, NMR, TLC, GLC, and optical rotation.

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8) From the mother liquor, the minor diterpenes, III and IV were obtained.[2]