[Chem. Pharm. Bull. 17(7)1438—1443(1969)]

UDC 581.19 : 582.717 : 547.597.02

Studies on the Constituents of Saxifragaceous Plants. I. Two Triterpene Acids of *Peltoboykinia tellimoides* (MAXIM.) HARA

MASAHIRO NAGAI, KAZUO IZAWA
and TAKAO INOUE

Hoshi College of Pharmacy[1]

(Received December 26, 1968)

From the rhizoma of *Peltoboykinia tellimoides* (MAXIM.) HARA, two triterpene acids were isolated. One of them, a new triterpene, named β-peltoboykinolic acid (I), mp 220—222° (decomp.), $[\alpha]_D^{24}+114°$, has been established to be olean-12-ene-3β-ol-27-oic acid and another, named α-peltoboykinolic acid (II), mp 234—236°, $[\alpha]_D^{17}+121°$, to be urs-12-ene-3β-ol-27-oic acid, which is identical with a compound derived from quinovic acid by L. Ruzicka, *et al.* in 1946.

These are the first examples of naturally occurring triterpene acids with the only carboxyl group at C–27 of ursane and oleanane types.

Peltoboykinia tellimoides (MAXIM.) HARA (Japanese mane "Yawataso") is a perennial herb of the family Saxifragaceae which is distributed on the pacific side of central Japan. In 1946 Tatsumaki, *et al.*[2] reported that the rhizoma of the plant contain bergenin which is often found in Saxifragaceae.

The two triterpene acids have now been isolated as the constituents of the rhizoma and their structures have been established by their chemical correlation with quinovic and cincholic acids.

The alkaline methanol soluble part of the ethereal extracts of the rhizoma was washed with petroleum ether and crystallized from aqueous methanol to give crude crystals of triterpenes. This crystalline mixture consisted mainly of two compounds which were chromatographically separated to afford a compound, $C_{30}H_{48}O_3$, mp 234—236° (decomp.) and another, $C_{30}H_{48}O_3$, mp 220—222° (decomp.). The content of the latter compound, named β-peltoboykinolic acid (I), was higher than that of the former, named α-peltoboykinolic acid (II), in the crude crystalline mixture. β-Peltoboykinolic acid showed negative Liebermann–Burchard reaction and gave the absorption bands of hydroxyl and carboxyl groups in the infrared (IR) spectrum. The acid (I) yielded a monoacetate (III), a monomethyl ester (IV) and a methyl ester acetate (V). In nuclear magnetic resonance (NMR) spectrum, V showed the following proton signals: τ 9.17 (15H, singlet), 9.03 (3H, singlet), 8.90 (3H, singlet), 7.97 (3H, singlet, $C\underline{H}_3COO$–), 6.34 (3H, singlet, –$COOC\underline{H}_3$), 5.53 (1H, triplet, $J=7.5$ cps), 4.42 (one olefinic proton, triplet, $J=2.3$ cps).

From these data, β-peltoboykinolic acid (I) was confirmed to be a monohydroxy–monocarboxylic acid with a trisubstituted double bond. The existence of the double bond was consistent with positive tetranitromethane tests of IV and V.

Oxidation of methyl ester (IV) with chromic acid gave a ketone (VI) which showed a positive Zimmermann color reaction. In the above NMR spectrum of V, one proton signal centered at τ 5.53 is analogous to the proton with α-configuration linking to C–3 bearing acetoxy group in usual cyclic triterpenes in its τ value and coupling pattern. These chemical and spectral evidence suggested that the hydroxy group of I is located at C–3 and orientated in β.

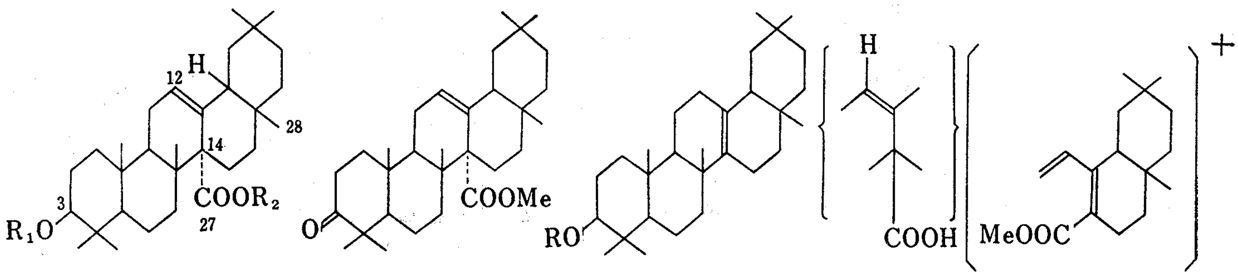
---

1) Location: *Ebara 2-chome, Shinagawa-ku, Tokyo.*

2) T. Tatsumaki and T. Inoue, *Sigenkagaku Kenkyusho Iho*, No. 10, 13 (1946) [*C. A.*, 42, 3034*h* (1948)].

β-Peltoboykinolic acid (I) bubbled actively by heating it at 225—230°, varying to a pyrocompound (VII), which on acetylation gave an acetate (VIII). The mass spectrum of VII showed an abundant molecular peak at *m/e* 412. These findings proved that VII was produced by loss of carbon dioxide from I. In NMR spectra of VII and VIII, no olefinic proton was observed but positive tetranitromethane test of VIII showed the existence of a double bond. The easy elimination of carbon dioxide and the concomitant migration of double bond from a "trisubstituted" to a "tetrasubstituted" can be reasonably explained by presuming the presence of a β, γ-unsaturated carboxylic acid system such as IX in the structure of the acid (I).

The mass spectrum of the methyl ester (IV) afforded the molecular peak at *m/e* 470 ($C_{31}H_{50}O_3$) and base peak at 262 ($C_{17}H_{26}O_2$) (X). The latter peak suggested that IV has a Δ^{12}-oleanene or -ursene skelton which are characterized by a peak resultant from retro-Diels–Alder fragmentation in mass spectroscopy.[3]



I : $R_1=R_2=H$
III : $R_1=Ac, R_2=H$
IV : $R_1=H, R_2=Me$
V : $R_1=Ac, R_2=Me$

VI

VII : R=H
VIII : R=Ac

IX

X

Chart 1

Considering that seven C–methyl signals of V and VII were observed uncoupled in their NMR spectra, the structure of β-peltoboykinolic acid was assumed as I in Chart 1.

Cinchona bark contains glycosides, the aglycones of which are well-known triterpenes, quinovic acid (XI)[4] and cincholic acid (XII). The latter acid was isolated from the bark and its structure was clarified by Tschesche, *et al.* in 1963.[5]

Chemical correlation of β-peltoboykinolic acid (I) with cincholic acid (XII) was achieved as follows. Dimethyl cincholate (XIII) was converted with sodium hydride, dimethyl sulfoxide and methyl iodide into the methyl ether (XIV),[6,7] which was partially reduced with $LiAlH_4$ to give a carbinol (XV). Carbomethoxy group at C–27 of XIV is sterically so hindered that it is not affected to the reduction.[5] Tosylate (XVI) of XV in tetrahydrofuran was refluxed with $LiAlH_4$ to yield the carbinol (XV), methoxy methyl ester (XVII) and methoxy carboxylic acid (XVIII). The formation of XVIII showed the hydrolysis of the C–27 ester group of XVII, which progressed in proportion to prolonged reaction times. It seems that the ester group was reduced with $LiAlH_4$ probably to form the acid (XVIII) and methane. To reconvert XVIII to XVII the mixture of the reduction products was treated with diazomethane and chromatographed on silica gel to afford a crystalline methoxy methyl ester (XVII), which was completely identical with methyl ether (XVII) derived from methyl β-peltoboykinolate (IV) in mixed melting point, IR spectra, thin-layer chromatography and

---

[3] H. Budzikiewicz, C. Djerassi and D.H. Williams, "Structural Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden–Day, Inc., San Francisco, 1964, p. 122.
[4] J.L. Simonsen and W.C. Ross, "The Terpenes," Vol. V, Cambridge Univ. Press, London, 1957, p. 75.
[5] R. Tschesche, I. Duphorn and G. Snatzke, *Ann. Chem.*, **667**, 151 (1963).
[6] S. Shibata, T. Ando and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **14**, 1157 (1966).
[7] S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

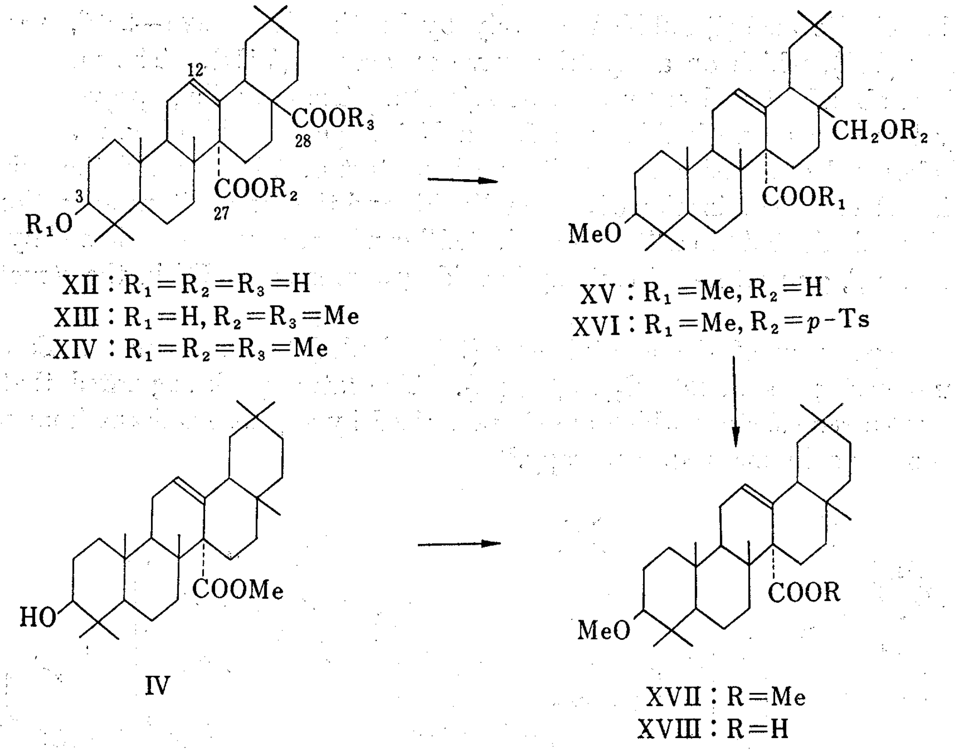


Chart 2

gas–liquid chromatography. Therefore β-peltoboykinolic acid (I) was concluded to be olean-12-ene-3β-ol-27-oic acid.

α-Peltoboykinolic acid (II) also decomposed at its melting point similarly to β-compound (I) and gave methyl ester (XIX) and methyl ester acetate (XX). Ruzicka, *et al.*,[8] on their research of quinovic acid (XI), reported to obtain a hydroxy acid and its methyl ester from XI through ten steps of reactions, whose structures were represented by II and XIX respectively.

The identity of the methyl ester (XIX) obtained from α-peltoboykinolic acid (II) with the authentic sample of XIX was established by mixed melting point and comparison of their thin–layer chromatograms. Consequently α-peltoboykinolic acid (II) was confirmed to be urs-12-ene-3β-ol-27-oic acid. It is of interest that the compound which was derived from quinovic acid in 1946, was now found to occur in nature.

Triterpene acids of oleanane and ursane types have often carboxyl groups at C–28 but α- and β-peltoboykinolic acids (II and I respectively) are the first examples of naturally occurring monohydroxy–monocarboxylic acids of these types with the only carboxyl group not at C–28 but at C–27.

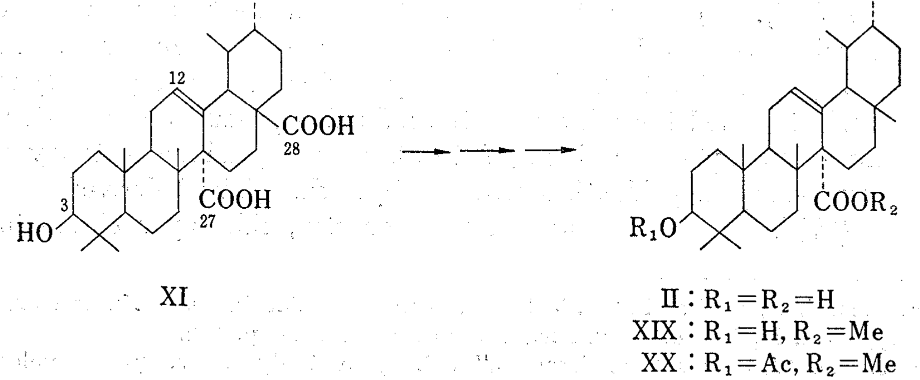


Chart 3

---

[8] L. Ruzicka, S. Szpilfogel and O. Jeger, *Helv. Chim. Acta*, 29, 1520 (1946).

Experimental

All melting points were determined on a micro hot–stage and are uncorrected. IR spectra were recorded with Koken–DS–301 Spectrometer. NMR spectra were measured in $CDCl_3$ using $(CH_3)_4Si$ as an internal reference with Hitachi Perkin–Elmer R–20 High Resolution NMR Spectrometer. Optical rotations were measured with an accuracy ± 0.05° in $CHCl_3$ as solvent unless otherwise stated. Gas–liquid chromatography was carried out with Shimadzu Gas Chromagraph GC–4APTF. Silica gel G was used for thin–layer chromatography and the detection was carried out by heating on a hot plate after spraying 10% H_2SO_4.

Isolation of α- and β-Peltoboykinolic Acids——*Peltoboykinia tellimoides* was collected from Tochigi Prefecture, Japan, in July of 1967. Air–dried, ground rhizoma (3 kg) were extracted three times with ether. The combined ether solution (6 liter) dried over anhyd. Na_2SO_4, was concentrated to dryness to yield brown residue (about 48 g), which was extracted with hot methanolic 2N NaOH solution (1 liter). The alkaline solution was filtered, concentrated *in vacuo* to 1/4 volume and acidified with 2N aq. HCl (1.2 liter) under cooling and allowed to stand overnight.

The resultant precipitate was collected by filtration, dried *in vacuo* and extracted for 1.5 hr with hot petroleum ether (bp 45—60°, 450 ml). After cooling the precipitate was collected and dissolved in hot aq. MeOH, then allowed to stand overnight in a refrigerator. The substance separated was collected and recrystallized from aq. MeOH to give crude crystals (2.5 g, yield: about 0.08%). The crystals were chromatographed on silica gel by using benzene, then benzene–ether (9:1) as solvents. Successive elution with benzene–ether (9:1) afforded initially α-peltoboykinolic acid and subsequently β-peltoboykinolic acid, which were recrystallized from MeOH to purify them. Yield of α- and β-peltoboykinolic acids: 0.5 g and 1.5 g respectively. Otherwise pure β-peltoboykinolic acid can be obtained by repeated recrystallization of the crude crystals from MeOH without chromatographic separation. On thin–layer chromatograms α- and β-peltoboykinolic acids give clearly separated spots with $CHCl_3$–EtOAc (2:1) as solvent.

β-Peltoboykinolic Acid (I): Colorless needles, mp 220—222° (decomp.), $[\alpha]_D^{25}$ +114° (c=1.6). *Anal.* Calcd. for $C_{30}H_{48}O_3$ (456.68): C, 78.89; H, 10.59. Found: C, 78.88; H, 10.88. Mol. wt.: 475 (Rast). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1686 (COOH).

α-Peltoboykinolic Acid (II): Colorless needles, mp 234—236° (decomp.), $[\alpha]_D^{17}$ +121° (c=0.91). *Anal.* Calcd. for $C_{30}H_{48}O_3$: C, 78.89; H, 10.59. Found: C, 78.91; H, 10.78 (lit.,[8] mp 235—236.5°, $[\alpha]_D$ +143° (c= 0.185)). IR ν_{max}^{KBr} cm^{-1}: 3370 (OH), 1730, 1680 (COOH).

Acetate of β-Peltoboykinolic Acid (I)——A mixture of I (0.3 g), pyridine (4 ml) and Ac_2O (2 ml) was allowed to stand overnight at room temperature and poured into dil. HCl. The precipitate was collected and washed with water. Recrystallization from MeOH gave colorless needles of acetate (III, 0.25 g), mp 219—222° (decomp.), $[\alpha]_D^{25}$ +111° (c=1.2). *Anal.* Calcd. for $C_{32}H_{50}O_4$: C, 77.06; H, 10.11. Found: C, 77.24; H, 10.17.

Methyl Ester of β-Peltoboykinolic Acid (I)——A solution of I (0.43 g) in ether was treated with CH_2N_2 in ether for 15 hr to give methyl ester (IV), which was recrystallized from MeOH as colorless prisms (0.35 g), mp 211—213°, $[\alpha]_D^{27}$ +137° (c=1.2). *Anal.* Calcd. for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71. Found: C, 79.31; H, 10.84. Mass spectrum m/e: 470 (M^+), 452 (M^+–H_2O), 411 (M^+–$COOCH_3$), 262 (base peak, ion X). Tetranitromethane test: yellow.

Methyl Ester Acetate of β-Peltoboykinolic Acid (I)——Methyl ester acetate (V) was obtained on methylation of III with CH_2N_2 or acetylation of IV with Ac_2O–pyridine, as colorless plates, mp 205—206.5° (from EtOH), $[\alpha]_D^{25}$ +131° (c=1.1). *Anal.* Calcd. for $C_{33}H_{52}O_4$: C, 77.29; H, 10.22. Found: C, 77.37; H, 10.39. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1740, 1250 (CH_3COO), 1725 ($COOCH_3$). Tetranitromethane test: yellow.

Oxidation of Methyl β-Peltoboykinolate (IV)——A solution of $K_2Cr_2O_7$ (0.1 g) in AcOH (2 ml) was added to methyl ester (IV, 0.2 g) in AcOH (8 ml). After the mixture was allowed to stand overnight at room temperature, water was added to it. The crystalline precipitate was collected, washed with water and recrystallized from MeOH to give a ketone (VI) as colorless cubics, mp 242.5—245°, $[\alpha]_D^{25}$ +176° (c=1.1). *Anal. Calcd.* for $C_{31}H_{48}O_3$: C, 79.43; H, 10.32. Found: C, 79.42; H, 10.50. IR ν_{max}^{KBr} cm^{-1}: 1717 ($COOCH_3$), 1702 (C=O). Zimmermann color test: blue–violet.

Methyl Ether of Methyl β-Peltoboykinolate (IV)——Commercial NaH (55%) (0.11 g) washed with petroleum ether, was warmed with abs. dimethyl sulfoxide (6 ml) at 70° for 2 hr under nitrogen stream. To this reagent thus prepared was added a solution of methylester (IV, 0.26 g) in abs. dimethyl sulfoxide (7 ml) and the mixture was kept at 70° for 20 min under nitrogen stream. CH_3I (0.7 g) was added under cooling in an ice-bath and the reaction mixture was allowed to stand overnight at room temperature.[6,7] The mixture was poured into water and extracted with ether. Ether layer was washed with water, dried and concentrated to dryness. The residue was recrystallized repeatedly from MeOH, affording prisms of methyl ether (XVII, 0.15 g), mp 206—208°, $[\alpha]_D^{27}$ +142° (c=1.1). *Anal.* Calcd. for $C_{32}H_{52}O_3$: C, 79.28; H, 10.81. Found: C, 79.35; H, 10.57. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1721, 1105 ($COOCH_3$). NMR τ: 9.23 (3H, singlet), 9.14 (9H, singlet), 9.04 (6H, singlet), 8.98 (3H, singlet), 6.61 ($OC\underline{H}_3$), 6.30 ($COOC\underline{H}_3$), 4.34 (1H, triplet, J=2.4 cps, olefinic). Mass spectrum m/e: 484 (M^+), 262 (ion X).

Pyrocompound (VII) and Its Acetate (VIII)——A round-bottomed flask containing β-peltoboykinolic acid (I, 0.1 g) was immersed into oil bath preheated at 225—230°. The content melted with bubbling. After heating for 15 min, the oily product crystallized by adding ether–MeOH, and recrystallized two times from ether–MeOH to afford pyrocompound (VII) as colorless needles, mp about 100°. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), no carbonyl absorption. Mass spectrum *m/e*: 412 (M$^+$, base peak, $C_{29}H_{48}O$), 397 (M$^+$–CH_3), 379 (M$^+$–CH_3–H_2O). NMR τ: 9.22 (6H, singlet), 9.14 (9H, singlet), 9.03 (6H, singlet), 6.80 (1H, multiplet).

Pyrocompound (VII) prepared from I (0.3 g) in the above method, was acetylated with Ac_2O (2 ml) and pyridine (4 ml). Working up in the usual way, repeated recrystallization from EtOH gave an acetate (VIII) as colorless plates, mp 182—185°, $[\alpha]_D^{26}$ $-25°$ (c=2.2). *Anal.* Calcd. for $C_{31}H_{50}O_2$: C, 81.88; H, 11.08. Found: C, 81.77; H, 11.30. NMR τ: 8.00 (3H, $C\underline{H}_3COO$), 5.52 (1H, quartet, J=6.1 and 9.7 cps). Tetranitromethane test: yellow.

Methyl Ester of α-Peltoboykinolic Acid (II)——II (0.1 g) was esterified with CH_2N_2 in the usual manner to obtain methyl ester (XIX, 0.09 g) as colorless prisms, mp 193—195° (from MeOH), $[\alpha]_D^{26}$ $+132°$ (c=1.0). (lit.,[8] mp 193.5—194.5°, $[\alpha]_D$ $+132°$ (c=0.94)). *Anal.* Calcd. for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71. Found: C, 79.39; H, 10.78. Mixed melting point of XIX with the authentic sample sent from Prof. O. Jeger showed no depression. Thin-layer chromatograms of both samples gave also their identity by using $CHCl_3$–ether (10:1) as solvent.

Methyl Ester Acetate of α-Peltoboykinolic Acid (II)——A mixture of II (0.2 g), Ac_2O (2 ml) and pyridine (4 ml) was allowed to stand overnight at room temperature and worked up in the usual manner. Resultant acetate was successively esterified with CH_2N_2 in ether to afford crude methyl ester acetate, which was filtered through a small amount of Al_2O_3 with benzene. On recrystallization from MeOH methyl ester acetate (XX) was obtained as fine colorless needles, mp 178—180°, $[\alpha]_D^{26}$ $+118°$ (c=1.1). (lit.,[8] mp 178—179°, $[\alpha]_D$ $+123°$ (c=1.2)).

Isolation of Dimethyl Cincholate (XIII) from *Cinchona* Barks——Aglycone mixture of quinovic acid (XII) and cincholic acid (XIII) were prepared from *cinchona* barks (5 kg) according to the method described by Tschesche, *et al.*[5] Under magnetical stirring, a solution of CH_2N_2 in ether was added portionwise into the aglycone mixture suspended in ether until almost all the solids disappeared and still slightly yellow color of CH_2N_2 was maintained. The crude methyl esters were chromatographed on Al_2O_3. Fractions eluted with benzene and benzene–ether afforded mixture of dimethyl quinovate and dimethyl cincholate, which were recrystallized several times from MeOH to obtain pure dimethyl cincholate (XIII, 1.2 g) on the basis of the property of easier crystallization than that of dimethyl quinovate. XIII, mp 212—214°, $[\alpha]_D^{27}$ $+121°$ (c=1.1, pyridine). (lit.,[5] mp 213—215°, $[\alpha]_D$ $+114°$ (pyridine)).

Methyl Ether of Dimethyl Cincholate (XIII)——The mixture of commercial NaH (55%) (0.13 g) washed with petroleum ether and abs. dimethyl sulfoxide (5 ml) was warmed at 70—75° for 3 hr. To thus prepared reagent a solution of XIII (0.40 g) in abs. dimethyl sulfoxide (8 ml) was added, the mixture was kept at 70—75° for 1 hr under nitrogen stream, to which CH_3I (0.31 g) was added under cooling in a ice-bath, and then the reaction mixture was allowed to stand overnight at room temperature.[6,7] The mixture was poured into water and extracted three times with benzene. The combined benzene layer was washed with water, dried over anhyd. Na_2SO_4 and concentrated *in vacuo* to a small volume. The solution in benzene was chromatographed on Al_2O_3 (grade II, 30 g). Fraction eluted with benzene gave methyl ether (XIV, 0.14 g), mp 202—204° (from MeOH), $[\alpha]_D^{27}$ $+131°$ (c=1.3). *Anal.* Calcd. for $C_{33}H_{52}O_5$: C, 74.96; H, 9.91. Found: C, 74.85; H, 9.94. IR ν_{max}^{KBr} cm^{-1}: 1725 ($COOCH_3$). Eluate with benzene–EtOH regenerated dimethyl cincholate (XIII).

Partial Reduction of Methyl Ether (XIV)——A mixture of XIV (0.25 g), abs. tetrahydrofuran (5 ml) and $LiAlH_4$ (0.1 g) was kept at 50° on oil bath for 2 hr. To the reaction mixture water, then 2N HCl were added, and the product was extracted with ether. The ether layer was washed with 2N NaOH, then water and dried over anhyd. Na_2SO_4. Ether was evaporated to afford crystalline residue, which was recrystallized from MeOH to give carbinol (XV) as colorless prisms (0.2 g), mp 244—246°, $[\alpha]_D^{26}$ $+139°$ (c=1.1). *Anal.* Calcd. for $C_{32}H_{52}O_4$: C, 76.75; H, 10.47. Found: C, 76.86; H, 10.44. IR ν_{max}^{KBr} cm^{-1}: 3588 (OH), 1710 ($COOCH_3$).

Tosylation of Carbinol (XV)——A mixture of XV (70 mg), p-toluene sulfonyl chloride (140 mg) and pyridine (3 ml) was kept at 70—80° for 4 hr. The mixture was poured into water and extracted with ether. The ether layer was washed with 2N HCl, then water and dried over anhyd. Na_2SO_4. Evaporation of the solvent yielded crystalline residue, which was recrystallized from MeOH to give prisms (XVI, 65 mg), mp 209—211°, $[\alpha]_D^{27}$ $+104°$ (c=0.7). *Anal.* Calcd. for $C_{39}H_{58}O_6S$: C, 71.53; H, 8.93. Found: C, 71.58; H, 8.81. IR ν_{max}^{KBr} cm^{-1}: 1719 ($COOCH_3$), 1598, 1362, 1173, 851 (p-Ts-O).

Reduction of Tosylate (XVI)——A mixture of XVI (55 mg), $LiAlH_4$ (155 mg) and abs. tetrahydrofuran (4 ml) was refluxed for 2 hr. Under cooling in an ice-bath, the reaction mixture was diluted with water, then 2N HCl and extracted three times with ether. The combined ether layer was washed with water, dried over anhyd. Na_2SO_4 and CH_2N_2 in ether was added to the above ether solution to give a slightly yellow-colored solution. The solvent was evaporated to give an oily residue, which was chromatographed on Al_2O_3 (15 g). Fraction eluted with benzene gave a methoxy methyl ester (XVII, 10 mg), mp 205—207°, after repeated recrystallization from MeOH, which was completely identical with that derived from β-peltoboykinolic acid (I) by mixed melting point, IR spectra, thin–layer chromatography (solvent: benzene or $CHCl_3$–benzene (3:1)) and gas–liquid chromatography (5% SE–30 on Shimalite W; column length 1.5 m; column temp. 237°; carrier gas, N_2; hydrogen flame ionization detector; r.r.t. to cholestane, 2.60).

Acknowlegement The authors thank Professor O. Jeger and Dr. H. Wehrli for the authentic sample of methyl urs-12-ene-3β-ol-27-oate. Mass spectral measurements were carried out by Nissei Sangyo Co., Ltd., to whom the authors are much indebted.