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Bitter Principles of *Pertya glabrescens*: Two Sesquiterpene Glucosides

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Two bitter principles, glucosyl pertate (I), $[\alpha]_D$ −48.6°, and glucosyl 3α-hydroxypertate (II), $[\alpha]_D$ −10.9°, were isolated from the leaves of *Pertya glabrescens* SCH. BIP. (Compositae).

On acid hdrolysis, I and II yielded new sesquiterpene acids, pertic acid (IV), $C_{15}H_{18}O_4$, mp 152—153°C (dec.), $[\alpha]_D$ −72.7°, and 3α-hydroxypertic acid (XI), $C_{15}H_{18}O_5$, mp 250—252°C (dec.), $[\alpha]_D$ +1.6°, as their genins, respectively. These genins were chemically correlated with pertilide (VI). The chemical structures of I and II were established as β-D-glucopyranosyl [1(10)*Z*, 4*E*]-(7*R*, 8*S*)-germacra-1(10), 4,11(13)-trien-12,8-olide-14-oate (I) and β-D-glucopyranosyl [1(10)*Z*, 4*E*]-(3*R*, 7*R*, 8*S*)-3-hydroxygermacra-1(10),4,11(13)-trien-12,8-olide-14-oate (II).

Keywords——*Pertya glabrescens*; Compositae; sesquiterpene; germacranolide; sesquiterpene glucoside; sesquiterpene acid; glucosyl pertate; pertic acid; glucosyl 3α-hydroxypertate; 3α-hydroxypertic acid

In the previous paper,[2] we reported the structure elucidation of a sesquiterpene dilactone, pertilide, isolated from the leaves of *Pertya glabrescens* SCH. BIP. (Compositae). In a continuation of our research on the bitter principles of the leaves, two amorphous compounds having a bitter taste, designated glucosyl pertate (I), $[\alpha]_D$ −48.6° and glucosyl 3α-hydroxypertate (II), $[\alpha]_D$ −10.9°, were isolated.

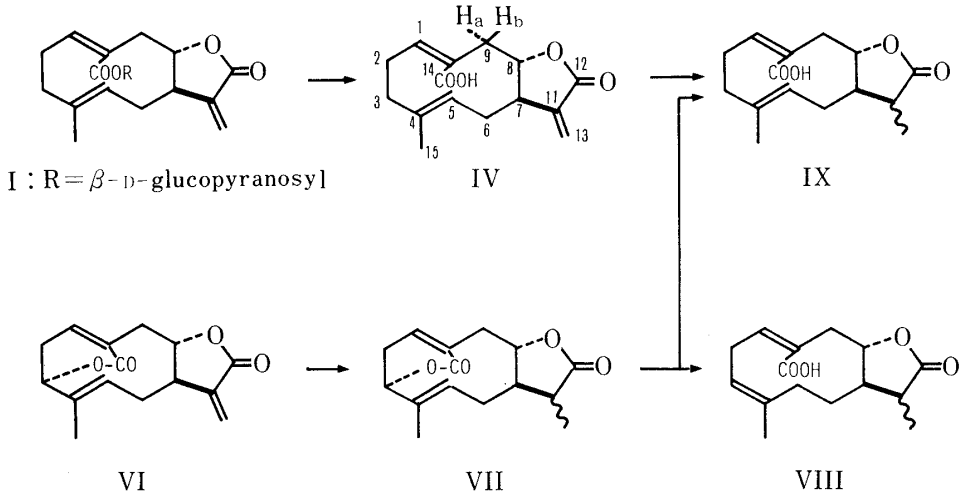
The molecular formula of I was deduced as follows. On acetylation I gave a tetraacetate (III), $[\alpha]_D$ −36.6°, the chemical ionization mass spectrum (CI-MS) (CH_4) of which exhibited the highest mass number at *m/z* 593 ($M^+ + 1$), corresponding to $C_{29}H_{36}O_{13}+H$. Other significant peaks at *m/z* 331, 271, 169 (base peak), and 109 implied the presence of a tetraacetylhexose residue in the molecule III.[3] In the mass spectrum of I, the fragment ion of the highest mass number, *m/z* 262, corresponds to $C_{15}H_{18}O_4$, *i.e.*, the genin moiety resulting from elimination of the hexose unit (180). From these results and the following chemical and spectral proofs (*vide infra*), we deduced the molecular formula of I to be $C_{21}H_{28}O_9$.

In the ^{13}C-nuclear magnetic resonance (NMR) spectrum of I (measured at room temperature) the six carbon atoms of the glucose moiety were readily assignable and the anomeric carbon (G-1′) resonated at δ_C 96.4 ppm, suggesting that a β-glucopyranose links with the genin through an ester bond.[4] As regards the genin part, only 13 of its 15 carbon atoms were observable, though several signals were extremely broadened. On measurement at 70 °C, the appearance of the spectrum was considerably improved. Fifteen carbon signals due to the genin part were clearly observed: six olefinic carbons (>C= ×3, =CH− ×2, $=CH_2$ ×1), two carbonyls, a methine joined to an oxygen atom, four methylene carbons, a methine carbon and a vinylic methyl group.

On acid hydrolysis, I afforded a genin designated here as pertic acid (IV), $C_{15}H_{18}O_4$, mp 152—153 °C (dec.),[5] $[\alpha]_D$ −72.7°, and glucose (identified by thin layer chromatography (TLC)). Pertic acid (IV) showed absorption bands at 3500—2600, 1680 cm^{-1} (carboxylic acid) 1730, and 1620 cm^{-1} (unsaturated lactone) in its infrared (IR) spectrum. It afforded a monomethyl ester (V), $C_{16}H_{20}O_4$, mp 87—88 °C, $[\alpha]_D$ −88.1°, on careful treatment with

diazomethane. Thus, the four oxygen atoms in the molecule IV are accounted for by the presence of a lactone group and a carboxy function.

The ^{1}H-NMR spectrum (at 70 °C) of pertic acid (IV) exhibited two distinctive doublets in lower field at δ_H 5.42 ppm (J=3.0 Hz) and δ_H 6.19 ppm (J=3.0 Hz), corresponding to the olefinic protons of an exo-cyclic methylene group conjugating with a *trans*-fused γ-lactone. Other signals included two olefinic proton signals at δ_H 7.01 ppm (1-H) and 5.10 ppm (5-H), and a slightly broadened vinyl methyl signal at δ_H 1.60 ppm (15-H_3). The spin decoupling (NMDR) experiments on pertic acid (IV) were performed at 70 °C. A proton at δ_H 4.19 ppm (8-H) coupled with three protons resonating at δ_H 3.13 ppm (9-H_b, J=3.5 Hz), 2.68 ppm (9-H_a, J=5.1 Hz) and 2.90 ppm (7-H, J=8.4 Hz). Moreover, allylic couplings were observed between 9-H_b and 1-H (J≒1.5 Hz), and between 15-H_3 and 5-H (J≒1 Hz). Taking into consideration that pertic acid (IV) possesses a carboxy group while pertilide (VI) has a δ-lactone in the molecule, the chemical structure of pertic acid was presumed to be IV (Chart 1).



I : R=β-D-glucopyranosyl IV IX

VI VII VIII

Chart 1

On catalytic hydrogenation, pertilide (VI) afforded an 11,13-dihydro derivative (VII) and a 1(10),3-diene-14-oic acid (VIII), as reported in the previous paper.[2] From the same reaction mixture, another acid (IX) $C_{15}H_{20}O_4$, mp 185—189 °C (dec.), $[\alpha]_D$ +154.5°, was newly isolated as a minor product (yield 14%). This product (IX) showed bands at 3650—2700, and 1690 cm^{-1} due to a carboxy group in its IR spectrum. On the basis of the ^{13}C-NMR spectrum of IX, its carbon system is the same as that of VIII, but it differs from VIII. NMDR experiments showed the absence of a 1,4-diene system in IX. Therefore the structure of IX, isomeric to VIII in double bond locality, was concluded to be IX (Chart 1), resulting from hydrogenolysis of the allylic oxygen-carbon bond of VI without migration of the double bond. When subjected to the same catalytic hydrogenation procedure, IV afforded a 11,13-dihydro derivative, mp 188—190 °C, $[\alpha]_D$ +157.7°, which was, on direct comparison, identical with IX. Consequently, the chemical structure of pertic acid (IV) was established as IV and that of I as β-D-glucopyranosyl [1(10)*Z*,4*E*]-(7*R*,8*S*)-germacra-1(10),4,11(13)-trien-12,8-olide-14-oate, illustrated as I (Chart 1).

The other bitter principle, glucosyl 3α-hydroxypertate (II) was acetylated to a penta-acetate (X), $C_{31}H_{38}O_{15}\cdot H_2O$, mp 123—126 °C (dec.), $[\alpha]_D$ +1.7°, so the molecular formula of II was concluded to be $C_{21}H_{28}O_{10}$. In the ^{13}C-NMR spectrum of II measured at room temperature, six carbon signals due to an ester-β-glucosyl residue were identified.[4] However, the signals of the genin moiety were indistinct.

On acid hydrolysis, II afforded glucose and a genin named 3α-hydroxypertic acid (XI), $C_{15}H_{18}O_5$, mp 250—252 °C (dec.),[5] $[\alpha]_D$ +1.6°. The latter was also obtained on enzymatic hydrolysis using crude hesperidinase. Compound XI showed absorptions in the IR spectrum assignable to a carboxy group (3300—2500, 1690 cm^{-1}), and yielded a methyl ester (XII), $C_{16}H_{20}O_5$, mp 202—204 °C (dec.),[5] $[\alpha]_D$ −4.5° on diazomethane treatment. The ester (XII) has a hydroxyl group (3500—3200 cm^{-1}) in addition to an ester function and an α,β-unsaturated γ-lactone group (1720, 1700, 1640, 1260, and 1240 cm^{-1}). The ^{1}H-NMR spectrum (in d_5-pyridine) of XI showed indistinguishable broad signals at room temperature, except for the following signals: the *exo*-cyclic methylene protons conjugating with a γ-lactone as two doublets at δ_H 6.4 ppm (J=3 Hz) and δ_H 5.7 ppm (J=2 Hz), two olefinic protons at δ_H 4.9 and δ_H 5.5 ppm, and a vinylic methyl signal δ_H 1.9 ppm. Variable-temperature (−30—110 °C) ^{1}H-NMR spectra of XI were measured, but the broad signals could not be substantially sharpened.

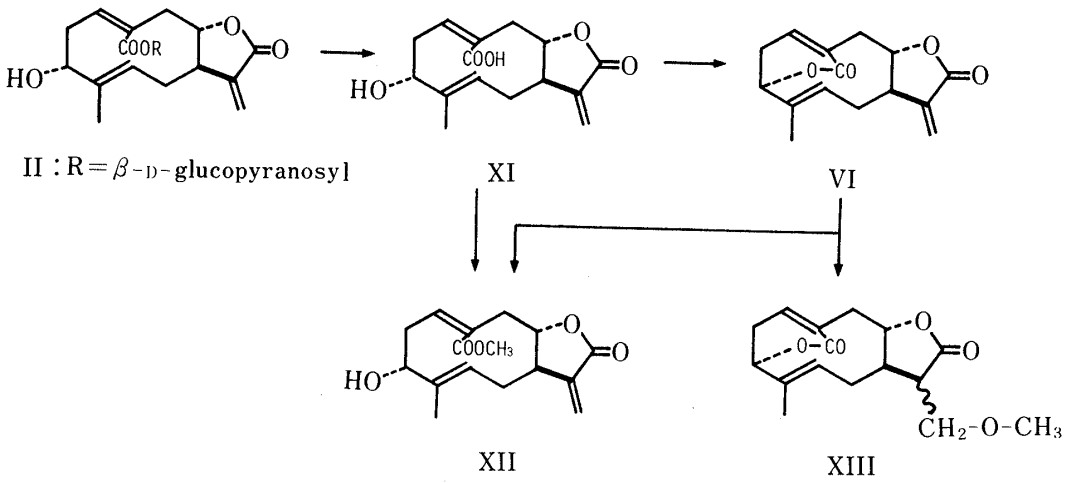


Chart 2

NMR spectroscopy is the most commonly-used technique in the structure elucidation of sesquiterpene lactones, but it unfortunately gave little information regarding the structure of XI. On the basis of the limited information from the ^{1}H-NMR spectrum and the molecular formula, we presumed XI to be a derivative of pertilide (VI) co-occurring in the same plant. In fact, XI could be identified as follows. Treatment of pertilide (VI) with potassium carbonate in aqueous methanol at room temperature gave two products: a methoxymethyl γ-lactone (XIII), $C_{16}H_{20}O_5$, mp 117—119 °C, $[\alpha]_D$ −42.3°, and a methyl ester mp 202—204 °C,[5] $[\alpha]_D$ −4.9°. The latter proved to be identical with XII obtained from XI. On the other hand, treatment of XI with *p*-bromobenzoyl chloride in pyridine provided pertilide (VI) as a main product. Consequently, these chemical correlations confirmed that the chemical structure of 3α-hydroxypertic acid is XI, and that of II is β-D-glucopyranosyl [1(10)*Z*, 4*E*]-(3*R*, 7*R*, 8*S*)-3-hydroxygermacra-1(10),4,11(13)-trien-12,8-olide-14-oate as illustrated in Chart 2.

The NMR spectra of III, XI, and some of their derivatives showed indistinct broad signals at room temperature as described above. That suggests the occurrence of inversion of the ten-membered ring, since several germacranolides with the α,β-unsaturated γ-lactone closure to C-8 have more flexible ten-membered medium rings than those with the lactone closure to C-6, and are known to exist in more than two conformations in solution.[6]

Experimental

All melting points were taken on a Shimadzu micro melting point determination apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 automatic polarimeter in a 1 dm tube. NMR spectra were recorded with a JEOL FX-100 spectrometer with tetramethylsilane as an internal standard, and were measured at room temperature unless otherwise stated. Chemical shifts are given on the δ scale (ppm) and coupling constants (J values) are expressed in Hz. The following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. MS were recorded with a JEOL JMS-D 300 machine. IR spectra were obtained with a Shimadzu IR-400 and a Hitachi IR-215 spectrometer. TLC was performed on Kiesel gel 60 F_{254} pre-coated plates (Merck) and detection was carried out by UV absorption measurement at 254 nm and by spraying 10% H_2SO_4 followed by heating.

Extraction and Separation——The leaves of *Pertya glabrescens* were collected in Agano, Saitama prefecture, Japan, in May 1981. The air-dried and powdered leaves (3.2 kg) were extracted six times with MeOH for 3 h each under reflux. The total MeOH solution was concentrated under reduced pressure as far as possible. The residue (610 g) was dissolved again in MeOH (1.2 l), and water (3.2 l) was added to the MeOH solution. The solution was allowed to stand at room temperature for a day, and then the precipitated matter was removed by filtration. The filtrate was concentrated under reduced pressure in order to evaporate off the MeOH present in it. The residual water solution (3 l) was successively extracted, once with hexane (1.8 l), and four times with EtOAc (total 10 l) in a separatory funnel. The hexane and the EtOAc extractives, after removal of the solvent, weighed 2 g and 116 g, respectively.

The aqueous layer was concentrated under reduced pressure to evaporate off the EtOAc present in it, and the concentrate was applied to a column of polyamide (350 g) (polyamide C-100 from Wako Pure Chemical Industries, Ltd.). The column was eluted with water (4 l) and the total eluate was applied to a column of Amberlite XAD-2 (1.1 kg). The column was washed with water (4 l), and then eluted with MeOH (3.6 l). The residue (31 g) obtained after concentration of the eluate was chromatographed over silica gel (600 g) and divided into the following five fractions (Fr.). Fr. 1 EtOAc (3 l) 1.1 g, Fr. 2 EtOAc–MeOH (9 : 1, 4.5 l) 1.5 g, Fr. 3 EtOAc–MeOH (4 : 1, 4 l) 5.4 g, Fr. 4 EtOAc–MeOH (1 : 1, 1.5 l) 13 g, Fr. 5 MeOH (1.5 l) 5 g.

Fr. 3 (5.4 g) was chromatographed on 10% $AgNO_3$-coated silica gel (150 g). The eluate with MeOH–$CHCl_3$ (1 : 5) was concentrated under reduced pressure, and the residue (3.5 g) was dissolved in water (15 ml). The solution was then applied to a column of Amberlite XAD-2 (50 g) for desalting. After being washed with water (200 ml), the column was eluted with MeOH (100 ml). The eluate was concentrated under reduced pressure, affording a gummy residue (2.9 g), which was rechromatographed on silica gel (70 g). Elution with $CHCl_3$–MeOH (9 : 1) afforded I as a colorless amorphous powder (2.2 g). $[\alpha]_D^{25}$ $-48.6°$ ($c=0.5$, EtOH). MS m/z: 262 ($M^+ -$(glucose + H_2O)), 244, 216. NMR (C_5D_5N, 70 °C) δ_H: 1.51 (3H, br s, 15-H_3), 4.96 (1H, br dd, $J=7$, $J=9$, 5-H), 5.39 (1H, d, $J=2.9$, 13-H_a), 6.14 (1H, d, $J=3.2$, 13-H_b), 6.29 (1H, d, $J=7.6$, G-1′-H), 7.17 (1H, br t, $J=10$, 1-H). δ_C: 18.3 (CH_3–), 28.6, 29.3, 33.8, 36.1 (–CH_2–), 46.5 (>CH–), 62.5 (C-6′ of the glucosyl residue (G-6′)), 71.3 (G-4′), 74.0 (G-2′), 78.2 (G-3′ or G-5′), 78.8 (G-5′ or G-3′), 83.7 (>CH–O–), 96.4 (G-1′), 119.0 (=CH_2), 122.2, 143.7 (–CH=), 166.1, 169.3 (>C=O), 131.3, 135.6, 140.7 (>C=). Further elution of the 10% $AgNO_3$-coated silica gel column with the same solvent afforded a syrupy material (620 mg), which was desalted by the same procedure as used for I. The chemical structure of this component is under investigation.

Fr. 4 (13 g) was chromatographed twice on silica gel with $CHCl_3$–MeOH–H_2O (280 : 60 : 1). The eluate (3.1 g) was further purified by preparative TLC (solvent: $CHCl_3$–EtOAc–MeOH–HCOOH (2 : 4 : 1 : 1)) and II was obtained as a colorless amorphous powder (one spot on TLC). $[\alpha]_D^{17}$ $-10.9°$ ($c=2.0$, pyridine). NMR (C_5D_5N) δ_H: 1.9 (3H, br s, 15-H_3), 5.7 (1H, d, 13-H_a), 6.4 (1H, d, 13-H_b). δ_C: 62.3 (G-6′), 71.0 (G-4′), 74.0 (G-2′), 78.8 (G-3′ or G-5′), 79.4 (G-5′ or G-3′), 96.1 (G-1′).

Acetylation of I——I (100 mg) was acetylated overnight with Ac_2O (1 ml) in pyridine (1 ml) at room temperature. After addition of ice and water to it, the reaction mixture was extracted with EtOAc (10 ml). The EtOAc layer was washed with water and dried over anhydrous Na_2SO_4. The product obtained after concentration of the EtOAc solution was applied to a column of silica gel (7 g) and eluted with benzene–EtOAc (9 : 1), providing a tetraacetate (III) (120 mg) as a colorless syrupy material. $[\alpha]_D^{24}$ $-36.6°$ ($c=1.2$, $CHCl_3$). CI-MS (CH_4) m/z: 593 ($M^+ + H$), 533, 437, 331, 271, 229, 211, 169 (base peak), 109. NMR ($CDCl_3$) δ_H: 1.67 (3H, br s, 15-H_3), 2.00, 2.02, 2.03, 2.09 (3H, s, CH_3COO–), 5.51 (1H, d, $J=2.9$, 13-H_a), 5.80 (1H, d, $J=8$, G-1′-H), 6.20 (1H, d, $J=3.2$, 13-H_b), 6.97 (1H, br t, $J=8$, 1-H).

Acid Hydrolysis of I——A solution of I (1.5 g) in 5% H_2SO_4 in 50% MeOH (110 ml) was refluxed for 1 h. The MeOH was removed *in vacuo*. The resulting solution was diluted with H_2O, and extracted with EtOAc (30 ml × 4). The total EtOAc solution was washed with H_2O, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue (0.8 g) was chromatographed over silica gel (30 g). Elution with $CHCl_3$–MeOH–HCOOH (400 : 6 : 1) gave pertic acid (0.4 g). Pertic acid (IV), colorless needles from acetone–isopropyl ether, mp 152—153 °C (dec.),[5] $[\alpha]_D^{19}$ $-72.7°$ ($c=0.4$, $CHCl_3$). *Anal.* Calcd for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92. Found: C, 68.66; H, 7.07. MS m/z: 262 (M^+), 244, 216. NMR (C_5D_5N, 70 °C) δ_H: 1.60 (3H, br s, 15-H_3), 2.68 (1H, dd, $J_{8,9a}=5.1$, $J_{9a,9b}=14.7$, 9-

H_a), 2.90 (1H, m, 7-H), 3.13 (1H, ddd, $J_{1,9b} \fallingdotseq 1.5$, $J_{8,9b}=3.5$, $J_{9a,9b}=14.7$, 9-H_b), 4.19 (1H, ddd, $J_{7,8}=8.4$, $J_{8,9a}=5.1$, $J_{8,9b}=3.5$, 8-H), 5.10 (1H, br dd, $J_{5,6a}=6$, $J_{5,6b}=10$, 5-H), 5.42 (1H, d, $J_{7,13a}=3.0$, 13-H_a), 6.19 (1H, d, $J_{7,13b}=3.0$, 13-H_b), 7.01 (1H, br t, $J_{1,2}=8.5$, $J_{1,9b} \fallingdotseq 1.5$, 1-H). δ_C: 18.7 (CH_3–), 29.1, 29.8, 34.7, 36.5 (–CH_2–), 47.0 (>CH–), 84.4 (>CH–O–), 119.2 (=CH_2), 122.2, 141.5 (=CH–), 132.9, 136.2, 141.4 (>C=), 170.0 (×2) (>C=O).

One-tenth of the water-soluble fraction of the above acid hydrolysate was passed through a column of Amberlite BM-3, and concentrated to a small volume. Glucose was detected on TLC (Cellulose F_{254} (Merck), BuOH–AcOH–H_2O (6:1:2), coloring with aniline-H_3PO_4).

Methylation of IV——Diazomethane in ether was added dropwise to a solution of IV (0.5 g) in EtOH (70 ml) under stirring at room temperature until TLC showed a single spot of the product. The reaction mixture was concentrated to dryness. The residue was chromatographed on silica gel using benzene–EtOAc (19:1) as the solvent. The eluate (320 mg) was recrystallized from acetone–isopropyl ether, furnishing a methyl ester (V) as colorless needles. mp 87—88 °C, $[\alpha]_D^{17}$ −88.1° ($c=0.5$, $CHCl_3$). High resolution MS *m/z*: Calcd for $C_{16}H_{20}O_4$ (M^+) 276.136. Found 276.138. NMR ($CDCl_3$) δ_H: 3.78 (3H, s, –$COOCH_3$).

Hydrogenation of IV——A solution of IV (50 mg) in 5 ml of EtOAc–EtOH (1:1) was hydrogenated for 3 h under atmospheric pressure in the presence of 10% Pd-C (45 mg). After removal of the catalyst by filtration, the solvent was evaporated off *in vacuo*. The residue was chromatographed over silica gel. Elution with benzene–EtOAc–AcOH (50:20:0.3) afforded a dihydro derivative (IX) as colorless needles (36 mg). mp 188—190 °C (dec.). $[\alpha]_D^{19}$ +157.7° ($c=0.1$, $CHCl_3$). High resolution MS *m/z*: Calcd for $C_{15}H_{20}O_4$ (M^+) 264.136. Found 264.137. NMR ($CDCl_3$) δ_H: 1.11 (3H, d, $J=6.8$, 13-H_3). δ_C: 13.15, 22.49 (CH_3–), 28.36, 28.47, 29.18 (×2) (–CH_2–), 41.86, 42.51 (>CH–), 85.01 (>CH–O–), 120.94, 143.37 (=CH–), 126.70, 135.86 (>C=), 172.54, 177.89 (>C=O).

Hydrogenation of VI——A solution of VI (210 mg) in 20 ml of EtOAc–EtOH (1:1) was hydrogenated for 3 h at atmospheric pressure in the presence of 10% Pd-C (120 mg). The solution was then processed as described in the previous paper.[2] The acidic reaction mixture (133 mg) was chromatographed over silica gel with benzene–dioxane–HCOOH (100:7:1) to afford an acid (VIII) as colorless needles (43 mg). mp 211—212 °C (dec.) (from acetone–isopropyl ether).

Further elution with the same solvents afforded colorless needles (30 mg). mp 185—189 °C (dec.) (acetone–isopropyl ether), $[\alpha]_D^{18}$ +154.5° ($c=0.1$, $CHCl_3$); this product was identical with IX on the basis of TLC, IR, NMR, and MS comparisons.

Acetylation of II——II (35 mg) was acetylated with Ac_2O (0.5 ml) in pyridine (0.3 ml). After work-up in the usual manner, the product was passed through a silica gel column (3 g) (eluent, benzene–EtOAc (5:2)). After recrystallization from EtOH, a pentaacetate (X) was obtained as colorless needles (28 mg). mp 123—126 °C (dec.). $[\alpha]_D^{19}$ +1.7° ($c=0.5$, $CHCl_3$). *Anal.* Calcd for $C_{31}H_{38}O_{15} \cdot H_2O$: C, 55.68; H, 6.03. Found: C, 55.38; H, 5.89. CI-MS (NH_3) *m/z*: 668 ($M^+ + NH_4$), 573, 366, 348, 331, 289, 271, 211, 169, 109, 108. NMR ($CDCl_3$) δ_H: 1.5 (3H, br s, 15-H_3), 2.03, 2.04, 2.05, 2.06, 2.09 (3H, s, CH_3COO–), 5.08 (1H, d, $J=7$, G-1′-H).

Acid Hydrolysis of II——A solution of II (0.5 g) in 5% H_2SO_4 in 50% MeOH (30 ml) was stirred for 1 h at 70 °C. The MeOH was removed *in vacuo*. The resulting solution was diluted with H_2O and extracted with EtOAc (5 ml × 4). The total EtOAc solution was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated. After recrystallization from acetone–isopropyl ether, 3α-hydroxypertic acid (XI) was obtained as colorless needles (150 mg). mp 250—252 °C (dec.).[5] $[\alpha]_{589}^{19}$ +1.6°, $[\alpha]_{577}^{19}$ +0.3°, $[\alpha]_{546}^{19}$ −1.5°, $[\alpha]_{435}^{19}$ −28.3°, $[\alpha]_{365}^{19}$ −115.6° ($c=1.0$, $CHCl_3$). *Anal.* Calcd for $C_{15}H_{18}O_5$: C, 64.73; H, 6.52. Found: C, 64.68; H, 6.63. MS *m/z*: 278 (M^+), 260 ($M^+ - H_2O$), 242.

The water-soluble fraction was processed in the same manner as I, and glucose was identified.

Methylation of XI——Diazomethane in ether was added to a solution of XI (0.5 g) in EtOH (70 ml) and the reaction mixture was worked up in the same way as in the case of the methylation of IV. The product (0.5 g) was recrystallized from acetone–EtOH and gave a methyl ester (XII) as colorless needles. mp 202—204 °C (dec.)[5] $[\alpha]_D^{20}$ −4.5° ($c=0.3$, EtOH). High resolution MS *m/z*: Calcd for $C_{16}H_{20}O_5$ (M^+) 292.131. Found: 292.131.

Treatment of XI with *p*-Bromobenzoyl Chloride——*p*-Bromobenzoyl chloride (165 mg) was added to a solution of XI (150 mg) in pyridine (3 ml), and the mixture was allowed to stand overnight at room temperature. After addition of ice and water, the reaction mixture was extracted with EtOAc (7 ml × 5). The total EtOAc layer was washed with 1 N HCl, 2 N Na_2CO_3 and then water, and dried over anhydrous Na_2SO_4. The products (93 mg) obtained after concentration of EtOAc solution were applied to a column of silica gel (5 g). Elution with benzene–EtOAc (4:1) provided pertilide (VI) (55.7 mg). mp 185—187 °C (dec.)[5] (acetone–isopropyl ether). $[\alpha]_D^{16}$ +1.3° ($c=0.6$, $CHCl_3$) which was identical with an authentic sample of VI on the basis of TLC, IR, ^{1}H-NMR, and MS comparisons.

Further elution with the same solvent afforded colorless needles (3.6 mg) (acetone–isopropyl ether). The chemical strucrure of this product is under investigation.

Methanolysis of Pertilide (VI)——VI (400 mg) was dissolved in a mixture of MeOH (88 ml) and aqueous solution (12 ml) saturated with K_2CO_3. The reaction mixture was allowed to stand for 12 h at room temperature with stirring. After addition of water (30 ml), the reaction mixture was concentrated under reduced pressure in order to evaporate off the MeOH present in it. The residual aqueous solution was extracted with EtOAc (20 ml × 4). The EtOAc layers were combined and concentrated. The residue was applied to a column of silica gel (75 g). Elution with benzene–

EtOAc (4 : 1) afforded a methyl ether (XIII) as colorless needles (100 mg) from acetone–isopropyl ether. mp 117—119 °C (dec.). $[\alpha]_D^{18}$ −42.3° (c=0.6, $CHCl_3$). *Anal.* Calcd for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90. Found: C, 65.81; H, 6.99. NMR ($CDCl_3$) δ_H: 3.36 (3H, s, CH_3–O–). Elution of the silica gel column with benzene–EtOAc (3 : 1) afforded a methyl ester (XII) as colorless needles (68 mg). mp 202—204 °C (dec.).[5] $[\alpha]_D^{20}$ −4.9° (c=0.5, $CHCl_3$). This product was identical with XII derived from 3α-hydroxypertic acid (XI) on the basis of TLC, IR, ^{1}H-NMR, and MS comparisons.

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