Table II. Physical Properties and Elemental Analysis of α-Nitroketones (2)

α-Nitro-ketone	mp (°C)	Recryst. solvent	IR ν_{max}^{Nujol} cm^{-1} CO, NO_2(as), NO_2(s)	NMR ($CDCl_3$) δ[ppm] α-CH_2
2a[a]	146.5—149	$CHCl_3$/*n*-C_6H_{14}	1690, 1550, 1330	5.88
2b[b]	156—161	$CHCl_3$	1690, 1550, 1330	6.30[c]
2c[d]	221.5—224[e]	$HCON(CH_3)_2/H_2O$	1690, 1560, 1325	6.44[f]
2d	54.5—55.5[g]	C_2H_5OH/H_2O	1700, 1550, 1315	5.75
2e	96—98[h]	C_2H_5OH	1700, 1570, 1320	5.90
2f[i]	169—170[e]	$CH_3CO_2C_2H_5$	1675, 1550, 1330	6.20[c]
2g[j]	124.5—127.5	$CHCl_3$/*n*-C_6H_{14}	1700, 1560, 1312	5.94

a) *Anal.* Calcd for $C_9H_9NO_3$: C, 60.33; H, 5.07; N, 7.82. Found: C, 60.23; H 4.83; N 7.98.
b) *Anal.* Calcd for $C_9H_9NO_4$: C, 55.38; H, 4.65; N, 7.18. Found: C, 55.28; H, 4.61; N, 7.02.
c) Dimethylsulfoxide-d_6 was used as a solvent.
d) *Anal.* Calcd for $C_{10}H_{10}N_2O_4$: C, 54.05; H, 4.54; N, 12.61. Found: C, 54.29; H, 4.32; N, 12.50.
e) Accompanied by decomposition.
f) A mixture of dimethylsulfoxide-d_6 and deuteriochloroform was used as a solvent.
g) Reported[4] mp 53.5—54.5°.
h) Reported[4] mp 96—97°.
i) *Anal.* Calcd for $C_9H_7NO_5$: C, 51.68; H, 3.37; N, 6.70. Found: C, 51.97; H, 3.23; N, 6.98.
j) *Anal.* Calcd for $C_{12}H_9NO_3$: C, 66.97; H, 4.22; N, 6.51. Found : C, 67.01; H, 4.13; N, 6.26.

with benzene–ethyl acetate (20—50: 1) and by recrystallization. The physical properties and the results of elemental analysis are summarized in Table II.

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Triterpenoids of the Bark of *Pieris japonica* D. Don

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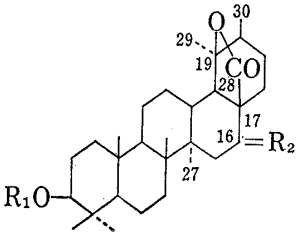
Triterpenoids in the bark of *Pieris japonica* D. Don were investigated. Oleanolic acid acetate, ursolic acid acetate, ursolic acid, β-sitosterol, lupeol and compound A (VI), mp 262—263°, $C_{32}H_{50}O_5$, were obtained. The physical properties of VI were elucidated by infrared absorption, nuclear magnetic resonance (NMR), ^{13}C-NMR and mass spectral studies. The structure of VI is probably 3β-acetoxy-16α-hydroxy-ursan-28,19-olide.

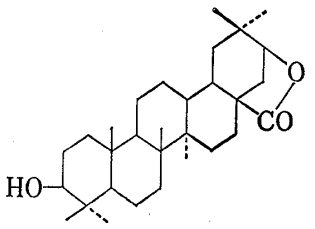
Keywords——*Pieri japonica*; bark; triterpenoid; ursan-type γ-lactone; 3β-acetoxy-16α-hydroxy-ursan-28,19-olide

Pieris japonica D. Don (asebi in Japanese) is a well known poisonous shrub of Ericaceae in Japan. A chemical study on the toxic components was reported by Eÿkman[1] in 1882. Kondo and Takemoto[2] identified ursolic acid, oleanolic acid, β-amyrin and lupeol as triterpenoid components of the flowers. However, the constituents of the bark were not studied. In this paper we describe some triterpenoids of the bark of asebi. A sample (800 g) of the dried bark which had been collected on Mt. Rokko, Hyogo-ken, was extracted with MeOH. Oleanolic acid acetate (I), ursolic acid acetate (II) and ursolic acid (III) were obtained from the extract. Solid material was filtered off, then the mother liquor was chromatographed on Al_2O_3 and subjected to preparative thin-layer chromatography (prep-TLC) to give β-sitosterol (IV), lupeol (V) and compound A (VI). The bark contained a large quantity of triterpenoids (13 g, 1.6%) and the major components were oleanolic acid and ursolic acid acetates. The leaves and flowers contained oleanolic and ursolic acids as major components with small amounts of oleanolic acid and ursolic acid acetates.

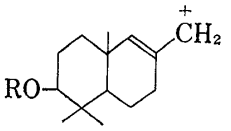
The molecular formula of $C_{32}H_{50}O_5$ was deduced for compound A (VI) from its elemental analysis data and the mass spectrum (M^+ at *m/e* 514). The infrared (IR) spectrum of VI showed absorptions at 3570 cm^{-1} (OH), 1755 cm^{-1} (γ-lactone) and 1725 and 1242 cm^{-1} (ester). The nuclear magnetic resonance (NMR) spectrum of VI showed signals for six tertiary methyl groups at 0.86, 0.87, 0.91, 1.15, 1.30 and 1.52 ppm (each 3H, s), a secondary methyl group at 0.95 ppm (3H, d, J=4.0 Hz), a hydroxy-methine group at 3.88 ppm (1H, t, J=1.4 Hz) in the environment $CH_2\underline{C}HOH$, an acetoxy-methine group at 4.58 ppm (1H, t, J=5.0 Hz, $\underline{C}HOCOCH_3$ assignable to the C-3α-proton) and one acetoxy at 2.04 ppm (3H, s), with no signal due to olefinic proton. Furthermore, the absence of signals at 2.5—3.5 ppm indicated that the C=O function of the lactone ring should be attached to a tertiary carbon atom, probably at the C-17 position. The carbon-13 nuclear magnetic resonance (^{13}C-NMR)[3] of VI showed the signals of an acetoxy group at 21.3 and 170.7, C-3 at 80.4, which was obviously attributable to a carbon bearing βOH (in 3α-hydroxy derivatives, it was displaced by ca. 2.8 ppm upfield[4]), C-16 at 75.7, C-17 at 49.6, and C-19 and C-28 in the lactone ring at 96.1 and 211.0 ppm, respectively. These data were obtained with a very small sample (approx. 3 mg), but identification of the signals was possible by comparison of the spectrum of VI with those of ursane derivatives reported by Seo *et al.*[5] and Doddrell,[6] and by the application of known chemical shift rules, such as the hydroxy substitution shifts presented by Grover *et al.*[7], the steric interactions described by Dalling *et al.*,[8] and the substitutions effects reported by Abraham *et al.*[9]. The mass spectrum of VI exhibited behavior similar to that recorded by Djerassi *et al.*[10] for dihydromachaeric acid lactone (VII). In VII, *m/e* 207 (species a, the cleavage in ring C) was lost (H_2O) to yield *m/e* 189 (species b, base peak), but in VI the relative intensity of *m/e* 207 was



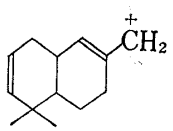
	R_1	R_2
VI	Ac	
VIII	Ac	=O
IX	H	=O



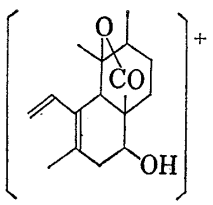
VII



species a



b



c

very low compared with that of VII. The fragment due to the corresponding species a must be *m/e* 250 (or 249), indicating the location of the OAc group in ring A or B, and of the lactone and hydroxyl groups in ring D and/or E. The presence of species c, c-H_2O and c-COOH+H at *m/e* 264, 246 and 218, respectively, supported the location in ring D and/or E. Chromic acid oxidation of VI in pyridine solution gave a ketone (VIII), colorless needles, mp 249—252°, and its IR spectrum showed bands at 1770 cm^{-1} (γ-lactone), 1730 and 1725 cm^{-1} (ester and cyclohexanone). There was no hydroxy group absorption, so that the hydroxy group in VI must be secondary. Alkaline hydrolysis of VIII gave a desacetyl compound (IX). In its mass spectrum, species a at *m/e* 207 was most abundant. The position of the hydroxyl and γ-lactone moieties in VI were assumed on the basis of following observations. In the NMR spectrum of VI, one (1.52 ppm, 3H, s) of the tertiary methyls must be on an oxygen-carrying carbon, and the absence of signals at 4.1—4.5 ppm[11] due to a proton on a carbon atom adjacent to the oxygen function of the lactone ring (CH–O–CO) indicates that only C-28→C-19-olide (CH_3–Ç–OCO–C–17) is possible. One methyl signal at 1.30 ppm (3H, s) appeared to be affected[12] by γ-lactone and hydroxy functions, and could be also assignable to the methyl group (C-27). This conclusion led us to consider the hydroxy group as being on ring D. On the assumption that the conformation of ring D is the usual chair form, the *J* value (1.4 Hz) of the triplet signal at 3.88 ppm suggests that this signal is assignable to 16βH (Calcd $J_{eé}=J_{eá}=$ 1.8 Hz). The downfield shift of the methyl group (C-27) can be explained as the results of 1,3-diaxial interaction. The secondary methyl group (C-30) at 0.95 ppm must be located at C-20. Thus, it seems most likely that VI is 3β-acetoxy-16α-hydroxy-ursan-28,19-olide, which has not previously been isolated in nature.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. IR absorption spectra were determined on Shimadzu IR-400 and IR-430 spectrometers. The NMR spectra were measured with a R-22 (Hitachi) and Varian EM-360 in $CDCl_3$ and chemical shifts are given in the δ (ppm) scale with tetramethylsilane as the internal standard (s, singlet; d, doublet; t, triplet). Mass spectra were recorded on a Japan Electron Optics Laboratory Co. JMS-01SG mass spectrometer.

Isolation of Triterpenoids——A sample (800 g) of the bark of asebi, collected on Mt. Rokko, Hyogo-ken, was extracted with MeOH at room temperature. The extract was concentrated under reduced pressure and 3 g of precipitate (ppt) A was obtained (0.37% yield). The residue was extracted with MeOH at room temperature and 12 g of ppt B was obtained (1.5% yield). After the removal of ppt A and ppt B, the mother liquor was chromatographed on Al_2O_3. The eluate with *n*-hexane–benzene was further subjected to prep-TLC on silica gel. The ppt B (3 g) was extracted with petroleum ether in a Soxhlet apparatus and the extract was concentrated. The resulting crystals were recrystallized from EtOH to give I and II.

Oleanolic Acid Acetate (I) and Ursolic Acetate (II)——Recrystallization of I from EtOH gave 600 mg of colorless needles, mp 289—290°. This product was identical (TLC, IR, NMR and mixed mp) with oleanolic acid acetate obtained from the asebi flowers. II was recrystallized from EtOH to give 90 mg of colorless needles, mp 268—269°. The material was identical (TLC, IR, NMR and mixed mp) with ursolic acid acetate obtained from the asebi flowers.

Ursolic Acid (III)——The ppt A was separated into acid and neutral fractions in the usual manner. From the acid fraction, III was obtained as colorless needles, mp 285—287°, this product was identical (TLC, IR and mixed mp) with ursolic acid obtained from the asebi flowers. From the neutral fraction, I and II were obtained.

β-Sitosterol (IV) and Lupeol (V)——After the removal of ppt A and ppt B from the MeOH extract by filtration, the mother liquor was subjected to prep-TLC on silica gel (layer thickness, 0.50 mm) with benzene–AcOEt (9: 1). Elution of material of *Rf* 0.50 with EtOH gave 18 mg of IV as colorless plates, mp 139—141°, this material was identical (IR, NMR, mass spectrum and mixed mp) with authentic β-sitosterol. Elution of material of *Rf* 0.71 with EtOH gave 20 mg of V as colorless needles, mp 213—214°. This product was identical (IR, NMR and mixed mp) with authentic lupeol.[13]

Compound A (VI)——Elution of material of *Rf* 0.67 with EtOH gave 15 mg of VI as a white crystalline powder, which was purified by recrystallization from $CHCl_3$ to give colorless needles, mp 262—263°. VI showed a yellowish-brown color in the Lieberman-Burchard reaction and was negative to the $FeCl_3$ test. *Anal.* Calcd for $C_{32}H_{50}O_5$: C, 74.67; H, 9.79. Found: C, 74.89; H, 9.76; C, 74.75; H, 9.69. M. S. *m/e*: 514 (M^+), 499 (M^+-CH_3), 496 (M^+-H_2O), 454 (M^+-HOAc), 453 ($M^+-COOH-CH_3-H$), 452 (M^+-COCH_3-

H_2O-H), 436 ($M^+-H_2O-HOAc$), 421 ($M^+-H_2O-HOAc-CH_3$), 300, 264 (c), 250 (a), 249, 246 (c-H_2O), 218 (c-COOH+H), 215, 189 (b, base peak), 175, 161, 147, 135, 121, 107. IR ν_{max}^{KBr} cm^{-1}: 3570 (OH), 1755 (γ-lactone), 1725, 1242 (ester). NMR: 0.86, 0.87, 0.91, 1.15, 1.30 and 1.52 (each 3H, s), 0.95 (3H, d, $J=4.0$ Hz), 2.04 (3H, s, $COCH_3$), 3.88 (1H, t, $J=1.4$ Hz, $CH_2\underline{C}HOH$), 4.58 (1H, t, $J=5.0$ Hz, $C\underline{H}OCOCH_3$). ^{13}C-NMR: 80.4 (C-3), 75.5 (C-16), 49.6 (C-17), 96.1 (C-19), 211.0 (C-28), 21.3 ($CO\underline{C}H_3$), 170.5 ($O\underline{C}OCH_3$). Chromic acid oxidation of VI in pyridine gave the ketone (VIII) as needles, mp 249—252°. IR ν_{max}^{KBr} cm^{-1}: 1770 (γ-lactone), 1730, 1725 (ester and cyclohexanone). *Anal.* Calcd for $C_{32}H_{48}O_5$: C, 74.96; H, 9.44. Found: C, 74.92; H, 9.59. VIII was hydrolyzed with 5% methanolic KOH to give the desacetyl compound (IX), amorphous. IR ν_{max}^{KBr} cm^{-1}: 1775 (γ-lactone), 1710 (C=O). MS *m/e*: 470 (M^+, 18%), 454, 426, 411, 300, 264, 262, 249, 248, 221, 218, 207 (100%), 205, 189, 175, 161, 149, 135, 121, 119, 107.

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Microencapsulation and Bioavailability in Beagle Dogs of Indomethacin[1,2]

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Indomethacin (IMC) suspended in soybean oil was microencapsulated in a gelatin-acacia system by a modified phase separation method from aqueous solution. The microcapsules were hardened with formaldehyde without the use of sodium hydroxide. Average particle diameter of the microcapsules was 111 μm, and the content of IMC in the microcapsules was more than 80% of the initial amount of IMC. The *in vitro* dissolution of IMC from the microcapsules was slower than that of intact IMC, and this indicated that the walls of the microcapsules affected the drug dissolution. The bioavailabilities in beagle dogs of the IMC microcapsules and of a soybean oil suspension of IMC administered in capsules were larger than that of intact IMC.

Keywords——indomethacin; microcapsule; soybean oil suspension; dissolution rate; bioavailability; beagle dogs