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The Structures of Camelliagenin A, B and C obtained from *Camellia Japonica* L.

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Camelliagenin A was oxidized by chromium trioxide to 28-nor-olean-12-ene-3,16,22-trione (VIII). From the data of the nuclear magnetic resonance (NMR) spectra, configuration of the C-3 hydroxyl group was decided as β. Formation of the acetonide and the NMR data also proved the configurations of other hydroxyl groups to be 16α,22α. The structures of the other two genins were also elucidated systematically in relation to camelliagenin A. Thus camelliagenin A, B and C were decided as 3β,16α,22α,28-tetrahydroxy-(I), 23-oxo-3β,16α,22α,28-tetrahydroxy-(II) and 3β,16α,22α,23,28-pentahydroxy-olean-12-one (III) respectively.

In the previous paper[2] the isolation of camelliagenin A (I), B (II) and C (III) from the fruits of *Camellia japonica* has been reported. The structures of sapogenins have been elucidated as reported in the preliminary communication[3] and this paper describes the details of the structural study.

The infrared (IR) spectra of cammelliagenins showed the presence of hydroxyl and the absence of carbonyl group. I, possessing four hydroxyls, easily forms the isopropylidene derivative (IV), mp 234—236°, $C_{33}H_{54}O_4$, which was further characterised by the diacetate (V), mp 193—195°, $C_{37}H_{58}O_6$. Acetylation of I gave diacetate (VII) in a crystalline state and tri- and tetra-acetate as amorphous compounds.

The glycol was assumed to be a 1,3-glycol, since I was stable for periodate oxidation. The oxidation of IV with chromium trioxide afforded the keto-aldehyde (XI), mp 223—225°, $C_{33}H_{50}O_4$, nuclear magnetic resonance (NMR) spectrum 9.42 ppm (1H), and the keto-carboxylic acid (XII), mp 240—246°, $C_{33}H_{50}O_5$. The Wolff–Kishner reduction of XI gave the isopropylidenederivative (XIII), mp 222°, $C_{33}H_{54}O_2$, which was hydrolysed into the diol (XIV), mp 243°, $C_{30}H_{50}O_2$. Similarly, the isopropylidene-carboxylic acid (XV), mp 237—239°, $C_{33}H_{52}O_4$, and the dihydroxy-carboxylic acid (XVI), mp 242°, $C_{30}H_{48}O_4$, were obtained from XII. These reactions characterised the nature of the hydroxyl groups in I. The oxidation of V with selenium dioxide in boiling acetic acid easily led to the formation of a heteroannular diene (VI), mp 220—223°, UV λ_{max}^{EtOH}: 243, 251, 262 mμ, NMR: 5.68 (C_{12}–H) doublet; 6.45 ppm (C_{11}–H) quartet. Tursch, *et al.*[4] reported on the influence of substitution at C-3 and C-17 on the methyl frequencies in the NMR spectrum of Δ^{12}-oleanene derivatives and there have been several reports

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2) H. Itokawa, N. Sawada and T. Murakami, *Yakugaku Zasshi*, **88**, 1463 (1968).
3) H. Itokawa, N. Sawada and T. Murakami, *Tetrahedron Letters*, **1967**, 597.
4) B. Tursch, R. Savoir, R. Ottinger and G. Chiurdoglu, *Tetrahedron Letters*, **1967**, 539.
5) S. Ito, M. Kodama and M. Konoike, *Tetrahedron Letters*, **1967**, 591.
6) S. Ito and T. Ogino, *Tetrahedron Letters*, **1967**, 1127.
7) S.G. Errington, D.E. White and M.W. Fuller, *Tetrahedron Letters*, **1967**, 1289.
8) S. Ito, T. Ogino, H. Sugiyama and M. Kodama, *Tetrahedron Letters*, **1967**, 2289.
9) R. Savoir and B. Tursch, *Tetrahedron Letters*, **1967**, 2129.

on the assignment of methyl signals of pentacyclic triterpenes.[5-9] The fact, along with the presence of seven tertiary methyl signals in the NMR spectra of I and the derivatives (Table I), suggested that I might be olean-12-ene derivatives.

Table I. Lower Field Singnals of Camelliagenin Derivatives (ppm)

	3H	16H	22H	23-H_2	28-H_2	12-H	-$OCOCH_3$		$-O\diagdown\!\diagup CH_3$ $-O\diagup\!\diagdown CH_3$
IV	3.1 3.4 (ol)	4.17 m	4.33 m		3.25 (b.s.)	5.33 m		1.39 1.44	
V	4.53 (q) (8.8, 9.3)	4.13 m	4.26 m		3.72 (11) 3.82 (11)	5.35 m	2.04 2.06	1.40 1.43	
VI	4.54 (q) (ol)	4.46 (b.s.)	4.18 m		3.96 (16) 4.02 (16)	5.68 (11) (d)	2.07 2.15		6.45 (q) (11, 3.6)
VII	4.52 (q) (8, 10)	3.95 (ol)	3.95 (ol)		3.38 (11) 3.70 (11)	5.32 m	2.04 2.09		
VIII						5.54 m			15.15 (1H)
XI		4.17 m	4.48 m		-CHO (9.42)	5.49 m		1.41 1.45	
XII		4.41 m	4.67 m		-COOH(a)	5.41 m		1.43 1.51	
XIII		3.78 m	3.90 m			5.31 m		1.43 1.43	
XIV		4.16 m	3.55 m			5.30 m			
XV		4.46 m	4.67 m		-COOH(a)	5.43 m		1.41 1.48	
XX	4.80 (q) (9.10)	4.11 m	4.25 m	3.76 (ol)	3.76 (ol)	5.37 m	2.00, 2.05 2.02	1.38 1.42	
XXI	3.5 (ol)	4.27 m	4.59 m	3.50 (ol)	3.25 (ol)	5.23 m		1.39, 1.40 1.43, 1.43	
II′	4.24 (b.s)	4.95 (t) (7)	5.33 (ol)	-CHO 9.24	3.67 (10) 3.77 (10)	5.33 (ol)	1.76, 1.95 2.05, 2.05		

t: triplet q: quartet d: doublet ol: overlap m: multiplet b.s.: broad singlet a: ambiguous

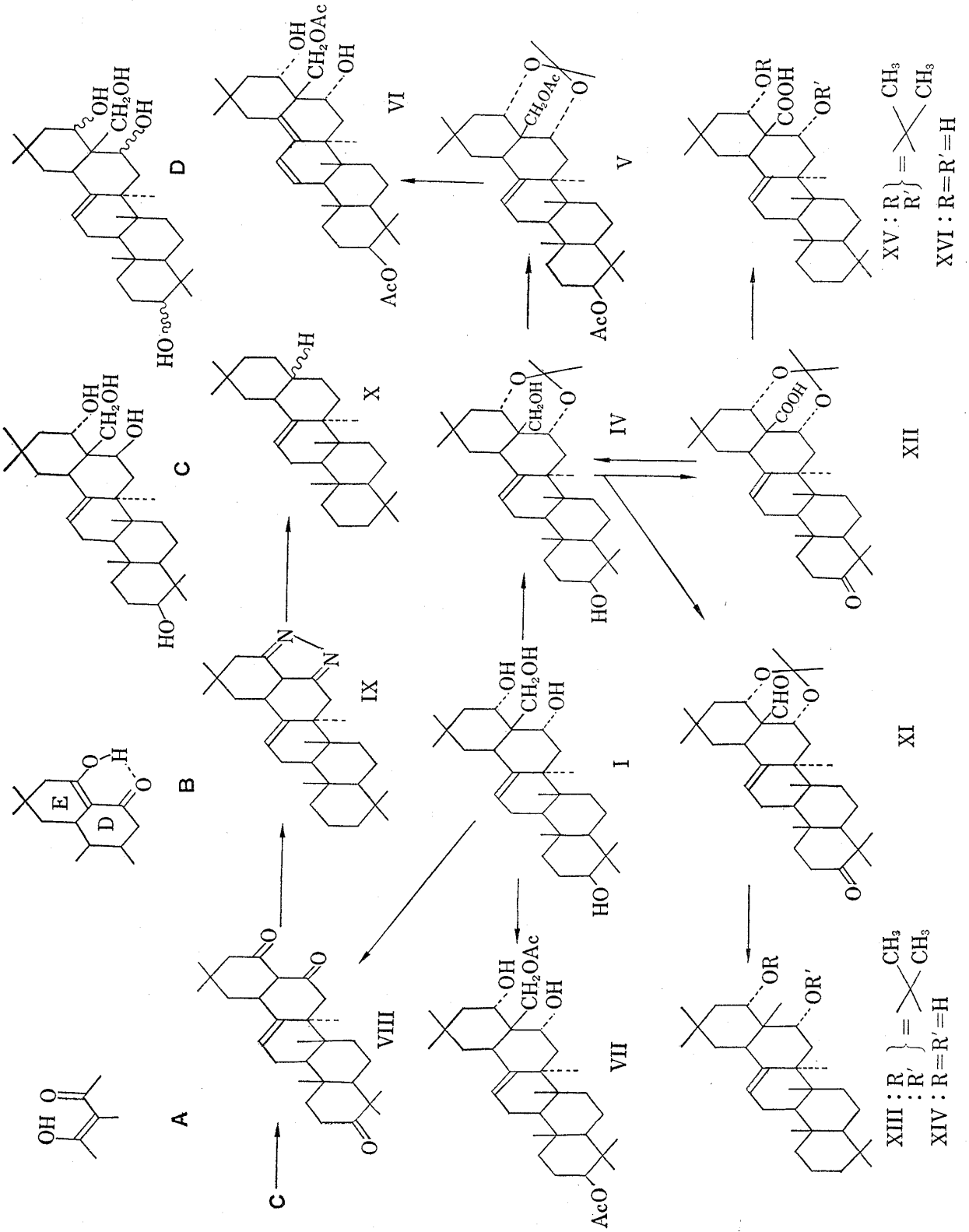
Table II. Methyl Signals of Camelliagenin Derivatives (ppm)

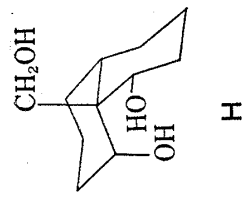
	23	24	25	26	27	28	29	30
IV	0.88	0.784	0.933	1.058	1.27		0.933	0.878
V	0.86	0.85	0.96	1.06	1.27		0.93	0.89
VI	0.87	0.87	0.95	1.07	1.30		0.90	0.76
VII	0.87	0.87	0.94	0.96	1.35		0.96	0.94
VIII	1.09	1.059	1.069	0.931	1.114		0.983	0.965
XI	1.10	1.06	1.06	0.83	1.26		1.06	1.00
XII	1.102	1.051	1.051	0.893	1.26		1.051	1.008
XIII	0.87	0.83[a]	0.93	1.07	1.28	0.78[a]	0.93	0.87
XIV	0.88	0.84[a]	0.88	0.90	1.36	0.80[a]	0.90	0.88
XV	0.853	0.805	0.924	0.798	1.23		1.05	0.979
XX		1.00	0.97	1.033	1.25		0.97	0.87
XXI		0.99	0.92	1.04	1.28		0.92	0.88
II′		1.09	1.00	1.00	1.48		0.86	0.86

a) tentative assignment

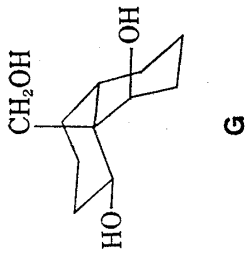
This has been firmly established by the chromium trioxide oxidation of camelliagenin A (I) itself. The product, mp 258°, was proved to be nor-triketone (VIII), $C_{29}H_{42}O_3$, (M^+)

438, IR 1710, 1620 cm^{-1}, UV λ_{max}^{EtOH} 291 mμ, λ_{max}^{KOH} 310 mμ, NMR 15.15 ppm (1H). These spectral data and the positive ferric chloride reaction of VIII show the presence of a β-diketone group, which exists in the enolised form (A) to form intramolecular hydrogen bond. The β-diketone system could be placed only in rings D and E as shown in the formula (B). A positive Zimmerman reaction of XI and XII suggested the presence of a carbonyl group in





H



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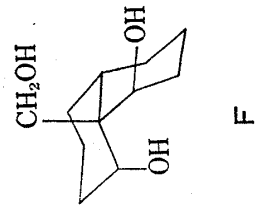

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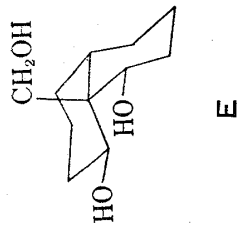


E

Chart 1

C-3 position. Thus the nor–triketone (VIII) might be 28-nor-olean-12-ene-3,16,22-trione. This compound had been known as the oxidation product of chichipegenin (C) and the identity was established by a mixed fusion and infrared spectra.[10] Now the structure of camelliagenin A (I) should be expressed by the formula (D). The loss of C-28 in the course of the oxidation will be explicable by the formulation.[11] The Wolff–Kishner reduction of VIII gave a compound, mp 175°, assumed to be expressed by the formula (IX), and the C_{29}-hydrocarbon(X), mp 148°.

The formation of the keto-aldehyde (XI) and the keto–acid (XII) from IV confines the location of the acetonide group between C-16 and C-22 hydroxyl groups. Of the four isomers (E-H) concerning with the hydroxyl groups, the acetonide formation is possible in E and H, in which the former should be excluded, because of the nonidentity of I with chichipegenin (C). Since lithium aluminum hydride reduction of XII regenerated IV, the orientation of the hydroxyl groups at C-3 was determined as equatorial (3β).[12] The chemical shifts and coupling constants of the hydrogenes attached to the carbon atoms bearing these hydroxyl groups in I and the derivatives are all consistent with these assignment of the configuration of the hydroxyl groups. Accordingly, camelliagenin A can be formulated as $3\beta,16\alpha,22\alpha$,28-tetrahydroxyolean-12-ene (I).

The NMR and IR spectra of camelliagenin B(II) and C(III) revealed the presence of an aldehyde group and an additional hydroxyl group in II and III, respectively, besides four hydroxyl groups in I. The interrelationship was firmly established by the Wolff–Kishner reduction of II to I and lithium aluminum hydride reduction of II to III, thus proving the presence of an aldehyde group in II and a carbinol group in III instead of one of the seven methyl groups in I. The formation of the diisopropylidene derivative (XXI), mp 160°, $C_{36}H_{58}O_5$, from III indicated the presence of the carbinol group at 4α or 4β position. II formed an oxime (XVII), and the monoisopropylidene derivative (XVIII). Lithium aluminum hydride reduction of XVIII afforded the acetonide pentaol (XIX), which formed the triacetate (XX).

The NMR spectra 9.24 ppm (CHO) in II and 3.76 ppm ($C\underline{H}_2$-OAc) in XX showed that these groups at C-4 position must have α-orientation.[13]

10) The identification has been carried out by the courtesy of Prof. C. Djerassi, Stanford University. A. Sandoval, A. Manjarrez, P.R. Leeming, G.H. Thomas and C. Djerassi, *J. Am. Chem. Soc.*, **79**, 4468 (1957).
11) J. Polonsky, *Bull. Soc. Chim. France*, **1953**, 173; C. Djerassi, D.B. Thomas, A.L. Livingston and C.R. Tompson, *J. Am. Chem. Soc.*, **79**, 5922 (1957); F.E. King and T.J. King, *J. Chem. Soc.*, **1956**, 4469.
12) O. Winterstein, G. Krakower and M. Moore, *J. Org. Chem.*, **30**, 2847 (1965).
13) M. Shamma, R.E. Glick and R.O. Mumma, *J. Org. Chem.*, 27 4512 (1962); T.J. King and J.P. Yardley, *J. Chem. Soc.*, **1961**, 4308.

Thus camelliagenin B and C must be represented 23-oxo-3β,16α,22α,28-tetrahydroxy-(II) and 3β,16α,22α,23,28-pentahydroxy-olean-12-ene (III) respectively.

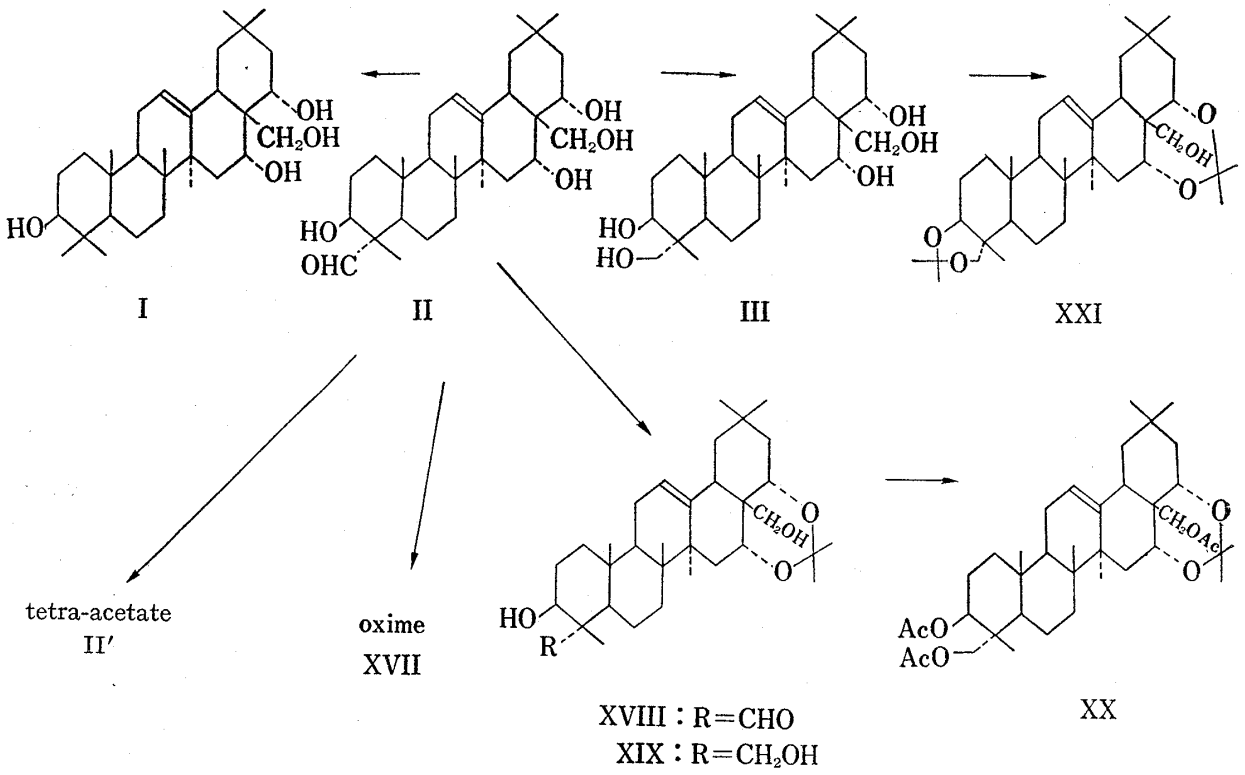
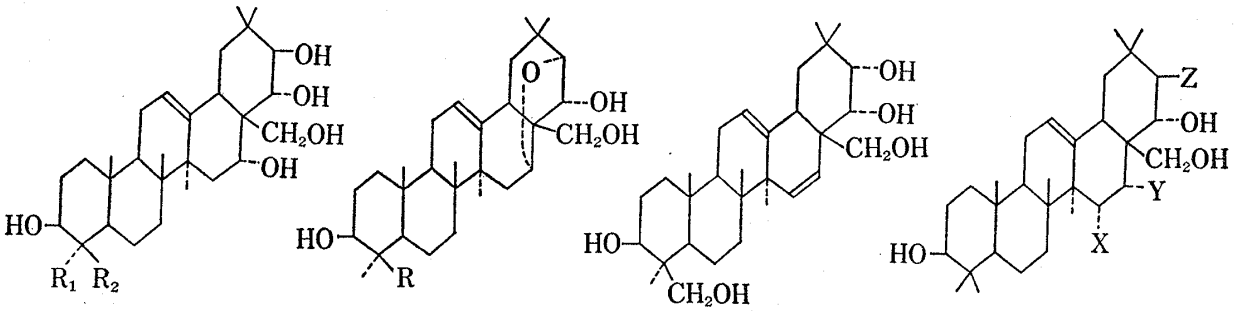


Chart 2

Ito, *et al.* reached to the same conclusion on the structures of camelliagenin A, B and C and we have reported the results simultaneously.[3,4]

After our publication, many results were reported about the related sapogenins. Camelliagenin A, B and C were also isolated from *Camellia sinensis* O. KUNTZE (*Thea sinensis*) and *C. sasanqua*, along with camelliagenin D(a) and E(b).[5] The identity of jegosapogenol, barringtogenol C(c), aescinidin and theasapogenol B and the configuration at C-21 and C-22 in barringtogenol C, barringtogenol D(d), protoaescigenin (e), and isoaescigenin (f) were



a : R_2=CHO
b : R_1=CHO
c : R_1=R_2=CH_3
e : R_2=CH_2OH
h : R_1=CH_2OH

d : R=CH_3
g : R=CH_2OH

f

i : X=Y=H, Z=OH
j : X=Y=OH, Z=H
k : X=Y=Z=OH

Chart 3

discussed.[14] About the structures of aescigenin (g) and protoaescigenin in relation to theasapogenols A(h) and B and genuine sapogenins, barringtogenol C, protoaescigenin, and 16-desoxy-barringtogenol C(i), of horse chestnut were clarified.[15-17] The structures of A_1-barrigenol (j) and R_1-barrigenol (k) were also elucidated.[7,8]

Experimental

The infrared spectra were measured with a Hitachi infrared spectrophotometer SPI-S2. The NMR spectra were run in $CDCl_3$ solution with a Varian HR-100 (100 Mc) spectrometer, serving tetramethylsilane as internal reference. The mass spectra were determined on a Hitachi RMU-6D or Japan Electron Optics Lab. JMS-O1SG spectrometer.

Chromium Trioxide Oxidation of Camelliagenin A (I)——Camelliagenin A[1] (1.4 g) was dissolved in 200 ml of acetic acid and a solution of 1.4 g of CrO_3 in 10 ml of acetic acid was added. After standing overnight, water was added and the product was extracted with $CHCl_3$ and chromatographed over silicagel (3×27 cm) by $CHCl_3$–MeOH (50:1). Recrystallization from acetone gave colourless needles (VIII), mp 254—257°. $[\alpha]_D^{24.5}$ −7.16 ($CHCl_3$). IR ν_{max}^{KBr} : 1703, 1621, 1600 (shoulder) cm^{-1}. IR $\nu_{max}^{CHCl_3}$: 1701, 1626, 1600 (shoulder) cm^{-1}. UV λ_{max}^{EtOH}: 291 mμ (log ε=3.767). UV λ_{max}^{KOH}: 310 mμ (log ε=4.118). NMR: 15.15 ppm (1H). Mass 438 (M^+). *Anal.* Calcd. for $C_{29}H_{42}O_3$: C, 79.40; H, 9.65. Found: C, 79.69; H, 9.69. VIII was identified as 28-nor-Δ^{12}-oleanene-3,16,22-trione by mixed melting point and IR. Oxidation of I by CrO_3–pyridine complex solution also yielded (VIII).

Wolff-Kishner Reduction of VIII——VIII (100 mg) was heated for 30 hr with 5 ml of diethylene glycol and sodium (0.3 g) and anhydrous hydrazine (0.8 ml) in a sealed tube at 230—250°. After cooling, water and hydrochloric acid were added, the precipitate was collected and purified with a column of slicagel by $CHCl_3$–MeOH (40:1). Recrystallization from EtOH gave colourless needles (IX), mp 170—175°. When the Wolff-Kishner reduction was continued for 100 hr, X was obtained, recrystallized from MeOH, colourless needles, mp 144—148°.

Chromium Trioxide Oxidation of IV——IV (1.5 g) was dissolved in pyridine (30 ml) and was added to 5 g of chromium trioxide in 30 ml of pyridine, and the mixture was kept at room temperature overnight. The solution was poured into ice-water and the solid product was filtered. Chromatography on silicagel (3×25 cm) and elution with $CHCl_3$–MeOH (40:1) provided 200 mg of solid which was recrystallized from MeOH to yield colourless needles of 3-oxo-16α,22α-acetonyl-olean-12-ene-28-carboxylic acid (XII), mp 243—249°. IR ν_{max}^{KBr} :1735, 1700 cm^{-1}, IR $\nu_{max}^{CHCl_3}$: 1702 (shoulder), 1691 cm^{-1}. *Anal.* Calcd. for $C_{33}H_{50}O_5$: C, 75.24; H, 9.57. Found: C, 75.61; H, 9.55. A by-product, 3,28-oxo-16α,22α-acetonyl-olean-12-ene (XI) was also obtained by this procedure. In order to obtain XI as a main product, this oxidation reaction was carried for 6 hr and treated as the same method mentioned above. The solid product (300 mg) was recrystallized from acetone to yield colourless needles (XI), mp 223—225.5°. IR ν_{max}^{KBr} :1700 (broad) cm^{-1}. IR $\nu_{max}^{CHCl_3}$: 1730 (shoulder), 1696 cm^{-1}. NMR: 9.42 ppm (1H). *Anal.* Calcd. for $C_{33}H_{50}O_4$: C, 77.60; H, 9.87. Found: C, 77.72; H, 9.92. XII was also obtained by this reaction as a by-product.

Wolff-Kishner Reduction of XI——XI (120 mg) was heated for 8 hr with 5 ml of diethylene glycol and sodium (0.3 g) and anhydrous hydrazine (1.0 ml) in a sealed tube at 200—220°. After cooling, water and hydrochloric acid were added, the precipitate was collected and purified on a column of silicagel with $CHCl_3$–MeOH (40:1). Recrystallization from benzene–MeOH gave colourless needles (XIII) (100 mg), mp 222°. *Anal.* Calcd. for $C_{33}H_{54}O_2$: C, 82.09; H, 11.27. Found: C, 81.99; H, 11.12. XIII was hydrolysed with 3% hydrochloric acid into the diol (XIV), colourless needles from MeOH, mp 243°. IR ν_{max}^{KBr}: 3420 (broad) cm^{-1}. *Anal.* Calcd. for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38. Found: C, 81.27; H, 11.41.

Wolff-Kishner Reduction of XII——XII (150 mg) was treated in the same way mentioned above and recrystallized from MeOH to colourless needles of 16α,22α-acetonyl-olean-12-ene-28-carboxylic acid (XV) (70 mg), mp 237—239°. *Anal.* Calcd. for $C_{33}H_{52}O_4$: C, 77.29; H, 10.22. Found: C, 77.24; H, 10.05. XV was further hydrolysed with 3% hydrochloric acid into XVI, recrystallized from MeOH, colourless needles, mp 234—242°. IR ν_{max}^{KBr}: 1700 cm^{-1}. *Anal.* Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 76.01; H, 10.15.

Oxime of Camelliagenin B (II)——Camelliagenin B (100 mg) in aqueous EtOH containing potassium carbonate (280 mg) and hydroxylamine hydrochloride (140 mg) was heated on the steam-bath for 5 hr. The reaction mixture was poured into crushed ice and the collected solid in EtOH was absorbed on a column of silicagel. Elution with $CHCl_3$–MeOH (40:1) yielded oxime XVII which was crystallized from

14) T. Nakano and M. Hasegawa, *Tetrahedron Letters*, **1967**, 1675.
15) I. Yoshioka, K. Imai, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **15**, 135 (1967).
16) I. Yoshioka, T. Nishimura, A. Matsuda, K. Imai and I. Kitagawa, *Tetrahedron Letters*, **1967**, 637.
17) I. Yoshioka, K. Imai and I. Kitagawa, *Tetrahedron Letters*, **1967**, 2577.

benzene–MeOH as colourless needles, mp 232—235°. *Anal.* Calcd. for $C_{30}H_{49}O_5N$: C, 71.53; H, 9.81; N, 2.78. Found: C, 71.36; H, 9.91; N, 2.82.

Monoacetonide of II (Monoisopropylidenecamelliagenin B)——II (200 mg) was shaken in 30 ml acetone containing 3 drops of concentrated sulfuric acid for 20 min and was kept at room temperature overnight. The solution was poured into ice–water and the solid product was filtered. The collected solid in acetone was adsorbed on a column of alumina. Elution with acetone yield XVIII (amorph.). *Anal.* Calcd. for $C_{33}H_{52}O_5$: C, 74.96; H, 9.91. Found: C, 74.63; H, 9.71.

$LiAlH_4$ Reduction of XVIII——XVIII (100 mg) in 50 ml of ether was stirred at room temperature for 5 hr with 400 mg lithium aluminum hydride. The excess reagent was decomposed with ethyl acetate followed by addition of a saturated aqueous solution of sodium sulfate. After drying with anhydrous sodium sulfate, the precipitate was filtered and the filtrate was evaporated to dryness. The collected solid in acetone was adsorbed on a column of alumina. Elution with acetone yielded 3β,23,28-trihydroxy-16α,22α-acetonyl-oleane-12-ene (XIX) (amorph.). *Anal.* Calcd. for $C_{33}H_{50}O_5$: C, 75.24; H, 9.57. Found: C, 75.05; H, 9.45.

Acetylation of XIX——XIX (50 mg) was acetylated with acetic anhydride–pyridine overnight at room temperature and the crude acetate was adsorbed on a column of silicagel. Elution with benzene yielded 3β,23,28-triacetyl-16α,22α-acetonyl-olean-12-ene (XX) (amorph.). *Anal.* Calcd. for $C_{39}H_{56}O_8$: C, 71.75; H, 8.65. Found: C, 71.62; H, 8.33.

Diisopropylidene-camelliagenin C (III)——III (200 mg) was shaken in 30 ml acetone containing 3 drops of concentrated sulfuric acid for 20 min, and was kept at room temperature overnight. The solution was poured into ice–water and the solid product was filtered. The collected solid in acetone was adsorbed on a column of alumina. Elution with acetone yielded XXI (amorph.). *Anal.* Calcd. for $C_{36}H_{58}O_5$: C, 75.74; H, 10.24. Found: C, 75.51; H, 10.14.

Acknowledgement The authors wish to express their appreciation to Prof. T. Okamoto of Tokyo University, for supplying plant materials; to Dr. Takamura of Chugai Co. Ltd., Prof. M. Fujita of Tokyo College of Pharmacy, Prof. M. Nagasawa of Science University of Tokyo and Dr. S. Natori of National Institute of Hygenic Sciences, for continued interest and encouragement; to Dr. M. Yamazaki of National Institute of Radiological Sciences for measurement of NMR spectra, to Dr. T. Miki of Takeda Co., Ltd. for measurement of Mass spectra.