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Saponin and Sapogenol. I. Seeds Sapogenols of *Thea sinensis* L. (1). Barringtogenol C (=Theasapogenol B)

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The structure of theasapogenol B, one of seeds sapogenols of *Thea sinensis* L. (Theaceae) has been established as 3β,16α,21β,22α,28-pentahydroxy-olean-12-ene (II). Based on the identity of theasapogenol B with barringtogenol C, a sapogenol of *Barringtonia acutangula* Gaertn. (Lecythidaceae) and its structure by the previous workers being (I), the structure of barringtogenol C has been revised to II incidentally along with the revision of the structure of barringtogenol D from (2) to XVI. In addition, the designations of seeds sapogenols of *Thea sinensis* L. have been proposed to be theasapogenol A (I), barringtogenol C(II), camelliagenin C (III), dihydropriverogenin A (IV), and theasapogenol E(V) respectively.

Seeds saponin of *Thea sinensis* L. (Theaceae) was initially isolated by Aoyama in 1931 and named "thea-saponin".[2] It was in 1952 when Ishidate and Ueda obtained the saponin in a crystalline form for the first time and elucidated the composition to be "thea-sapogenol" linked by one mole each of glucuronic acid, galactose, arabinose, xylose, and angelic acid.[3] Later on, Ueda reported the chemical study on the structure of "thea-sapogenol" in which he proposed it to be a new amyrin-type triterpenoid.[4] With the generous consent of Professor Y. Ueda, we took over the investigation with the hope of clarifying the chemical structure of "thea-sapogenol.[5]

The chromatographic separation of the crude sapogenol obtained by the procedure after Ishidate and Ueda[3] led the isolation of then new sapogenols provisionally named theasapogenols A, B, C, D, and E respectively. Among them, at first the chemical constitutions of theasapogenols A (I)[6] and B (II)[7] were elucidated as presented in our communications in 1966. In particular of importancy was that the established identity of theasapogenol B with barringtogenol C, a sapogenol of *Barringtonia acutangula* Gaertn. (Lecythidaceae) and whose structure proposed by Barua and Chakrabarti being (1),[8] resulted the revision of the structure (I) incidentally. As to the remaining theasapogenols, theasapogenol E was next determined as V[9] and subsequently the structures of theasapogenols C (III) and D (IV) were elucidated by the respective identification with camelliagenins C[10,11] and A[10,11] and also by our own effort.[12] The structure elucidation of these camelliagenins, seeds sapogenols of *Camellia japonica* L.

1) Location: *Toneyama, Toyonaka, Osaka.*
2) S. Aoyama, *Yakugaku Zasshi*, **51**, 367 (1931).
3) *a*) M. Ishidate and Y. Ueda, *Yakugaku Zasshi*, **72**, 1523 (1952); *b*) Y. Ueda, *ibid.*, **72**, 1525 (1952).
4) Y. Ueda, *Chem. Pharm. Bull.* (Tokyo), **2**, 175 (1954).
5) A sample of "thea-sapogenol" cordially provided by Professor Ueda appeared to consist of theasapogenols A, B, and E as disclosed by TLC.
6) I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Tetrahedron Letters*, **1966**, 5979.
7) I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Tetrahedron Letters* **1966**, 5973.
8) A.K. Barua and P. Chakrabarti, *Tetrahedron*, **21**, 381 (1965).
9) I. Yosioka, A. Matsuda, T. Nishimura, and I. Kitagawa, *Chem. Ind.* (London), **1966**, 2202.
10) H. Itokawa, N. Sawada, and T. Murakami, *Chem. Pharm. Bull.* (Tokyo), **17**, 474 (1969) (*cf. Idem, Tetrahedron Letters*, **1967**, 597).
11) S. Ito, M. Kodama, and M. Konoike, *Tetrahedron Letters*, **1967**, 591.
12) I. Yosioka, T. Nishimura, N. Watani, and I. Kitagawa, *Tetrahedron Letters*, **1967**, 5343.

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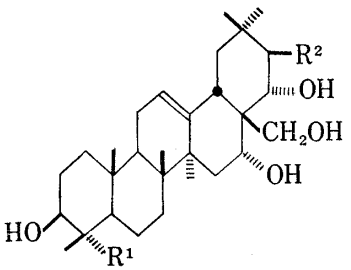
and *C. sasanqua* THUNB.[13,14], is credited to two independent works by Murakami, *et al.*[10] and Ito, *et al.*[11] Interestingly in addition, theasapogenol D(=camelliagenin A) was found identical[12,15] with dihydropriverogenin A obtainable from some European[16] and Japanese[17,18] *Primulaceous* plants, and this caused the revision of the latter structure. Furthermore it is of note mentioning that barringtogenol C has been revealed by several groups to distribute as the sapogenol in some other plant sources such as *Aesculus hippocastanum* L. (initially proposed name was aescinidin[19]), *A.turbinata* BLUME[20] (Hippocastanaceae), *Styrax japonica* SIEB. et ZUCC. (jegosapogenol[21-23]) (Styracaceae).

Taking into the consideration of the precedency and for the simplicity in some cases, we would like to designate the compounds expressed by I, II, III, IV, and V as theasapogenol A, barringtogenol C, camelliagenin C, dihydropriverogenin A and theasapogenol E respectively hereafter. In the present paper, we describe the details of the study on barringtogenol C (=theasapogenol B) leading the structure II.



I: $R^1=CH_2OH$, $R^2=OH$ theasapogenol A

II: $R^1=CH_3$, $R^2=OH$ barringtogenol C (=theasapogenol B, aescinidin, jegosapogenol)

III: $R^1=CH_2OH$, $R^2=H$ camelliagenin C (=theasapogenol C)

IV: $R^1=CH_3$, $R^2=H$ dihydropriverogenin A (=camelliagenin A, theasapogenol D)

V: $R^1=CHO$, $R^2=OH$ theasapogenol E

Barringtogenol C(=theasapogenol B) (II), $C_{30}H_{50}O_5$, mp 278—284°, $[\alpha]_D$ +11° (pyridine), exhibits the absorption bands at 3367 cm^{-1} (hydroxyl) and 1643 cm^{-1} (double bond) in its infrared (IR) spectrum (KBr). On acetylation with acetic anhydride and pyridine at room temperature, it afforded a triacetate (VI), $C_{30}H_{47}O_2(OCOCH_3)_3$, mp 244—246°, and a tetraacetate (VII), $C_{30}H_{46}O(OCOCH_3)_4$ (molecular ion at *m/e* 658 in its mass spectrum), mp 228—229°, whose IR spectrum (KBr) shows the absorption bands at 3448 (hydroxyl), 1733, 1245 cm^{-1} (acetate), while the acetylation of II using *p*-toluenesulfonic acid as a catalyst gave a pentaacetate (VIII), $C_{30}H_{45}(OCOCH_3)_5$, mp 147—150°, which lacks the hydroxyl absorption band in its IR spectrum (CCl_4). Alkaline hydrolysis of the latter resumed II smoothly, thus suggesting that all five oxygen functions of II are hydroxyls.

In the nuclear magnetic resonance (NMR) spectrum of VII (Table I), a broad singlet at 6.30 τ (2H) is ascribed to methylene hydrogens of an acetylated primary alcohol attached

13) I. Yosioka, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **15**, 547 (1967).
14) S. Ito and T. Ogino, *Tetrahedron Letters*, **1967**, 1127.
15) M. Kodama and S. Ito, *Chem. Ind.* (London), **1967**, 1647.
16) *a*) R. Tschesche, B.T. Tjoa, and G. Wulff, *Ann.*, **696**, 160 (1966); *b*) *Idem*, *Tetrahedron Letters*, **1968**, 183.
17) I. Kitagawa, A. Matsuda, T. Nishimura, S. Hirai, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **15**, 1435 (1967).
18) I. Kitagawa, A. Matsuda, and I. Yosioka, *Tetrahedron Letters*, **1968**, 5377.
19) R. Tschesche and G. Wulff, *Tetrahedron Letters*, **1965**, 1569.
20) I. Yosioka, K. Imai, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **15**, 135 (1967).
21) *a*) T. Nakano, M. Hasegawa, T. Fukumaru, S. Tobinaga, C. Djerassi, L.J. Durham, and H. Budzikiewicz, *Tetrahedron Letters*, **1967**, 365; *b*) T. Nakano, M. Hasegawa, T. Fukumaru, L.J. Durham, H. Budzikiewicz, and C. Djerassi, *J. Org. Chem.*, **34**, 3135 (1969).
22) T. Hayashi, C. Koshiro, T. Adachi, I. Yosioka, and I. Kitagawa, *Tetrahedron Letters*, **1967**, 2353.
23) N. Sugiyama, H. Aoyama, T. Sayama, and K. Yamada, *Nippon Kagaku Zasshi*, **88**, 1316 (1967).

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to a tertiary carbon, whereas, an AB type quartet appearing at 4.40 (1H) and 4.54 τ (1H) with a coupling constant of 10 cps is assigned to two carbinyl hydrogens of an acetylated α-glycol. The signal pattern of the AB quartet suggests that two hydroxyls concerned are in the *trans*-diequatorial orientation and in addition the carbons neighboring both side of α-glycolic carbons possess no hydrogen. A multiplet with rather small half-band width ($\sim$10 cps) at 5.77 τ (1H) is predictable to a carbinyl hydrogen of an axial secondary alcohol. The assignment is in accordance with the resistance of the hydroxyl function against the ordinary acetylation. A triplet-like signal at 5.45 τ (1H) has a feature characteristic in the triterpenoid sapogenol due to the carbinyl α-axial hydrogen at C-3. The biogenetic consideration would also support the assumption. Accordingly, it follows that all five hydroxyl functions in II consist of one primary and four secondary hydroxyls among which two constitute an α-glycol moiety. A remaining signal at 4.60 τ (1H, multiplet) in the lower region is assignable to a hydrogen on a trisubstituted olefin.

Table I. The NMR Data given in τ Values(J Values in the Parentheses are in cps)[a]

Compound	C-3H	C-16H	C-21H	C-22H	C-28H$_2$	C-12H[b]	Lowest CH$_3$[c]	Others
VI	5.48 (t.l)	5.82 (m)	6.06 (d, 10)	4.76 (d, 10)	6.30 (br. s)	4.65	8.53	
VII	5.45 (t.l w1/2 =17 cps)	5.77 (m w1/2=10)	4.40, 4.54 (2H, AB q, 10)		6.30 (br.s)	4.60	8.53	9.10(4 Me), 9.03(1 Me), 8.93(1 Me), 8.53(1 Me) (all s): C-CH$_3$
IX	5.48 (t.l)	—	4.45, 4.83 (2H, AB q, 10)		5.68 (q, 12)	4.52	8.72	
XII	5.49 (t.l)	6.28 (t)	4.79 (d,9)	5.98 (d, 9)	5.96 (br.s)	4.66	8.76	8.63 (2 Me, s): acetonide methyls
XIII	5.48 (t.l)	5.15 (m)	4.38 (d, 10)	6.14 (d, 10)	6.50 (br.s)	4.68	8.55	8.55 (2 Me, s): acetonide methyls
XIV	5.50 (t.l)	4.16 (m)	4.61 (d, 11)	6.19 (d, 11)	6.47 (q, 11)	4.64	8.70	8.61 (2 Me, s): acetonide methyls
XV	5.47 (t.l)	5.71 (m)	6.39 (s)	4.71 (s)	6.06 (q. 12)	4.71	8.47	
XVII	—	5.25 (d[d],4)	6.45 (s)	5.74 (d[e])	—	4.72	8.54	6.34 (s): COOCH$_3$
XXII	5.50 (t.l)	5.77 (m)	4.63 (d, 10)	6.16 (d, 10)	6.17 (br.s)	4.68	8.56	

a) br. s=broad singlet, d=doublet, m=multiplet, q=quartet, s=singlet, t=triplet, t. l=triplet like
b) All the signals were observed as multiplets.
c) Appeared as singlets.
d) with further coupling
e) Collapsed to a singlet by D_2O addition.

The chromium trioxide-pyridine oxidation of VII yielded a monoketo derivative (IX), $C_{38}H_{56}O_9$, (M^+: *m/e* 656), mp 281—283°, whose IR spectrum (KBr) discloses a newly generated six-membered carbonyl (1718 cm^{-1}) in addition to the original acetyl carbonyl absorption band at 1739 cm^{-1}. Alkaline treatment of IX liberated formaldehyde presumably *via* reverse aldolization as depicted in Chart 1.

These observations are consistent with a partial constitution (a) for II where the correlation of the primary carbinol and the secondary axial hydroxyl function is revealed. The methylene formulation next to the carbon bearing the axial hydroxyl is substantiated by the multiplet signal pattern at 5.77 τ in VII as mentioned in the above NMR analysis.

On treatment with 2,2-dimethoxypropane and *p*-toluenesulfonic acid in acetone, barringtogenol C(=theasapogenol B) furnished two isomeric monoacetonides, $C_{33}H_{54}O_5$: X, mp 206—208°, and XI (major), mp 255—262°. Ordinary acetylation of XI at room temperature yielded a monoacetonyl-diacetate (XIII), $C_{37}H_{58}O_7$, mp 237—239°, whereas the acetylation at reflux gave a monoacetonyl-triacetate (XIV), $C_{39}H_{60}O_8$, mp 249—250°, whose IR spectrum

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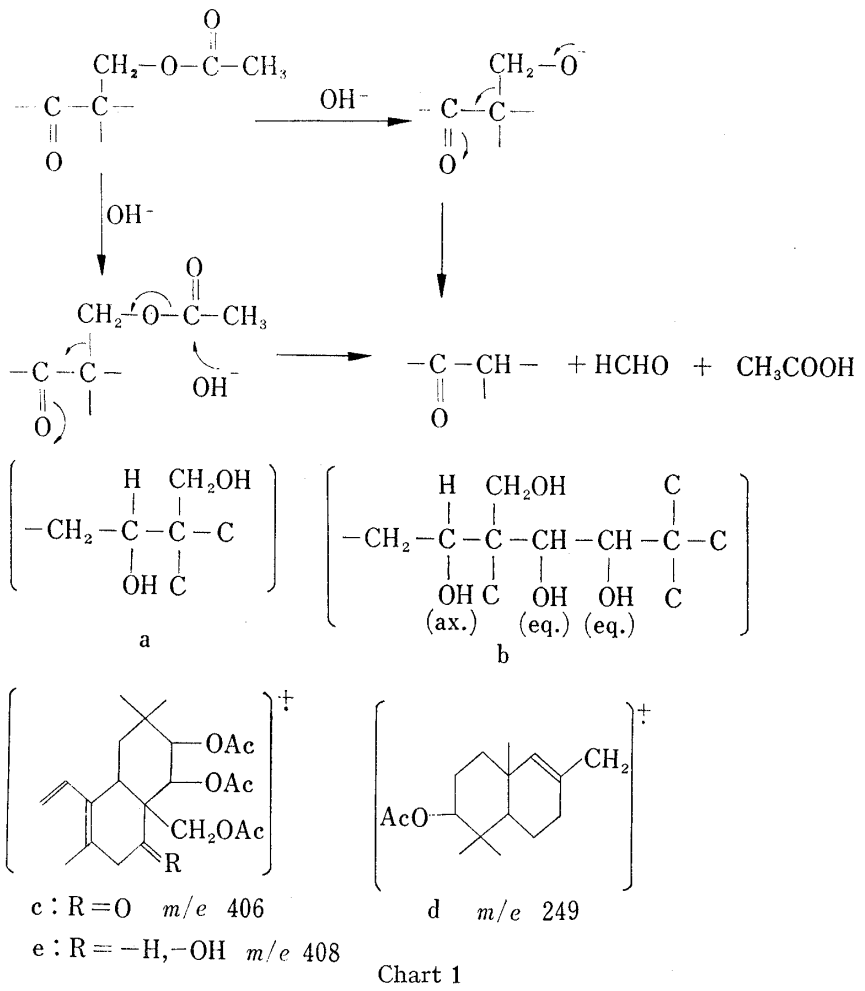

Chart 1

(CCl_4) indicates the absence of free hydroxyl function in XIV. The acetonide linkage in XI is formed between the primary alcohol and one of the α-glycolic hydroxyls as based on the NMR comparison of XIV and VII. Thus, a signal (at 6.47 τ, 2H) due to methylene hydrogens of primary carbinol in XIV is found diamagnetically shifted as compared with that (at 6.30 τ) in VII, and in addition the signal due to one of the carbinyl hydrogens of α-glycolic function appears markedly in the higher field in XIV (at 6.19 τ) than in VII (at 4.40 or 4.54 τ). As disclosed analogously by the NMR analysis of the peracetyl derivative (XII), $C_{39}H_{60}O_8$, of the other acetonide (X), the acetonide formation in X is allocated between one of the α-glycolic hydroxyls and the axial hydroxyl, namely the signals ascribable to the hydrogens attached to the carbons bearing the hydroxyls involved are found at diamagnetically shifted position (at 5.98 and 6.28 τ) respectively. The evidence thus far results in the expansion of the partial structure of II from a to b (Chart 1).

The carbon framework of barringtogenol C(=theasapogenol B) is deduced mass spectrometrically as based on the fragmentation pattern of the monoketone (IX) and tetraacetate (VII). In the former, the significant peaks at *m/e* 406 and 249 are ascribed to fragment ions (c) and (d) probably derived through retro Diels-Alder type fragmentation which is predominant and characteristic among Δ^{12}-oleanene or Δ^{12}-ursene triterpenoids.[24] The assumption alike is also made on the mass spectrum of the latter. Furthermore, the mass number of ions (c) and (d) endorses to locate one hydroxyl in the ring A or B (presumably at C-3) and the residual four hydroxyls in the rings C, D, and E. In addition, all the C-methyl signals given as singlets in the NMR spectra of all the derivatives of II (for instance, VII in Table I) prefer oleanene

24) H. Budzikiewicz, J.M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 3688 (1963).

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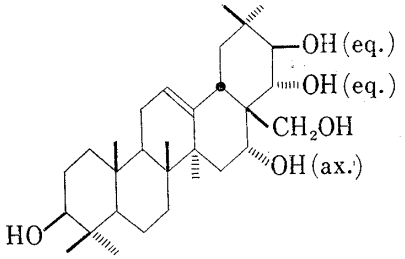
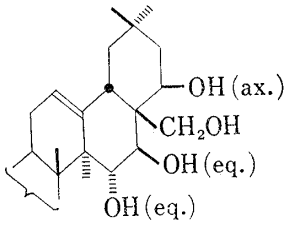


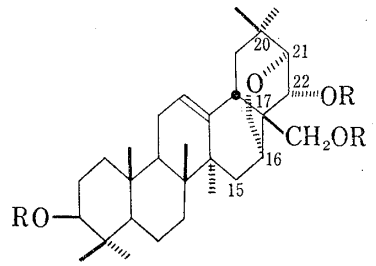
II



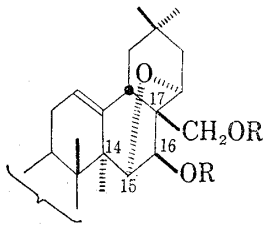
II'

rather than ursene as the possible skeleton of II. The combined observations above accordingly put forward two plausible structures either II or II' for barringtogenol C(=theasapogenol B).

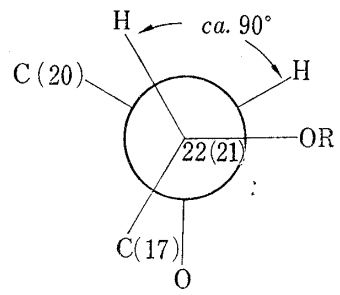
The triacetate (VI) carries two hydroxyls intact, for, as shown in its NMR spectrum, the carbinyl hydrogen of the axial secondary alcohol appears at 5.82 τ and that of one of the α-glycol at 6.06 τ. On refluxing in a mixture of phosphorus oxychloride and pyridine for two hours, VI afforded an acetyl-anhydro derivative (XV), $C_{36}H_{54}O_7$ mp 221.5—223°, as a sole product, which in turn on alkaline hydrolysis gave an anhydro derivative (XVI), $C_{30}H_{48}O_4$, mp 303—309°, $[\alpha]_D$ +73.5° (ethanol). The anhydro compound (XVI) was then converted to two methyl esters: XVII, $C_{31}H_{46}O_5$, mp 273—276°, and XVIII, $C_{31}H_{44}O_5$, mp 214—216°, by the Kiliani oxidation followed by the diazomethane treatment. The IR spectrum (CCl_4) of XVIII reveals three carbonyl absorption bands at 1773, 1743, and 1710 cm^{-1} assignable to five-membered ketone, methyl ester, and six-membered ketone respectively. It is therefore reasonably understood that the reaction from VI to XV proceeded through dehydration between two free hydroxyls in VI resulting a five-membered ether ring. The other ester is given structure XVII, in which one of the parent α-glycolic hydroxyls remained intact according to the physical data in particular as follows: *i.e.* IR ($CHCl_3$): 3580, 3420 (hydroxyl), 1727 (ester carbonyl), 1698 cm^{-1} (carbonyl at C-3) and NMR: 5.74 τ (1H, singlet, due to the hydrogen at C-22 as discussed later). When XVII is once formed, the hydroxyl function on



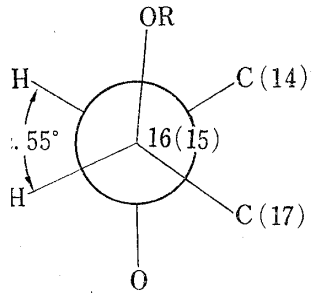
XV :R=Ac
XVI:R=H



XV' : R=Ac
XVI': R=H



XV : R=Ac
XVI: R=H



XV' : R=Ac
XVI': R=H

Fig. 1

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the ether ring resists against the Kiliani oxidation. This probably is reasoned by the significant intramolecular hydrogen bonding between the hydroxyl and the neighboring ester carbonyl.

Assuming the structure of barringtogenol C(=theasapogenol B) as II, the anhydro derivative is formulated as XVI. On the other hand, the structure XVI′ could be approved, if barringtogenol C(=theasapogenol B) were represented by II′. The Dreiding model examination in connection with the NMR analysis rationalizes the structure XVI in preference of XVI′.

As illustrated in Fig. 1, the dihedral angle between C-21H and C-22H in XVI falls into *ca.* 90°, so that the neglisible coupling of these two hydrogenswould result the signals due to each hydrogens to appear singlet like. Meanwhile, in case of XVI′, the angle is estimated *ca.* 55° and hence the signals due to the respective hydrogens would be expected as a pair of doublets with a coupling of *ca.* 5.4 cps as calculated with the Karplus equation.[25] In reality, these hydrogens in XV are given as two singlets at 4.71 and 6.39 τ respectively (Table I).

The structure of barringtogenol C(=theasapogenol B) is therefore formulated as 3β, 16α,21β,22α,28-pentahydroxy-olean-12-ene (II) and the anhydro derivative as 3β, 22α, 28-trihydroxy-16α,21α-oxido-olean-12-ene (XVI).

In regard to the hydroxyl configuration in the ring E, the structures, II and XVI, are diastereoisomeric respectively to those, **1** and **2**, of barringtogenols C and D initially proposed by Barua and Chakrabarti.[8] However, the direct comparison (mixed mp, IR, and TLC) of the acetyl-anhydro derivative (XV) with authentic barringtogenol D triacetate kindly provided by Dr. Barua revealed the unexpected identity of both. Moreover, the tetraacetate (VII) was also identified in all respects with a tetraacetyl derivative of aescinidin(=barringtogenol C) isolated from *Aesculus turbinata* BLUME.[20]

The most decisive basis for the structure (**1**)[8] was depending on the facts that the derivatives of barringtogenol C, (**4**) and (**5**), were identical with corresponding those of aescigenin, whose structure had already been presented as **3** by Jeger, *et al.*[26] at that time. As was disclosed in our previous paper[27], the C-22 hydroxyl of aescigenin possesses α-equatorial (as XIX) rather than β-axial orientation (as **3**) and consequently the structures, II and XVI, have been proved correct for barringtogenol C(=theasapogenol B) and barringtogenol D respectively. Although the full account of the above evidence will be reported in our forthcoming paper, the structures of the related compounds, named protoaescigenin and isoaescigenin have also been revised from **6** (21α-OH, 22β-OH: by Jeger, *et al.*[26] and Kuhn, *et al.*[28]) and **7** (21α-OH, 22β-OH: by Thomson[29]) to XX (21β-OH, 22α-OH) and XXI (21α-OH, 22α-OH) at the same time.[27] Recently, the correctness of our presentation have been demonstrated X-ray crystallographically by Hoppe, *et al.*[30]

Next, the evidence concerning to the acyl migration is mentioned. As described above, the mild acetylation of II furnished the partially acetylated derivative VI in addition to VII, thus indicating that C-22 hydroxy is readily acetylated as compared with C-21 hydroxyl. On the other hand, Barua, *et al.* reported[31] the isolation of a 3,21,28-tri-O-benzoyl derivative of II on mild benzoylation (benzoyl chloride and pyridine at 0°). The isolation of the acetates, VI and VII, was achieved by silicic acid column chromatography as given in the experimental section and no change occurring during the procedure was confirmed by TLC, while in case of benzoylation, Barua, *et al.* applied "acid washed alumina" as the adsorbent. Since alumina

25) M. Karplus, *J. Am. Chem. Soc.*, **85**, 2870 (1963).
26) G. Cainelli, A. Melera, D. Arigoni, and O. Jeger, *Helv. Chim. Acta*, **40**, 2390 (1957).
27) I. Yosioka, T. Nishimura, A. Matsuda, K. Imai, and I. Kitagawa, *Tetrahedron Letters*, **1967**, 637.
28) R. Kuhn and I. Loew, *Ann.*, **669**, 183 (1963).
29) J.B. Thomson, *Tetrahedron*, **22**, 351 (1966).
30) W. Hoppe, A. Gieren, N. Brodherr, R. Tschesche, and G. Wulff, *Angew. Chem.*, **80**, 563 (1968).
31) A.K. Barua, S.K. Chakraborti, P. Chakrabarti, and P.C. Maiti, *J. Indian Chem. Soc.*, **40**, 483 (1963).

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has been known to catalyze the acyl migration,[32] if it is assumed that the benzoyl group migration from C-22 to C-21 hydroxyl (*i.e.* from 3,22,28-tri-O-benzoate to 3,21,28-tri-O-benzoate of II) was inevitable during the isolation by them, the apparently different reactivity of C-21 and C-22 hydroxyls towards acetylation and benzoylation is explicable without discrepancy.

To verify the assumption, the following experiment was undertaken. Thus, the benzene solution of the triacetate (VI) was once adsorbed on neutral alumina column (Woelm grade

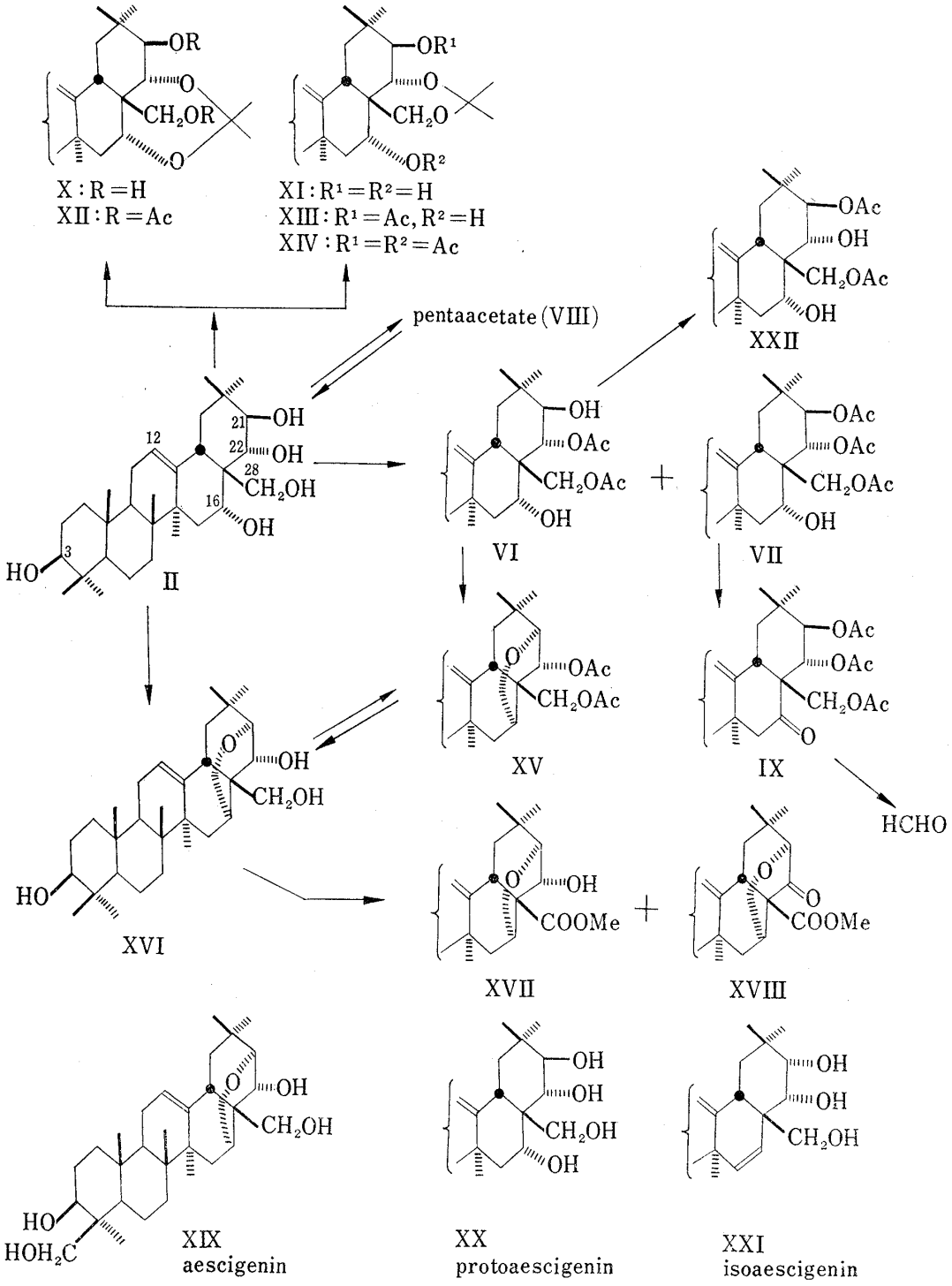

Chart 2

32) General review on the acyl migration: *cf.* R.U. Lemieux, "Molecular Rearrangement," Part II, ed. by P. de Mayo, Interscience Publishers, Inc., New York, N.Y. 1964, p. 763.

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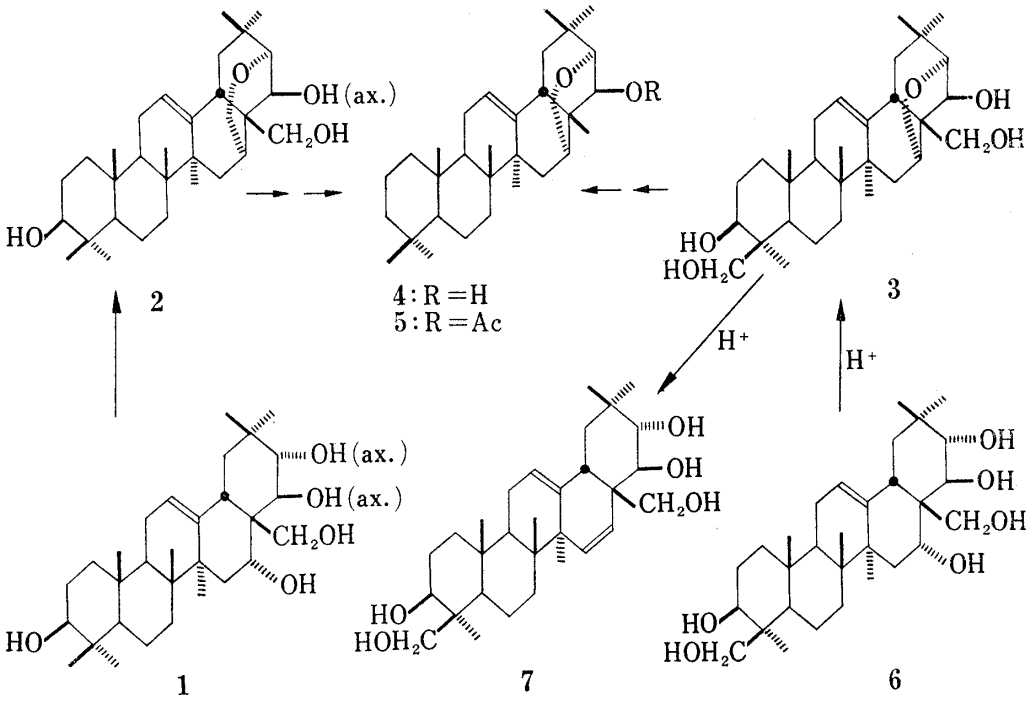

Chart 3. Proposed Structures by the Previous Workers

I), and then after standing on overnight at room temperature, the total compound was eluted out using a chloroform–methanol mixture. The product was revealed by TLC to consist mostly of a new triacetate contaminated with a minor amount of the parent triacetate (VI). The newly derived triacetate, $C_{30}H_{47}O_2(OCOCH_3)_3$, mp 158—166° (unsharp), was isolated by silicic acid column and the NMR examination of which agrees with the formulation XXII, so that it follows that acetyl migration from C-22 hydroxyl to C-21 hydroxyl has been illustrated. Considering the mechanism through the cyclic intermediate (f) as shown below (Chart 4), the triacetate (XXII) appears more favored than VI in the equilibrium. These findings

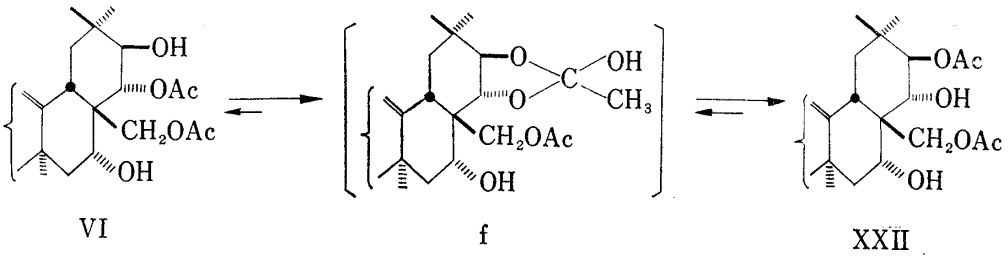

Chart 4

seem to corroborate the assumption above since the benzoyl migration has also been accepted to occur analogously.[32] It should be postulated here that the acetyl migration from C-22 to C-16 hydroxyl was not observed in the present experiment and furthermore the similar treatment of the tetraacetate (VII), where only C-16 hydroxyl is free, resulted the complete recovery of VII. The acetyl migrations in the similar environment have been reported in case of protoaescigenin (XX),[33] dihydropriverogenin A (IV)[12,16b] (=camelliagenin A, theasapogenol D) and primulagenin A (IV, without OH at C-22).[34]

33) I. Loew, *Z. Physiol. Chem.*, **348**, 839 (1967).
34) O.D. Hensens and K.G. Lewis, *Tetrahedron Letters*, **1968**, 3213.

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Experimental[35]

Isolation of Sapogenol——1) A solution of "teaponin"[3] (available from Izome Yuka Kogyo Co., Shizuoka) (250 g) in methanol (3750 ml) and water (750 ml) containing conc.HCl (300 ml) was refluxed for 10 hr on a boiling water-bath. Methanol was removed under reduced pressure while the addition of water to keep the low acidity of the mixture. The precipitate was filtered, washed with water and dried *in vacuo* to give grayish dark-brown powder (=acid hydrolysate, 85 g).

2) After refluxing a mixture of the acid-hydrolysate (120 g), methanol (3 liter), water (600 ml) and KOH (90 g) for 3 hr, the product was acidified with conc.HCl (170 ml) and evaporated under reduced pressure to remove methanol giving yellow brown precipitate, which was collected by filtration, washed with water and dried, giving a mixture of crude sapogenols (97 g). On repeating the above procedure, 1 and 2, "teaponin" (4 kg) furnished 1.15 kg of the crude sapogenol mixture (*ca.* 29% from "teaponin").

3) After removing the resinous insolubility, an acetone solution (*ca.* 5—7% (w/v)) of the crude sapogenol mixture was passed through a column of active carbon (3.5 times to the sapogenol mixture by weight) and the elution with the same solvent was succeeded to give three fractions, which on evaporation of the solvent gave the following residues: Fr.-1: 273 g, Fr.-2: 155 g, Fr.-3: 208 g (from 1.15 kg of the crude sapogenol mixture). The initial acetone insoluble residue was 220 g. TLC (Camag silica gel D-5: $CHCl_3$: MeOH =10:1)[36] disclosed the composition of Fr.-1 as theasapogenol A(I), theasapogenol E(V), barringtogenol C(II) and dihydropriverogenin A(IV).[37] As Fr.-2 and Fr.-3 were shown by TLC to contain rich amount of the more polar components (prosapogenin alike), the later experiment was performed by using Fr.-1 as the starting mixture.

4) A solution of sapogenol mixture (Fr.-1) (30 g) in 10% KOH–EtOH (800 ml) was reflxed for 2 hr, and worked up in a usual manner giving brown powder (16 g) (=final hydrolysate).[38]

5) The final hydrolysate was chromatographed on alumina (using 50—60 times by weight) eluting with benzene, benzene–$CHCl_3$, and $CHCl_3$–MeOH successively to give the following compounds as the major.

i) $CHCl_3$–MeOH (97:3) eluates: dihydropriverogenin A (colorless needles)

ii) $CHCl_3$–MeOH (90:10) :a) camelliagenin C+barringtogenol C+others;

b) barringtogenol C (colorless needles)

iii) $CHCl_3$–MeOH (80:20)—(50:50): theasapogenol A (coloress needles)

For instance, one gram of the final hydrolysate gave dihydropriverogenin A (trace), barringtogenol C (172 mg), theasapogenol A(637 mg), and camelliagenin C(trace). Theasapogenol E was not obtained in this case as explained below,[38] while silicic acid (Mallinckrodt) column chromatography of the crude sapogenol mixture (Fr.-1) (3 g) developing with $CHCl_3$–MeOH (95:5) gave theasapogenol E(200 mg). The pure sample of camelliagenin C was obtained by preparative TLC of the fractions around ii).

6) The recrystallization of the compound obtained above (ii, b) gave barringtogenol C(II), mp 278—284° (colorless needles), $[\alpha]_D$ +11° (c=0.47, pyridine). *Anal.* Calcd. for $C_{30}H_{50}O_5$: C, 73.43; H, 10.27. Found: C, 73.12; H, 9.94. IR ν_{max}^{KBr} cm^{-1}: 3367 (OH), 1634 (C=C).

3,22,28-Tri-O-acetyl- and 3,21,22,28-Tetra-O-acetyl-barrigtogenol C(VI and VII)——Barringtogenol C(II) (508 mg) was treated with pyridine (10 ml) and Ac_2O (2.5 ml) at room temperature for 5 hr as usual followed by silica gel column chromatography to give VI (174 mg, recrystallized from acetone–*n*-hexane, and VII (68 mg, recryst. from aq. EtOH). The analytical sample of VI was obtained by further recrystalli-

35) Melting points were taken on the Yanagimoto Micromelting-point Apparatus (a hotstage type) and the Ishii Highmelting-point Apparatus (a capillary type) and recorded as read. Specific rotations were measured with the Rex Photoelectric Polarimeter NEP-2(l=1 dm), the IR spectra were taken with the Hitachi EPI-2 and EPI-S2 IR Spectrophotometer, the NMR spectra were with the Hitachi–Perkin Elmer H-60 NMR Spectrometer (tetramethylsilane as the internal standard), and the Mass spectra with the Hitachi RMU-6D Spectrometer. TLC plates were developed by spraying 1% $Ce(SO_4)_2$/10% H_2SO_4 solution followed by heating.

36) The *Rf* values of theasapogenols with this solvent mixture were: theasapogenol A(I) 0.21, barringtogenol C(=theasapogenol B) (II) 0.37, camelliagenin C(=theasapogenol C) (III) 0.41, dihydropriverogenin A(=camelliagenin A, theasapogenol D) (IV) 0.67, and theasapogenol E(V) 0.34.

37) Due to the low content, a spot of camelliagenin C(III) was not detected on TLC at this stage.

38) As will be detailed in our forthcoming paper, at the early stage of our investigation on theasapogenols, we assumed that the crude sapogenol (Fr.-1) yet contained some esterified theasapogenols as revealed by its IR carbonyl absorption band, and therefore the further alkaline treatment on Fr.-1 was performed. In fact, the carbonyl absorption band of Fr.-1 was largely due to theasapogenol E(V), however, this additional alkaline treatment interestingly resulted the ready conversion of theasapogenol E(V) to theasapogenol A(I). *cf.* *a*) I. Yosioka, A. Matsuda, M. Fujio, T. Nishimura, and I. Kitagawa, The 87th Annual Meeting of Pharmaceutical Society of Japan, Kyoto, April, 1967 (abstract Paper, p. 464); *b*) I. Yosioka, A. Matsuda, and I. Kitagawa, The 88th Annual Meeting of the same Society, Tokyo, April, 1968 (abstract Paper, p. 256).

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zation with CH_2Cl_2–*n*-hexane, mp 244—246°, $[\alpha]_D$ +15° (*c*=0.50, $CHCl_3$). *Anal.* Calcd. for $C_{36}H_{56}O_8$: C, 70.10; H, 9.15. Found: C, 70.08; H, 9.15. IR ν_{max}^{KBr} cm^{-1}: 3434, 3388 (OH), 1716, 1273, 1241 ($OCOCH_3$). VII, mp 228—229° (recryst. from $CHCl_3$–MeOH), $[\alpha]_D$ +20° (*c*=0.53, $CHCl_3$). *Anal.* Calcd. for $C_{38}H_{58}O_9$: C, 69.27; H, 8.87; mol. wt., 658.84. Found: C, 69.56; H, 9.14; mol. wt. (Mass) M^+=658 *m/e*. IR ν_{max}^{KBr} cm^{-1}: 3448 (OH), 1733, 1245 ($OCOCH_3$). Mass Spectrum *m/e* (relative peak intensity, assignment): 658 (M^+), 598 (4, M^+-AcOH), 538 (7, M^+-2AcOH), 478 (5, M^+-3AcOH), 465 (6, M^+-2AcOH–CH_2OAc), 436 (5, M^+-3AcOH–CH_2CO), 405 (4, M^+-3AcOH–CH_2OAc), 390 (11, e-H_2O), 348 (30, e-AcOH), 288 (17, e-2AcOH), 270 (19, e-2AcOH–H_2O), 257 (40, e-AcOH–CH_2OAc–H_2O), 249 (23, d) 228 (38, e-3AcOH), 215 (77, 2AcOH–CH_2-OAc), 197 (72, e-2AcOH–CH_2OAc–H_2O), 190 (100, d-AcOH+H), 189 (67, d-AcOH). VII, thus obtained here, was identified with 3,21,22,28-tetra-O-acetyl-aescinidin (=3,21,22,28-tetra-O-acetyl-barrigtogenol C)[20] by mixed mp, IR ($CHCl_3$), and TLC.

3,16,21,22,28-Penta-O-acetyl-barringtogenol C(VIII)——A mixture of barringtogenol C(II) (150 mg) in Ac_2O (10 ml) with a catalytic amount of *p*-TsOH·H_2O was kept stirring at room temperature for 3 hr to give a clear solution, which was then let stand for further 25 hr, poured into ice–water and treated as usual. The product (205 mg, single spot on TLC) was crystallized from aq. EtOH repeatedly(to give colorless needles (VIII) of mp 147—150°, $[\alpha]_D$ −10° (*c*=1.03, $CHCl_3$). *Anal.* Calcd. for $C_{40}H_{60}O_{10}$: C, 68.54; H, 8.63. Found: C, 68.53; H, 8.53. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1745, 1243, 1225 ($OCOCH_3$), no hydroxyl. Alkaline hydrolysis of VIII (17 mg) by refluxing 1 hr in 5% KOH–MeOH (4 ml) gave II, mp 277—284° (recryst. from MeOH), identified with barringtogenol C by mixed mp, IR (KBr), and TLC comparisons.

Oxidation of VII giving IX——To an ice-cooled pyridine solution (5 ml) of VII (200 mg), was added CrO_3–pyridine complex (300 mg–5 ml) and the total mixture was stirred for 8 hr at R.T. and treated as usual to give 155 mg of a crude product. The silica gel chromatography followed by recrystallization with CH_2Cl_2–MeOH yielded IX, mp 281—283°, $[\alpha]_D$ −34° (*c*=0.41, $CHCl_3$). *Anal.* Calcd. for $C_{38}H_{56}O_9$: C, 69.48; H, 8.59; mol. wt., 656.83. Found: C, 69.37; H, 8.56; mol. wt. (Mass) M^+=656 *m/e*. IR ν_{max}^{KBr} cm^{-1}: 1739 ($OCOCH_3$), 1718 (CO). Mass Spectrum *m/e*: 656 (3, M^+), 523 (31, M^+-AcOH–CH_2OAc), 463 (18, M^+-2AcOH–CH_2OAc), 406 (19, c), 346 (15, c-AcOH), 304 (26, c-AcOH–CH_2CO), 249 (11, d), 231 (10, c-AcOH–CH_2OAc–CH_2CO), 213 (13, c-2AcOH–CH_2OAc), 190 (100, d+H-AcOH), 189 (33, d-AcOH).

Alkaline Treatment of IX——A solution of IX (102 mg) in 95% EtOH (70 ml) was added with aq. solution (30 ml) of KOH (4 g) under ice cooling. After keeping overnight at 25°, the mixture was adjusted to pH 3 with 33% aq. H_2SO_4 and then with 1% aq. H_2SO_4 (Fair amount of Na_2SO_4crystallized out at this stage). The total mixture was then distilled on a boiling water-bath. The distillate was treated with dimedone (70 mg), let stand overnight (25°), and concentrated to a volume of 10 ml, when the colorless needles (4.3 mg) separated out. Recrystallization once with aq. EtOH gave a pure sample of mp 189—191°, which was identified with dimethone by mixed mp, IR(KBr), and TLC. The blank test (the procedure as above without IX) gave no indication of dimethone.

Acetonide Derivatives, X and XI, from Barringtogenol C(II)——A suspension of barringtogenol C(II) (514 mg) in anhydrous acetone (100 ml) containing 2,2-dimethoxy propane (0.5 ml) was added with *p*-TsOH·H_2O (0.15 g) and kept stirring at R.T. Within several minutes, the reaction mixture turned to a clear solution. After 1 hr, the solution was treated with anhydrous pyridine (1 ml), evaporated under reduced pressure to remove acetone and diluted with water. The colorless precipitate was then filtered, washed successively with water, aq. K_2CO_3, water and dried *in vacuo* to yield a crude product (554 mg). Washing the product with benzene recovered II (91 mg) as an insoluble portion. The benzene soluble part was chromatographed on alumina (Merck, standard, 50 g). The $CHCl_3$ eluted fractions gave XI (230 mg, after acetone–*n*-hexane recrystallization), while the later fractions eluted by $CHCl_3$–MeOH (100:2) furnished X (67 mg after CH_2Cl_2–MeOH recrystallization). Analytical sample of X was obtained by repeated recrystallization with CH_2Cl_2–MeOH, colorless needles, mp 206—208°, $[\alpha]_D$ +4° (*c*=1.04, $CHCl_3$). *Anal.* Calcd. for $C_{33}H_{54}O_5 \cdot \frac{1}{2}H_2O$: C, 73.43; H, 10.27. Found: C, 73.40; H, 10.45. IR ν_{max}^{KBr} cm^{-1}: 3413 (OH), 1650 (C=C). XI, mp 255—262° (recryst. from ether–*n*-hexane). *Anal.* Calcd. for $C_{33}H_{54}O_5$: C, 74.67; H, 10.26. Found: C, 74.57; H, 10.07.

Acetylation of X and XI yielding XII, XIII and XIV——A usual treatment of X (50 mg) with pyridine (2.5 ml) and Ac_2O (1.5 ml) at 35° for seven days afforded a crude product (57 mg), which was purified by preparative TLC (Camag D-5, $CHCl_3$:MeOH=50:1) to give XII (colorless, amorphous; many attempts for crystallization being without success). *Anal.* Calcd. for $C_{39}H_{60}O_8$: C, 71.31; H, 9.21. Found: C, 71.06; H, 9.24. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1743, 1237 ($OCOCH_3$), no hydroxyl.

A similar treatment of XI (52 mg) with pyridine (2.5 ml) and Ac_2O (1.5 ml) at 35° for 2 days gave a product, which on crystallization with aq. MeOH furnished XIII (53 mg). Analytical sample of XIII obtained by recrystallization with aq. MeOH showed mp 237—239°, $[\alpha]_D$ +19° (*c*=1.21, $CHCl_3$). *Anal.* Calcd. for $C_{37}H_{58}O_7$: C, 72.27; H, 9.51. Found: C, 72.09; H, 9.54. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1733, 1242 ($OCOCH_3$).

Refluxing a mixture of XI (102 mg), pyridine (0.5 ml), and Ac_2O (5 ml) for 3 hr in an oil–bath, followed by a usual working-up gave a crude product (120 mg), which was purified by passing through alumina column (Woelm, grade I, neutral) with the aid of ether and recrystallized repeatedly with ether–*n*-hexane giving XIV, mp 249—250°. *Anal.* Calcd. for $C_{39}H_{60}O_8$: C, 71.31; H, 9.21. Found: C, 71.03; H, 9.05. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1743, 1237 ($OCOCH_3$), no hydroxyl.

16,21-Anhydro-3,22,28-tri-O-acetyl-barringtogenol C (=3,22,28-Tri-O-acetyl-barringtogenol D) (XV)——

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A mixture of VI (155 mg), pyridine (25 ml) and $POCl_3$ (2.5 ml) was refluxed 2 hr and treated as usual giving a slightly discolored product (125 mg, single spot on TLC). After the alumina column chromatography (Woelm, grade I, neutral, 3 g) of the product with benzene, pure sample of XV was obtained, mp 221.5—223° (recryst. from *n*-hexane), $[\alpha]_D$ +65° (c=0.54, $CHCl_3$). *Anal.* Calcd. for $C_{36}H_{54}O_7$: C, 72.21; H, 9.09. Found: C, 72.36; H, 9.04. IR ν_{max}^{KBr} cm^{-1}: 1745, 1240 ($OCOCH_3$).

16,21-Anhydro-barringtogenol C (=Barringtogenol D) (XVI)——i) From XV: Alkaline hydrolysis of XV (114 mg) with 5% KOH–MeOH (30 ml) by refluxing 2 hr afforded 72 mg of crystalline product. Recrystallization with acetone–*n*-hexane and then with AcOEt gave colorless leaflets (XVI), mp 301—312°, $[\alpha]_D$ +73.5° (c=0.35, EtOH). IR ν_{max}^{KBr} cm^{-1}: 3401 (OH), 1629 (C=C). The sample was identical with barringtogenol D by mixed mp, IR (KBr), and TLC. ii) From Barringtogenol C (II): Barringtogenol D (XVI) was also obtained along with one new dioxane compound directly from II by heating in DMSO which will be discussed in the following paper.[39]

Acetylation of XVI (28 mg) with pyridine (2.5 ml)–Ac_2O (1 ml) in a usual manner gave XV, mp 221.5—223° (colorless needles from *n*-hexane), which was identified with authentic barringtogenol D triacetate[8] by mixed mp, IR (KBr), and TLC.

Oxidation of 16,21-Anhydro-barringtogenol C (XVI) followed by Methylation yielding XVII and XVIII—— To an ice-cooled suspension of XVI (220 mg) in acetone (15 ml) was added Kiliani reagent dropwise (total 1.5 ml) with stirring during 1 hr. The mixture was poured into ice–water and extracted with ether. The ether solution was treated with 1% NaOH to separate acidic part. The ether soluble neutral portion was oxidized again with the Kiliani reagent as above. The combined acidic part (198 mg, amorphous) was chromatographed on silica gel (Merck, 15 g) developing with (i) $CHCl_3$, (ii) $CHCl_3$–MeOH (100:1), (iii) $CHCl_3$–MeOH (10:1) successively. The fractions from (i) and early part of (ii) gave a diketo-acid, while from the later (ii) and (iii) a monoketo-acid (major) was obtained. The monoketo-acid (153 mg) was again subjected to the Kiliani oxidation (acetone 8 ml, Kiliani reagent 0.5 ml) as above, however it was found on TLC that most of the monoketo-acid was unchanged. The reaction product was treated with diazomethane in ether and recrystallized with ether and acetone successively to give 53 mg of the monoketo-methylester (XVII). The mother liquor was chromatographed on alumina (Merck, neutral, grade I, 15 g) eluting with benzene to yield the diketo-methylester (XVIII), which was combined with the diketo-methylester prepared by diazomethane treatment of the above diketo-acid (obtained from (i) and early (ii)) and recrystallized with EtOH to afford colorless needles of XVIII. The $CHCl_3$–benzene (1:1) and $CHCl_3$ eluting portion of the above alumina chromatography furnished additional crop (44 mg) of XVII (Total yield of XVII was 97 mg). Analytical sample of XVII was obtained by recrystallization with MeOH and then with AcOEt, mp 273—276° (colorless needles), $[\alpha]_D$ +77° (c=1.05, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{46}O_5$: C, 74.66; H, 9.30. Found: C, 74.40; H, 9.14. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 3420(OH), 1727 ($COOCH_3$), 1698 (CO). Recrystallization with *n*-hexane and then with MeOH gave XVIII, mp 214—216° (colorless needles). *Anal.* Calcd. for $C_{31}H_{44}O_5$: C, 74.96; H, 8.93. Found: C, 75.05; H, 8.96. IR $\nu_{max}^{CCl_4}$: cm^{-1}: 1773 (CO), 1743 ($COOCH_3$), 1710 (CO).

Acetyl Migration of 3,22,28-Tri-O-acetyl-barringtogenol C (VI) giving 3,21,28-Tri-O-acetyl-barringtogenol C (XXII)——A solution of VI (139 mg) in benzene (dried over $CaCl_2$) was adsorbed on a column of alumina (Woelm, neutral, grade I, 14 g) and let stand at R. T. for 24 hr. The column was then developed with $CHCl_3$ and $CHCl_3$–MeOH (3:1) successively to elute out all the components. Next, the crude eluates was chromatographed on silicic acid column (Mallinckrodt, 15 g) developing with $CHCl_3$ to afford XXII (101 mg) and VI (19 mg). The analytical sample of XXII (colorless needles) was obtained by recrystallization with aq. EtOH, mp 158—166° (unsharp), $[\alpha]_D$ +39° (c=1.08, $CHCl_3$). *Anal.* Calcd. for $C_{36}H_{56}O_8$: C, 70.10; H, 9.15. Found: C, 70.30; H, 9.29. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (shoulder), 3510 (OH), 1745 (sh.), 1733, 1720 (sh.) ($OCOCH_3$).

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39) I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **18** 1621 (1970).

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