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Saponin and Sapogenol. IV.[1] Seeds Sapogenols of *Aesculus turbinata* Blume. On the Configuration of Hydroxyl Functions in Ring E of Aescigenin, Protoaescigenin, and Isoaescigenin in Relation to Barringtogenol C and Theasapogenol A

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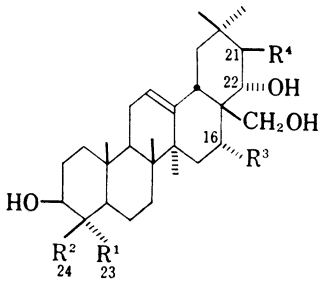
1) The sapogenol composition of Japanese horse chestnuts (*Aesculus turbinata* Blume, Hippocastanaceae) has been clarified as barringtogenol C (I), aescigenin (VI), protoaescigenin (VII) and 16-desoxy-barringtogenol C (IX). 2) The structure elucidation of the new sapogenol named 16-desoxy-barringtogenol C (IX) has been described in detail. 3) The full account on the configuration of hydroxyl functions in ring E of aescigenin (VI; 22α-OH), protoaescigenin (VII; 21β-OH, 22α-OH) and isoaescigenin (VIII; 21α-OH, 22α-OH) in relation to barringtogenol C (I) and theasapogenol A (II) has been presented.

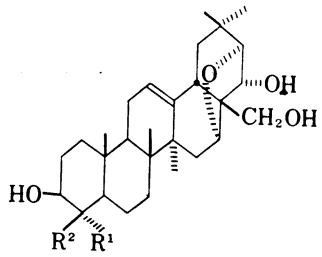
In the series of papers on saponin and sapogenol, we have so far detailed the investigations on five tea seeds sapogenols, *i.e.* barringtogenol C (I),[3] theasapogenol A (II),[4] theasapogenol E (III),[1] dihydropriverogenin A (IV),[1] and camelliagenin C (V).[1] As mentioned briefly in Part I,[3] the structures of aescigenin, protoaescigenin, and isoaescigenin, which are closely related to barringtogenol C particularly on the hydroxyl configuration in ring E, have been revised from **1**, **2**, and **3** to VI, VII, and VIII respectively in our previous communication.[5] Later, Nakano, *et al.* have also communicated the same conclusion.[6] Since then, the correctness of our presentation has been approved by Barua, *et al.*,[7] Tschesche, *et al.*[8,9] and finally concluded X-ray crystallographically by Hoppe, *et al.*[10]

In this paper, we describe the study on the sapogenol constituents of Japanese horse chestnuts (*Aesculus turbinata* Blume, seeds, Hippocastanaceae),[11] which has enabled us to examine aescigenin, protoaescigenin and isoaescigenin from the stereochemical view-point, and also detail the structure elucidation of a minor sapogenol, named 16-desoxy-barringtogenol C(IX).[12] In addition, we present the full account of our effort on the configuration of hydroxyl functions in ring E of aescigenin (VI), protoaescigenin (VII), and isoaescigenin (VIII) in relation to barringtogenol C (I)[3] and theasapogenol A (II).[4]

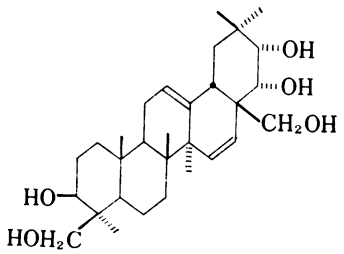
1) Part III: I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 1186 (1971).
2) Location: *Toneyama, Toyonaka, Osaka.*
3) I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **18**, 1610 (1970).
4) I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **18**, 1621 (1970).
5) I. Yosioka, T. Nishimura, A. Matsuda, K. Imai, and I. Kitagawa, *Tetrahedron Letters*, **1967**, 637.
6) *a*) T. Nakano, M. Hasegawa, and J.B. Thomson, *Tetrahedron Letters*, **1967**, 1675; *b*) T. Nakano, M. Hasegawa, T. Fukumaru, L.J. Durham, H. Budzikiewicz, and C. Djerassi, *J. Org. Chem.*, **34**, 3135 (1969).
7) A.K. Barua, S.P. Dutta, and B.C. Das, *Tetrahedron*, **24**, 1113 (1968).
8) R. Tschesche, B.T. Tjoa, and G. Wulff, *Tetrahedron Letters*, **1968**, 183.
9) G. Wulff and R. Tschesche, *Tetrahedron*, **25**, 415 (1969).
10) W. Hoppe, A. Gieren, N. Brodherr, R. Tschesche, and G. Wulff, *Angew. Chem.*, **80**, 563 (1968).
11) I. Yosioka, K. Imai, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **15**, 135 (1967) (Preliminary report on the sapogenol constituents.)
12) I. Yosioka, K. Imai, and I. Kitagawa, *Tetrahedron Letters*, **1967**, 2577.



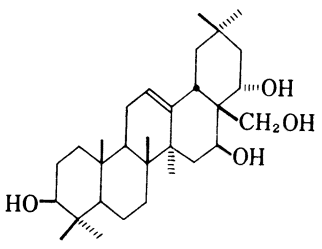
	R^1	R^2	R^3	R^4	
I	CH_3	CH_3	OH	OH	barringtogenol C
II	CH_2OH	CH_3	OH	OH	theasapogenol A
III	CHO	CH_3	OH	OH	theasapogenol E
IV	CH_3	CH_3	OH	H	dihydropriverogenin A
V	CH_2OH	CH_3	OH	H	camelliagenin C
VII	CH_3	CH_2OH	OH	OH	protoaescigenin
IX	CH_3	CH_3	H	OH	16-desoxy-barringtogenol C



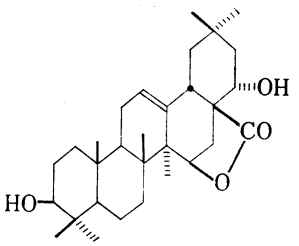
	R^1	R^2	
VI	CH_3	CH_2OH	aescigenin
X	CH_3	CH_3	barringtogenol D
XIII	CH_2OH	CH_3	16,21-anhydro-theasapogenol A



VIII isoaescigenin



XI chichipegenin



XII dumortierigenin

Chart 1

Sapogenol Constituents of *Aesculus turbinata* Blume Seeds

On European horse chestnuts sapogenols (*A. hippocastanum* L., seeds), Jeger and his co-workers investigated the composition and proposed the structure of aescigenin as (**1**) in 1957.[13] Later on, Kuhn and Loew have succeeded[14] in the isolation of protoaescigenin (**2**) which was presumably considered as a genuine sapogenol form of aescigenin before[13] and also have isolated some acylated derivatives of protoaescigenin.[15] Furthermore, Kuhn and Loew[16] and Tschesche and Wulff[17] independently have elucidated the identity of a minor sapogenol, then named aescinidin, with barringtogenol C (the proposed structure at that time was **4**,[18] *cf.* Part I[3]). In 1966, Thomson presented the investigation on isoaescigenin proposing the structure **3**.[19] It should be noticed that all the structures mentioned above have more or less been based on the structure of aescigenin (**1**),[13] which, however, has now been

13) G. Cainelli, A. Melera, D. Arigoni, and O. Jeger, *Helv. Chim. Acta*, **40**, 2390 (1957).
14) R. Kuhn and I. Loew, *Ann.*, **669**, 183 (1963).
15) R. Kuhn and I. Loew, *Tetrahedron*, **22**, 1899 (1966).
16) R. Kuhn and I. Loew, *Tetrahedron Letters*, **1964**, 891.
17) R. Tschesche and G. Wulff, *Tetrahedron Letters*, **1965**, 1569.
18) A.K. Barua and P. Chakrabarti, *Tetrahedron*, **21**, 381 (1965).
19) J.B. Thomson, *Tetrahedron*, **22**, 351 (1966).

revised to VI as clarified in the later part of this paper. As for the saponin named aescin, there have been presented the extensive studies by Tschesche and his co-workers upon the structure.[9,20]

Meanwhile, concerning Japanese horse chestnuts (*A. turbinata* BLUME, seeds), there were two reports in 1958 by Kariyone and Tobinaga on the isolation[21] and the chemical study[22] of sapogenol named japoaescigenol, for which they claimed the nonidentity with aescigenin. Thereafter, however, no conclusive investigation on the structure of the sapogenol has been provided.

In view of the comparative study and expecting the isolation of aescigenin and protoaescigenin, we have examined the sapogenol constituents of Japanese horse chestnuts.

Hot methanolic extract of air dried seeds was at first partitioned with butanol and water. Crude saponin mixture (6.7% from seeds) obtained by defatting the butanol soluble portion with ether was then subjected to acid and alkaline hydrolysis followed by alumina column chromatography, furnishing protoaescigenin, aescigenin, and barringtogenol C with the respective yields as shown in Table I. The identification of these sapogenols with the authentic specimen was attained by direct comparison or by comparison of the corresponding acetates as described in experimental section through the courtesy of Professor Tschesche to whom we are highly obliged. Moreover, the column chromatography has led the isolation of a new sapogenol, designated Rx, in 0.8% yield. The structure elucidation assigning 16-desoxybarringtogenol C (IX) to it is detailed later. Although there was a difference in the hydrolysis condition between two experiments as explained in Table I, the results indicate the significant resemblance of the both sapogenol compositions.

TABLE I[a]

	A. hippocastanum L. seeds[b] (%)	*A. turbinata* BLUME seeds[c] (%)
Protoaescigenin (VII)	63	50
Aescigenin (VI)	14	7.8
Barringtogenol C (I)	19	3.0
Barringtogenol D (X)	4	—[d]
16-Desoxybarringtogenol C (IX)	—[d]	0.8

a) TLC comparison disclosed that the compositions of both sapogenol mixtures were almost identical except a minor spot having lower *Rf* value than protoaescigenin in European horse chestnuts sapogenols.
b) According to Tschesche and Wulff.[17] The saponin was hydrolyzed with 1N HCl in EtOH/H_2O at 90° for 14 hr.
c) by us. The saponin was hydrolyzed with 2N HCl in EtOH/H_2O at reflux for 2 hr followed by 5% KOH-MeOH treatment at reflux for 1 hr.
d) Not isolated, but detected on TLC.

On the Hydroxyl Configuration in Ring E of Protoaescigenin and Aescigenin

As reported in Part I,[3] the assignment of the hydroxyl configuration in ring E of barringtogenol C(I) was done on the basis of the nuclear magnetic resonance (NMR) examination. Thus, (i) the signals due to C-21H and C-22H of barringtogenol D triacetate (Xa) have been seen as two singlets at 6.39 and 4.71 τ, and (ii) two hydrogens at C-21 and C-22 of barringtogenol C tetraacetate (Ib) have been observed as an AB quartet at 4.40 and 4.54 τ with a coupling constant of 10 Hz, while a pair of doublets at 6.06 and 4.76 τ with a splitting of 10 Hz have been ascribed to C-21H and C-22H in the triacetate (Ia) respectively. These observations have enabled us to assign the structures of barringtogenol D (X) possessing C-22αOH

20) R. Tschesche, U. Axen, and G. Snatzke, *Ann.*, **669**, 171 (1963).
21) T. Kariyone and S. Tobinaga, *Yakugaku Zasshi*, **78**, 531 (1958).
22) S. Tobinaga, *Yakugaku Zasshi*, **78**, 534 (1958).

and barringtogenol C(I) with C-21βOH and C-22αOH. It should be stated here that the above conclusion has been based on Karplus equation[23] with the assumption that the ring E of Ia, b, and Xa lie in the chair conformation.

On the other hand, the reasons by Barua and his co-workers providing the structures (**4** and **5**) to barringtogenols C and D were as follows. (iii) The reactivity of C-22 hydroxyl function of barringtogenol C(**4**) as compared with that of chichipegenin (XI), which possesses C-22αOH,[24] is significantly different[18]: *e.g.* the less reactivity towards benzoylation and the facile acetonide formation between C-22 and C-28 hydroxyls in the former contrary to the latter.[24] (iv) Barringtogenols C and D were chemically correlated[18,25] to aescigenin derivatives (**6** and **7**), whose configuration at C-22 had already been proposed as β by Jeger, *et al.*[13] at that time. (v) The lead tetraacetate oxidation rate of barringtogenol C was quite slow thus suggesting the trans glycol configuration.[18]

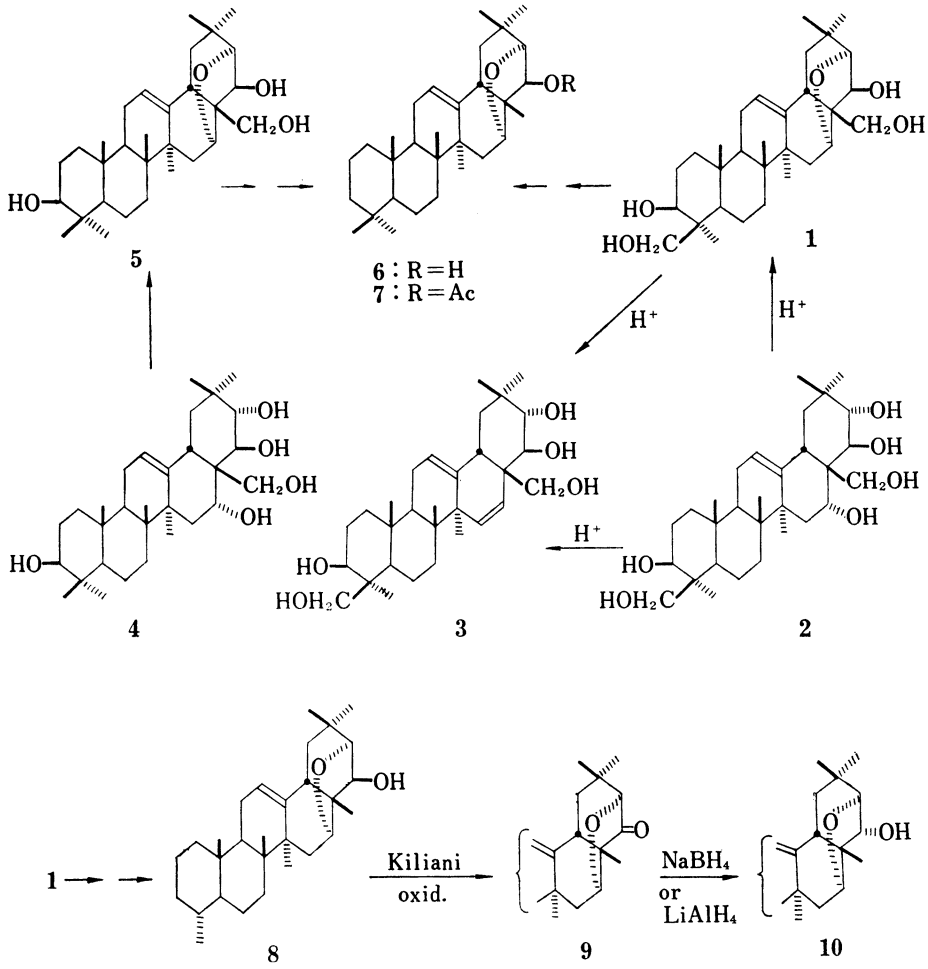
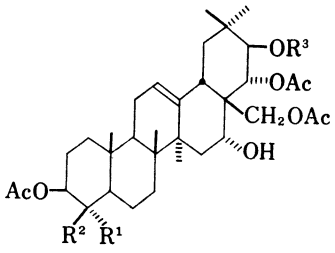


Chart 2. Proposed Structures by Previous Workers

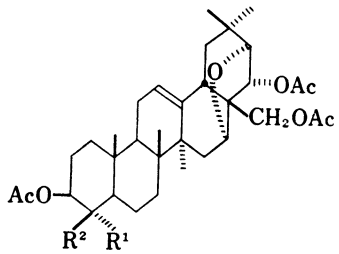
23) M. Karplus, *J. Am. Chem. Soc.*, **85**, 2870 (1963).
24) A. Sandoval, A. Manjarrez, P.R. Leeming, G.H. Thomas, and C. Djerassi, *J. Am. Chem. Soc.*, **79**, 4468 (1957).
25) S.K. Chakraborti and A.K. Barua, *Tetrahedron*, **19**, 1727 (1963).

Among the above three major reasons (iii—v), the less reactive benzoylation of C-22OH in barringtogenol C could not be a definite evidence for β(axial) configuration as discussed in Part I[3] in terms of acyl migration. The acetonide formation between C-22 and C-28 hydroxyls of barringtogenol C seems to be incomparable[8] with chichipegenin since the latter differs in the hydroxyl configuration at C-16. Chichipegenin (XI) possesses C-16βOH and therefore the acetonide bonding in case of chichipegenin either between C-16β and C-28 hydroxyls or between C-16β and C-22α hydroxyls appears more feasible as compared with that between C-22α and C-28 hydroxyls. Because in the latter as revealed by Dreiding model inspection, the 1,3-dioxane ring of 22,28-acetonide moiety seems to suffer the peri interactions caused by C-16β hydroxyl and two oxygen functions attached to C-28 and C-22α provided that the dioxane ring is in the chair conformation as depicted in Fig. 1A (*cf.* Part III[1]). Accordingly, it follows that the reason (iii) seems inapplicable to determine the hydroxyl configuration at C-22 of barringtogenol C. In regard to the reason (iv) by Barua, *et al.*, the consideration of the work on aescigenin presenting the structure(**1**) done by Jeger, *et al.*[13] should be made. According to their presentation as schemed in Chart 2, a ketone (**9**) derived through the Kiliani oxidation of 24-nor-22ξ-hydroxy-16α,21α-epoxy-olean-12-ene (**8**), which was prepared from aescigenin(**1**) and retains its C-22 configuration, furnished an epimer upon $NaBH_4$ or $LiAlH_4$ reduction. The assignment(**10**) (with C-22αOH) for the epimer was made on the basis of assuming that as the 22-ketone derivative of dumortierigenin (XII) was known to afford an equatorial hydroxyl derivative *via* metal hydride reduction,[26] the ketone(**9**) would also give a thermodynamically favored equatorial derivative(**10**). Therefore, the parent alcohol(**8**), which is epimeric to the product(**10**), has been assigned with β-axial hydroxyl at C-22 and consequently the structure(**1**) has been given to aescigenin. However, in relation to the assigned structure of barringtogenol C (I)[3] and theasapogenol A (II),[4] it seemed worthwhile to re-examine the assumption in particular since there has been no appropriate example for the metal hydride reduction of the carbonyl function located in such a strained system. Our effort along this line is given later. The determination of α-glycol configuration by employing the lead tetraacetate oxidation method (reason v) brought out in consequence the correct assumption in the sense of trans glycol, but as was experienced by Thomson[19] in case of the assigning structure (**3**) for isoaescigenin, the method could not always be crucial especially when lacking the systematic study using the ample examples of oleanene derivatives possessing the E ring glycol.

To settle the above described problem, we have engaged in the investigation given below.



Ia: $R^1=R^2=CH_3$, $R^3=H$
Ib: $R^1=R^2=CH_3$, $R^3=Ac$
IIa: $R^1=CH_2OAc$, $R^2=CH_3$, $R^3=H$
IIb: $R^1=CH_2OAc$, $R^2=CH_3$, $R^3=Ac$
VIIa: $R^1=CH_3$, $R^2=CH_2OAc$, $R^3=H$
VIIb: $R^1=CH_3$, $R^2=CH_2OAc$, $R^3=Ac$



VIa: $R^1=CH_3$, $R^2=CH_2OAc$
Xa: $R^1=R^2=CH_3$
XIIIa: $R^1=CH_2OAc$, $R^2=CH_3$

Chart 3

26) C. Djerassi, C.H. Robinson, and D.B. Thomas, *J. Am. Chem. Soc.*, **78**, 5685 (1956).

TABLE II. NMR Data at 60 MHz given in τ Values, J Values in the Parentheses are given in Hz[a)]

	C-21H	C-22H
Protoaescigenin tetraacetate (VIIa)[b)]	6.09 (1H, d, 10)[c)]	4.86 (1H, d, 10)[c)]
Barringtogenol C triacetate (Ia)[3)]	6.06 (1H, d, 10)	4.76 (1H, d, 10)
Theasapogenol A tetraacetate (IIa)[4)b)]	6.06 (1H, d, 10)[c)]	4.82 (1H, d, 10)[c)]
Protoaescigenin pentaacetate (VIIb)[b)]	4.58, 4.70 (2H, ABq, 10)	
Barringtogenol C tetraacetate (Ib)[3)]	4.40, 4.54 (2H, ABq, 10)	
Theasapogenol A pentaacetate (IIb)[4)b)]	4.48, 4.59 (2H, ABq, 10)	
Aescigenin tetraacetate (VIa)[b)]	6.49 (1H, s)	4.80 (1H, s)
Barringtogenol D triacetate (Xa)[3)]	6.39 (1H, s)	4.71 (1H, s)
16, 21-Anhydro-theasapogenol A tetraacetate (XIIIa)[4)]	6.39 (1H, s)	4.70 (1H, s)

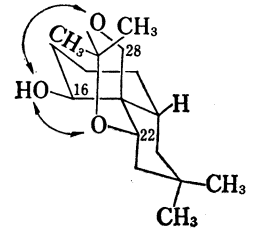
a) abbreviation: d=doublet, ABq=AB type quartet, s=singlet
b) measured at 100 MHz
c) Confirmed by the decoupling experiment.

At first, the NMR examination of the acetyl derivatives of protoaescigenin(VII) and aescigenin (VI) has been performed in comparison with those of barringtogenol C (I) and theasapogenol A(II). On acetylation with acetic anhydride and pyridine at room temperature, protoaescigenin (VII) afforded a tetraacetate(VIIa), while the acetylation of VII at reflux for 50 minutes yielded a pentaacetate(VIIb). In the NMR data as given in Table II, the signals due to C-21H and C-22H of VIIb are observed as an AB quartet at 4.58 and 4.70 τ with a coupling constant of 10 Hz and those of VIIa are found as a pair of doublets at 6.09 and 4.86 τ (J=10 Hz). These coupling patterns are consistent with the assignment VII (*cf.* Fig. 1B) where hydrogens at C-21 and C-22 lie in the *trans*-diaxial orientation similarly as in case of the acetyl derivatives of barringtogenol C (I)[3)] and theasapogenol A (II).[4)] However, these data are not necessarily sufficient to fix conclusively the configuration of C-21OH and C-22OH as being VII, since if ring E constitutes a boat conformation (Fig. 1C) the assignment giving C-21αOH and C-22βOH (as the structure (**2**)) would not be inconsistent with the data. Strictly speaking, it follows that the above NMR data are only valid for assigning the *trans* α-glycol at C-21 and C-22 of protoaescigenin unless the conformation of ring E is established.

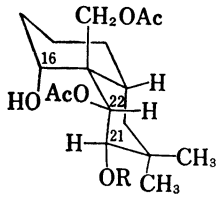
In the NMR spectrum of aescigenin tetraacetate (VIa) (Table II), in which the chair conformation of ring E is considered certain due to the 16,21-epoxy moiety, two singlets at 6.49 and 4.80 τ ascribable respectively to C-21H and C-22H are seen, as were experienced in case of barringtogenol D triacetate (Xa)[3)] and 16, 21-anhydro-theasapogenol A tetraacetate (XIIIa).[4,27)] According to the Dreiding model inspection, the dihedral angle between C-21βH and C-22βH in VIa (Fig. 1D) is found *ca.* 90°, while in case of a tetraacetate derived from the structure (**1**) (with C-22βOH) the dihedral angle between C-21βH and C-22αH (Fig. 1E) becomes *ca.* 30°. If the Karplus equation[23)] holds good in this system, the structure (**1**) for aescigenin should be replaced by VI which is in good accord with the observed data and consequently the structure VII possessing C-21βOH and C-22αOH becomes reasonable for protoaescigenin and the formation of aescigenin from protoaescigenin under the acid treatment is rationalized in terms of the intramolecular S_{N2} type mechanism as depicted in Fig. 1F analogously as for the formation of 16, 21-anhydro-theasapogenol A(XIII).[4,28)]

Secondly, to shed light on the validity of the application of Karplus equation for the strained system as in VI and further, to clarify the mode of metal hydride reduction of 22-ketone function of (**9**), which has been the rationale of the assignment (**1**)[13)] as mentioned

27) Previously designated as anhydrotheasapogenol A tetraacetate (*cf.* I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Tetrahedron Letters*, **1966**, 5979).
28) Although no comment on the configuration of hydroxyls at C-21 and C-22 has been provided in their study on some acylated protoaescigenin derivatives, Kuhn and Loew[15)] have described the NMR data which are sufficient to support our presentation here.

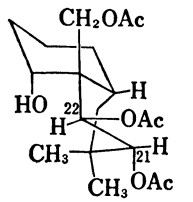


1A



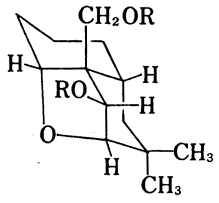
R=H or Ac
dihedral angle(θ) between
C-21αH, C-22βH= *ca.* 180°

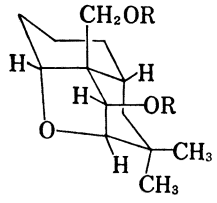
1B

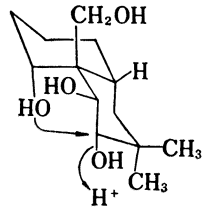


θ(C-21βH, C-22αH)
= *ca.* 180°

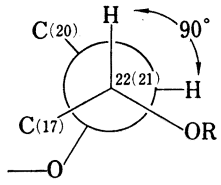
1C



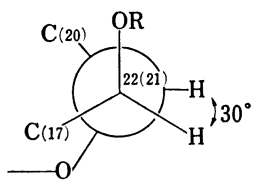




1F



1D



1E

Fig. 1A—1F

above, we have prepared two epimers, *i.e.* 22α- and 22β-hydroxy-24-nor-16α,21α-epoxy-olean-12-enes (XIV and XVI) and examined the NMR spectra of their acetates.

Thus, 24-nor-22α-hydroxy-16α,21α-epoxy-olean-12-ene(XIV) prepared from aescigenin (VI) through the modified procedure (see experimental section) was subjected to the Kiliani oxidation giving a ketone (XV).[13] $LiAlH_4$ reduction of the ketone gave rise to an epimeric compound (XVI). These results are fully consistent with those described by Jeger, *et al.*[13]

In the NMR data of the acetates as given in Table III, the signals due to C-21H and C-22H of XIVa are observed as two singlets at 6.53 and 4.97 τ, whereas the corresponding signals of the epimeric acetate (XVIa) are found as a pair of doublets at 6.33 and 5.09 τ with a coupling constant of 6 Hz. The calculation based on the Karplus equation[23] leads the assumption that the dihedral angle between C-21H and C-22H in XIVa comes to 90°, while that in XVIa comes to *ca.* 30°, and both of which coincide nicely with the Dreiding model construction of XIVa and XVIa as shown in Chart 4. Hence, it follows that the Karplus equation is valid as expected in the system concerned. In addition, the more deshielded line position of C-16βH in XIVa (5.92 τ) as compared with that of XVIa (6.30 τ) is also in accordance with the assignment, since C-16βH in XIVa falls in a peri disposition as to the oxygen function at C-22α.

Therefore, the structures of aescigenin and protoaescigenin have now been confirmed as VI and VII respectively, and further, it has been clarified that the metal hydride reduction of 22-ketone in XV proceeds from rear (probably less hindered) side furnishing the axial hydroxyl derivative (XVI) contrary to the previous assumption.[13] The similar mode of metal

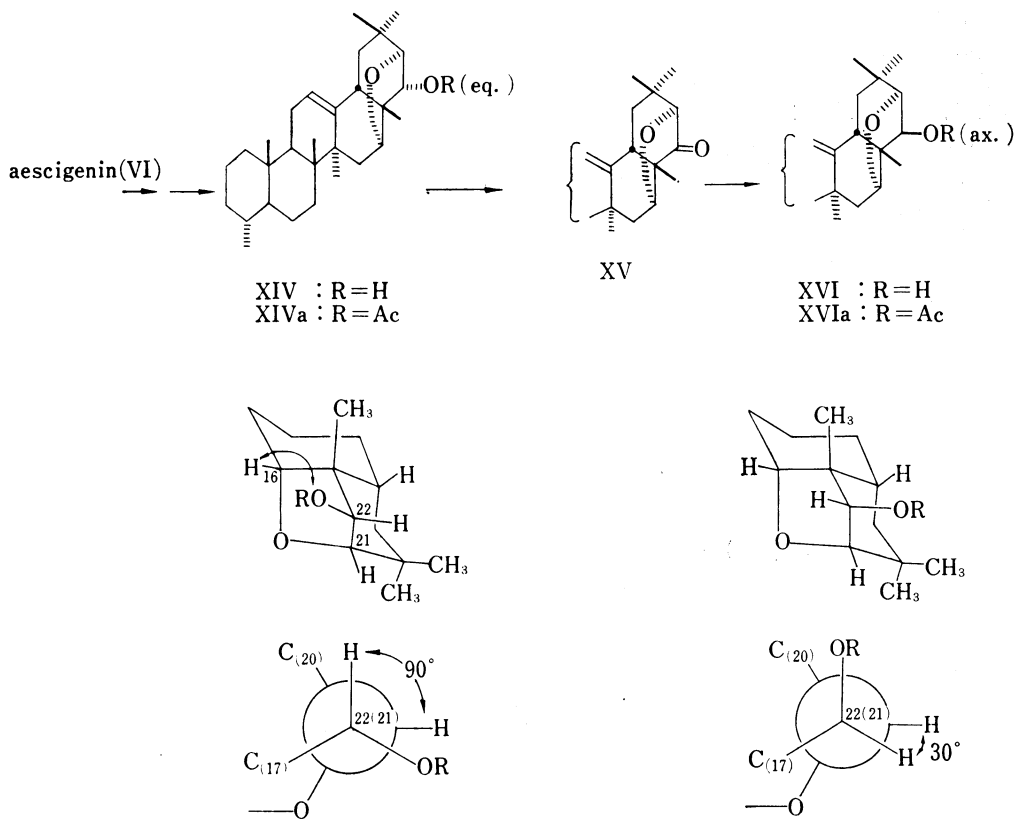


Chart 4

TABLE III. NMR Data at 100 MHz given in τ Values[a)]

	XIVa	XVIa
C-21H	6.53 (1H, s)	6.33 (1H, d, J=6 Hz)[b)]
C-22H	4.97 (1H, s)	5.09 (1H, d, J=6 Hz)[b)]
C-16βH	5.92 (1H, m)	6.30 (1H, m)

a) abbreviation: d=doublet, m=multiplet, s=singlet
b) Confirmed by the decoupling experiment.

hydride reduction of 22-ketone in dihydropriverogenin A (IV) derivative has also been described in the preceding paper.[1)]

Isoaescigenin

In 1966 Thomson proposed[19)] the structure (**3**) for isoaescigenin which was obtained by the drastic acid treatment of either aescigenin or protoaescigenin (*cf.* Chart 2). The structure (**3**) corresponds to the 16(15)-anhydro derivative of then proposed structure (**2**) of protoaescigenin. By the way, if in fact aescigenin and protoaescigenin possess the structures VI and VII respectively as elucidated above, the structure of isoaescigenin must be formulated as VIII having C-21αOH and C-22αOH on the basis of its formation mechanism as was proposed by Thomson[19)] (Chart 5), and it must be nonidentical with the 16(15)-anhydro derivative of protoaescigenin(VII).

To clarify the subject, the 16(15)-anhydro derivative[29] was prepared under the similar condition as applied for theasapogenol A(II).[4] Thus, treatment of protoaescigenin pentaacetate (VIIb) with thionyl chloride in pyridine furnished the 16(15)-anhydro derivative (XVIIa), whose NMR spectrum (Table IV) discloses the disappearance of hydroxyl function at C-16 and instead a newly formed disubstituted olefin at C-15 and C-16 by an AB quartet signal (2H) at 4.30 and 4.43 τ with a coupling constant of 10 Hz. Furthermore, another AB quartet (2H) at 4.68 and 5.28 τ (J=11 Hz) is ascribed to C-21H and C-22H which ar ein the trans diaxial orientation as in the parent acetate (VIIb). The results are in parallel with those for theasapogenol A derivatives (IIb→XIX, Table IV).[4] Importantly, the physical data (mp, IR, $[\alpha]_D$, and NMR) of 16(15)-anhydro-protoaescigenin pentaacetate (XVIIa) thus prepared above are completely distinguishable from those reported for isoaescigenin

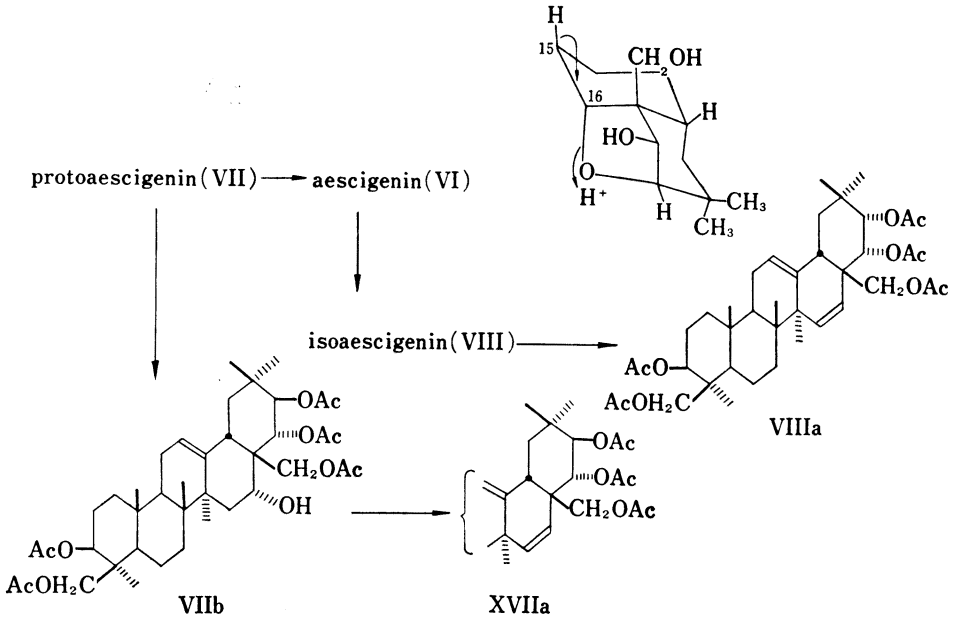


Chart 5

TABLE IV. NMR Data at 100 MHz given in τ Values, J Values in the Parentheses are in Hz[a]

	C-16H (carbinyl)	C-15H,-16H (olefinic)	C-21H,-22H
Isoaescigenin pentaacetate (VIIIa)[b]	—	4.30, 4.51 (ABq, 10.8)	4.68, 5.04 (ABq, 2.8)
16(15)-Anhydro-protoaescigenin pentaacetate (XVIIa)	—	4.30, 4.43 (ABq, 10)	4.68, 5.28 (ABq, 11)
Protoaescigenin pentaacetate (VIIb)	5.95(m)	—	4.58, 4.70 (ABq, 10)
16(15)-Anhydro-theasapogenol A pentaacetate (XIX)[c]	—	4.20, 4.34 (ABq, 11)	4.57, 5.17 (ABq, 10)
Theasapogenol A pentaacetate (IIb)	5.81(m)	—	4.48, 4.59 (ABq, 10)

a) abbreviation: ABq=AB type quartet, m=multiplet
b) data by Thomson[19]
c) measured at 60 MHz

29) Previously designated as Δ^{15}-16-desoxy derivative (I. Yosioka, T. Nishimura, A. Matsuda, K, Imai, and I. Kitagawa, ref. 5).

pentaacetate by Thomson.[19] Therefore, it is concluded that the structure of isoaescigenin could not be expressed by the 16(15)-anhydro formulation(as **3**) of protoaescigenin but is best delineated as VIII, for if the structure (**3**) were correct, 16(15)-anhydroprotoaescigenin must be identical with isoaescigenin. The coupling pattern (J=2.8 Hz) of C-21H and C-22H in the NMR data of isoaescigenin pentaacetate presented by Thomson[19] are explicable nicely by either the structure (**3**) or VIII.

In conclusion, it has been established that the structures of aescigenin, protoaescigenin and isoaescigenin are expressed as VI, VII, and VIII and hence it follows that the structures of barringtogenol C (I),[3] barringtogenol D (X),[3] theasapogenol A (II)[4] along with the related compound, *e.g.* theasapogenol E (III)[1] have also been reconfirmed.

16-Desoxy-barringtogenol C(IX)

Finally, the structure elucidation of a newly isolated minor sapogenol, designated provisionally Rx[11] and now 16-desoxy-barringtogenol C (IX),[12] is detailed. The sapogenol, $C_{30}H_{50}O_4$, mp 288—290.5°, $[\alpha]_D$ +50° (EtOH), gave a tetraacetate (IXa), $C_{30}H_{46}(OCOCH_3)_4$, mp 225—226.5°, on ordinary acetylation with acetic anhydride and pyridine. The tetraacetate lacks free hydroxyl absorption band as revealed by its IR spectrum (KBr). The NMR data of IXa (Table V) discloses the existence of seven tertiary methyls, one olefinic hydrogen and four acetoxyl functions, among which one is attached to primary and the other three are connected to secondary alcohols. The primary acetoxyl function is deduced to possess equatorial orientation as based on its chemical shift,[30] and a triplet-like signal (1H) at 5.49 τ is assigned to the carbinyl hydrogen at C-3α as judged by its characteristic signal pattern.[3,4] Remaining two carbinyl hydrogens giving signals of an AB quartet at 4.71 and 5.06 τ with a coupling constant of 10 Hz are located in a *trans*- diequatorial α-glycol system. Comparison of these data with barringtogenol C tetraacetate (Ib) along with the comparison of hydroxylation pattern with the other co-existing sapogenols from the biogenetic view-point, and especially the absence of a signal ascribable to hydrogen at C-16 carrying α-OH in IXa, have led us to assign the structure (IX= 16-desoxy-barringtogenol C) for the minor sapogenol.

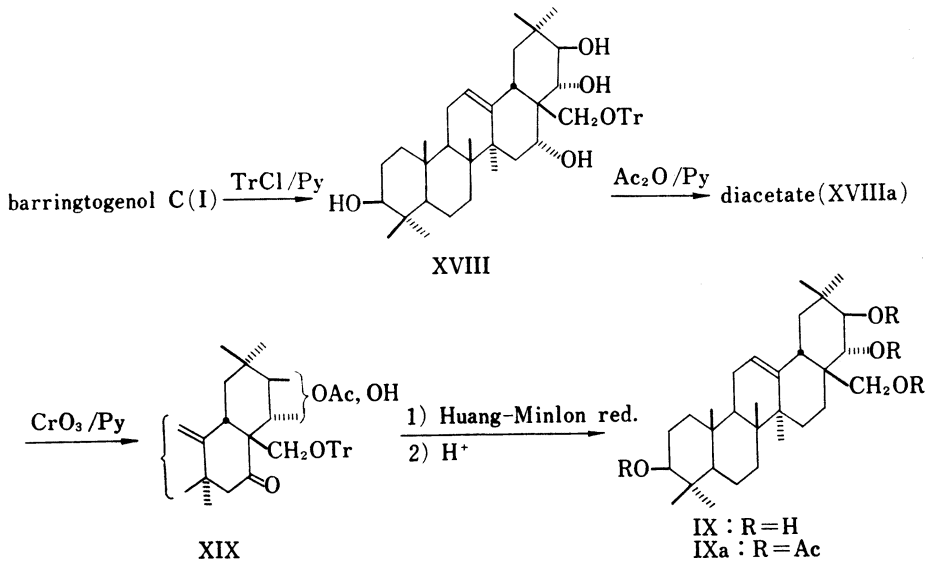


Chart 6

30) A. Gaudemer, J. Polonsky, and E. Wenkert, *Bull. Soc. Chim. France*, **1964**, 407.

Table V. NMR Data in τ Values[a)]

	IXa (100 MHz)	Ib (60 MHz)[3)]
$C_{(3)}$HOAc	5.49 (t-like)	5.45 (t-like)
$C_{(16)}$HOH	—	5.77 (m)
C=$C_{(12)}$H	4.70 (m)	4.60 (m)
—$C_{(17)}H_2$OAc	6.16 (br. s)	6.30 (br. s)
$C_{(22)}$HOAc, $C_{(21)}$HOAc	4.71, 5.06 (ABq, J=10 Hz)	4.40, 4.54 (ABq, J=10 Hz)

a) abbreviation: ABq=AB type quartet, br.s.=broad singlet, m=multiplet, t-like=triplet like

The assignment was corroborated through the conversion of barringtogenol C(I) to the sapogenol as given in Chart 6. Thus, monotrityl-barringtogenol C (XVIII) was acetylated to give a diacetate (XVIIIa), in which it should be noticed that one of the α-glycolic hydroxyls resists both for acetylation and oxidation and remained unattacked against chromic anhydride-pyridine complex oxidation as ascertained by the NMR examination of XIX (see experimental section). The monoketone (XIX) was then subjected to Huang-Minlon reduction followed by detritylation with acid affording 16-desoxy-barringtogenol C(IX), which was identified with the sapogenol concerned in all respects (mixed mp, IR, and TLC).

It should be noted finally that among four sapogenols, *i.e.* protoaescigenin (VII), aescigenin (VI), barringtogenol C(I) and 16-desoxy-barringtogenol C(IX) isolated from Japanese horse chestnuts as described in this paper, protoaescigenin, barringtogenol C and 16-desoxy-barringtogenol C have been shown to be genuine sapogenols by virtue of the soil bacterial hydrolysis method[12)] which has been developed in this laboratory for these years,[31)] thus proving the artefact of aescigenin. Wulff and Tschesche,[9)] on the other hand, have similarly disclosed that European horse chestnuts saponin (aescin) afforded neither aescigenin nor barringtogenol D(X) under the snail enzyme hydrolysis.

Experimental[32)]

Sapogenols of *Aesculus turbinata* Blume Seeds——1) Saponin: Concentrated syrupy extract of crushed Japanese horse chestnuts (1.0 kg) prepared by boiling methanol extraction 3 times, was dissolved in water and treated with *n*-butanol saturated with water. The butanol soluble portion was evaporated *in vacuo* to give resinous residue, which was then treated with ether at reflux giving crude saponin (67 g) as the deposit.

2) Hydrolysis of Saponin: A mixture of 4N HCl (130 ml) and EtOH (130 ml) containing crude saponin (25 g) was refluxed 2 hr, diluted with water and distilled *in vacuo* to remove 130 ml of the solvent. Further dilution of the residue with water yielded a precipitate, which was saponified with 5% KOH-MeOH (400 ml) at reflux for 1 hr. Dilution of the reaction mixture with water gave a precipitate, which after washing with methanol yielded crude sapogenol mixture (7.57 g).

3) Isolation of Sapogenols: Alumina (Sumitomo, acid washed, 130 g) column chromatography of the crude sapogenol mixture (2.48 g) developing with $CHCl_3$ containing MeOH (1—15 v/v%) gave four sapogenol components successively designated as Rx (20 mg, 0.8% from the crude sapogenol mixture), Ra (196 mg, 7.8%), Rb (47 mg, 3%), and Rp (1.25 g, 50%).

31) I. Yosioka, M. Fujio, M. Osamura, and I. Kitagawa, *Tetrahedron Letters*, **1966**, 6303.

32) Melting points, IR, NMR, and Mass Spectra were taken as described in the preceding paper.[1)]

The major sapogenol R_P was recrystallized with MeOH to give colorless needles of mp 300—307°, which was identified[33] with authentic protoaescigenin (VII) by mixed mp, IR(KBr), and TLC.

Recrystallization of R_A with aq. MeOH afforded colorless needles of mp 295—301°, which was acetylated with acetic anhydride and pyridine at reflux 45 min and crystallized with MeOH giving colorless needles of melting at 205—207°. NMR ($CDCl_3$, 100 MHz) τ: 9.15, 9.05 (3H each, s), 8.98 (6H, s), 8.92, 8.53 (3H each, s) (totally six methyls), 7.98 (12H, s, four acetoxyls), 6.49 (1H, s, $>C_{(21)}H$–O–), 6.27, 5.98 (2H, AB quartet, J=11 Hz, $-C_{(28)}H_2OAc$), 5.95, 5.71 (2H, AB quartet, J=11 Hz, $-C_{(24)}H_2OAc$), 5.82 (1H, m, $>C_{(16)}H$–O–), 5.49 (1H, t-like, $>C_{(3)}HOAc$), 4.80 (1H, s, $>C_{(22)}HOAc$), 4.80 (1H, m, $=C_{(12)}H$–). The product thus obtained was identified[33] with authentic aescigenin tetraacetate (VIa) by mixed mp, IR (KBr), and TLC.

R_B was recrystallized with EtOH–MeOH mixture to give colorless needles of mp 293—295°. Acetylation of the sapogenol with acetic anhydride and pyridine by keeping at 90—100° for 6 hr followed by TLC (Merck, SiO_2) separation and recrystallization with aq. EtOH, furnished an acetate (mp 227—228°), which was identified[33] with authentic aescinidin tetraacetate (=barringtogenol C tetraacetate (Ib)) by mixed mp, IR (KBr) and TLC.

Recrystallization with aq. MeOH gave an analytical sample of R_X (=16-desoxy-barringtogenol C (IX)) as colorless needles of mp 288—290.5°, $[\alpha]_D$+50° (c=0.4, EtOH). *Anal.* Calcd. for $C_{30}H_{50}O_4$: C, 75.90; H, 10.62. Found: C, 75.80; H, 10.46. IR ν_{max}^{KBr} cm^{-1}: 3378 (OH), 1630 (C=C).

Protoaescigenin Tetraacetate (VIIa)——A mixture of protoaescigenin (VII) (196 mg), Ac_2O (0.5 ml) and pyridine (1.25 ml) was let stand at 10° for 21 hr. The crude product (243 mg) obtained by usual work-up was purified by SiO_2 (Mallinckrodt) column to give protoaescigenin tetraacetate (103 mg). Analytical sample (37 mg) was prepared by repeated recrystallization with ether–*n*-hexane and then with aq. MeOH, mp 229—232°, $[\alpha]_D$+10.4° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{38}H_{58}O_{10}$: C, 67.63; H, 8.66. Found: C, 68.00; H, 8.84. NMR ($CDCl_3$, 100 MHz) τ: 9.11 (3H, s), 9.01 (6H, s), 8.99 (6H, s), 8.60 (3H, s) (totally six methyls), 7.99 (12H, s), 7.93 (3H, s) (totally four acetoxyls), 6.44, 6.36 (2H, AB quartet, J=11 Hz, $-C_{(28)}H_2OAc$), 6.09, 4.86 (2H, AB quartet, J=10 Hz, $>C_{(21)}H$–$C_{(22)}H\langle$), 5.94, 5.72 (2H, AB quartet, J=10 Hz, $-C_{(24)}H_2OAc$), 5.89 (1H, m, $>C_{(16)}HOH$), 5.48 (1H, t-like, $>C_{(3)}HOAc$), 4.71 (1H, m, $=C_{(12)}H$–).

Protoaescigenin Pentaacetate (VIIb)——Acetylation of protoaescigenin (VII) (136 mg) with Ac_2O (1 ml) and pyridine (2.5 ml) at reflux 50 min followed by TLC (Merck, SiO_2) separation afforded a crude pentaacetate 74 mg). Analytical sample (49 mg) was prepared by further recrystallization with *n*-hexane–ether mixture giving crystals (VIIb) of mp 205—211°. *Anal.* Calcd. for $C_{40}H_{60}O_{11}$: C, 67.01; H, 8.44. Found: C, 66.82, H, 8.54. NMR ($CDCl_3$, 100 MHz) τ: 9.13 (6H, s), 9.04, 9.00, 8.97, 8.61 (3H each, s) (totally six methyls), 8.05, 8.03 (3H each, s), 8.00 (9H, s) (totally five acetoxyls), 6.43 (2H, s, $-C_{(28)}H_2OAc$), 5.95 (1H, m, $>C_{(16)}HOH$), 5.96, 5.73 (2H, AB quartet, J=11 Hz, $-C_{(24)}H_2OAc$), 5.49 (1H, t-like, $>C_{(3)}HOAc$), 4.73 (1H, m, $=C_{(12)}H$–), 4.58, 4.70 (2H, AB quartet, J=10 Hz, $>C_{(21)}H$–$C_{(22)}H\langle$).

24-Nor-22α-hydroxy-16α,21α-epoxy-olean-12-ene (XIV) from Aescigenin (VI)——1) Tritylation: A mixture of aescigenin (VI) (1.6 g), pyridine (30 ml) and trityl chloride (5 g) was refluxed 3 hr. During the period, the reaction mixture was monitored by TLC and it was disclosed that the major product consisted of the

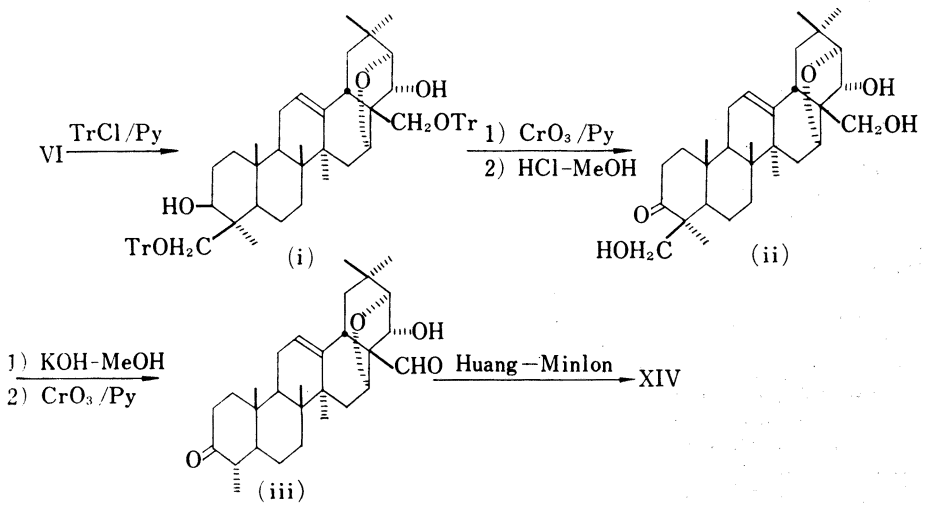


Chart 7

33) Through the courtesy of Professor Tschesche of Bonn University, Germany.

ditrityl derivative. After working up in a usual manner, the product was chromatographed on neutral alumina to isolate the major portion, which was then recrystallized with acetone–*n*-hexane affording 24, 28-di-O-trityl-aescigenin (i) (1.5 g), mp 189—192° (single spot on TLC). IR ν_{max}^{KBr} cm^{-1}: 3367, 1616, 1595, 1493, 1451, 776, 764, 748.

2) Oxidation followed by Detritylation: Pyridine solution (10 ml) of the ditrityl ether (i) (1.2 g) was treated with CrO_3–pyridine complex (CrO_3 2 g, pyridine 30 ml) at room temperature 3 hr. Work up in a usual manner gave a product (1.1 g), which showed single spot on TLC. Since C-22α hydroxyl in 16α,21α-epoxy-olean-12-ene system resists the oxidation under the applied condition as mentioned before,[3] the product is considered to be 3-ketone derivative. Next, the monoketone was detritylated with 1% HCl–MeOH (conc. HCl 2 ml, MeOH 70 ml) at reflux for 20 min. Subsequent chromatography using neutral alumina gave a product (475 mg, single spot on TLC), which was recrystallized with MeOH affording the 3-ketone (ii), mp 245—246°. IR ν_{max}^{Nujol} cm^{-1}: 3350 (OH), 1705 (six-membered carbonyl).

3) Reverse Aldolization and Oxidation followed by Huang–Minlon Reduction: The 3-ketone derivative (ii) (435 mg) was treated with 1% KOH–MeOH (50 ml) at reflux 10 min yielding a reverse aldolization product (370 mg, nearly single spot on TLC). The product (330 mg) in pyridine (10 ml) was then oxidized with CrO_3–pyridine complex (CrO_3 1 g, pyridine 15 ml) at room temperature 1.5 hr and treated as usual. The product (275 mg, nearly single spot on TLC) is assigned as the aldehyde–ketone (iii) based on its IR spectrum: $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450(OH), 2740, 1720(sh), 1700(CHO and CO). Finally, the adlehyde–ketone (iii) was subjected to Huang–Minlon reduction. A mixture of the aldehyde–ketone (iii) (275 mg) in EtOH (8 ml), diethylene glycol (30 ml), and 80% hydrazine hydrate (12 ml) was refluxed in an oil bath and added with KOH (2.4 g). Then, the oil bath temp. was raised gradually to 230° fitted with a downward condenser to distill off the low boiling component. The mixture was refluxed further 4 hr in the oil bath (temp. 230—240°) and worked up in a usual manner followed by recrystallization with MeOH giving 24-nor-22α-hydroxy-16α,21α-epoxy-olean-12-ene (XIV) (127 mg, single spot on TLC), mp 240° (lit.[13]: mp 254—255°). *Anal.* Calcd. for $C_{29}H_{46}O_2$: C, 81.63; H, 10.87. Found: C, 81.32; H, 10.84. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH).

Acetylation of XIV (28 mg) with Ac_2O (1 ml) and pyridine (2.5 ml) in a usual manner at room temperature furnished 24-nor-22α-acetoxy-16α,21α-epoxy-olean-12-ene (XIVa) (25 mg). NMR ($CDCl_3$, 100 MHz) τ: 9.14, 9.08, 9.00, 8.94 (total 18H), 8.76 (3H, s) (totally seven methyls), 7.95 (3H, s, $-OCOCH_3$), 6.53 (1H, s, $>C_{(21)}H$–O–), 5.92 (1H, m, $>C_{(16)}H$–O–), 4.97 (1H, s, $>C_{(22)}HOAc$), 4.82 (1H, m, $=C_{(12)}H$–).

24-Nor-22β-acetoxy-16α,21α-epoxy-olean-12-ene (XVIa)——1) To a solution of XIV (89 mg) in acetone (12 ml) and benzene (6 ml) was added Kiliani reagent (1.5 ml) (composition: CrO_3 2.66 g, conc. H_2SO_4 2.3 ml, H_2O 7.7 ml) under ice-cooling and the mixture was stirred further 20 min, poured into water, taken up with ether, and chromatographed over SiO_2 to give a ketone (XV) (39 mg, single spot on TLC).

2) The ketone (39 mg) was reduced by $LiAlH_4$ (200 mg) in anhydrous dioxane (12 ml) at reflux 5 hr and treated as usual. The reduction product was shown nearly single spot on TLC (XVI, 40 mg).

3) The product (XVI, 35 mg) was then acetylated with Ac_2O (9 ml) and pyridine (1 ml) at reflux 1.5 hr in a usual manner followed by recrystallization with MeOH affording XVIa (20 mg), mp 179—180. *Anal.* Calcd. for $C_{31}H_{48}O_3$: C, 79.43; H, 10.32. Found: C, 79.54; H, 10.27. NMR ($CDCl_3$, 100 MHz) τ: 9.18, 9.14, 9.10, 9.07, 8.98 (total 18H), 8.76 (3H, s) (totally seven methyls), 7.92 (3H, s, $-OCOCH_3$), 6.33 (1H, d, J=6 Hz, $>C_{(21)}H$–O–), 6.30 (1H, m, $>C_{(16)}H$–O–), 5.09 (1H, d, J=6 Hz, $>C_{(22)}HOAc$), 4.77 (1H, m, $=C_{(12)}H$–).

16(15)-Anhydro-protoaescigenin Pentaacetate (XVIIa)——To pyridine solution (5 ml) of protoaescigenin pentaacetate (VIIb) (210 mg) was added $SOCl_2$ (1 ml) under ice-cooling and the total mixture was let stand at room temperature overnight. After dilution with the additional amount of pyridine, the reaction mixture was filtered and the filtrate was poured into ice–water, extracted with ether and treated as usual. The product (200 mg) was crystallized from ether–*n*-hexane and then repeatedly recrystallized with $CHCl_3$-MeOH to give crystals (36 mg) of XVIIa, mp 272—273°, $[\alpha]_D$+10.1 (c=0.7, $CHCl_3$). *Anal.* Calcd. for $C_{40}H_{58}O_{10}$: C, 68.74; H, 8.37. Found: C, 68.98; H, 8.18. IR ν_{max}^{KBr} cm^{-1}: 1730, 1233 ($OCOCH_3$), 1629 (C=C). NMR ($CDCl_3$, 100 MHz) τ: 9.23, 9.14, 9.03 (3H each, s), 8.99 (6H, s), 8.78 (3H, s) (totally six methyls), 8.02, 8.00, 7.99, 7.96 (totally five acetoxyls), 6.29 (2H, s, $-C_{(28)}H_2OAc$), 5.69, 5.94 (2H, AB quartet, J=11 Hz, $-C_{(24)}H_2OAc$), 5.48 (1H, t-like, $>C_{(3)}HOAc$), 5.28, 4.68 (2H, AB quartet, J=11 Hz, $>C_{(21)}H$–$C_{(22)}H<$), 4.66 (1H, m, $=C_{(12)}H$–), 4.43, 4.30 (2H, AB quartet, J=10 Hz, $-C_{(15)}H$=$C_{(16)}H$–).

16-Desoxy-barringtogenol C Tetraacetate(IXa)——Acetylation of 16-desoxy-barringtogenol C (IX) (=R_x) (20 mg) with Ac_2O (0.4 ml) and pyridine (1 ml) under refluxing 1.5 hr followed by the usual work-up afforded a crude product, which was purified by preparative TLC (Camag, SiO_2) giving 16 mg of IXa. Analytical sample of IXa, mp 225—226.5°, $[\alpha]_D$+50° (c=0.83, $CHCl_3$), was obtained by recrystallization with EtOH. *Anal.* Calcd. for $C_{38}H_{58}O_8$: C, 70.90; H, 9.09. Found: C, 70.55; H, 9.08. IR ν_{max}^{KBr} cm^{-1}: 1730, 1238 ($OCOCH_3$), 1631 (C=C). NMR ($CDCl_3$, 60 MHz) τ: 9.11 (9H, s), 9.06, 9.03, 8.95 (3H each, s), 8.89 (3H, s) (totally seven methyls), 8.03, 7.99, 7.96, 7.95 (3H each, s, totally four acetoxyls), 6.16 (2H, s, $-C_{(28)}H_2OAc$), 5.49 (1H, $>C_{(3)}HOAc$), 5.06, 4.71 (2H, AB quartet, J=10 Hz, $>C_{(21)}H$–$C_{(22)}H<$), 4.70 (1H, m, $=C_{(12)}H$–).

Conversion of Barringtogenol C(I) to 16-Desoxy-barringtogenol C(IX)——1) Tritylation followed by Acetylation: A mixture of barringtogenol C (I) (2 g), TrCl (3 g), and pyridine (50 ml) was refluxed 5 hr and

treated in a usual manner giving a crude product (2.9 g), which was crystallized from benzene–*n*-hexane–ethanol mixture to give 2 g of 28-O-trityl-barringtogenol C (XVIII).[1] Acetylation of the monotrityl ether (200 mg) using Ac_2O (1.6 ml) and pyridine (4 ml) by keeping at 28° two overnights, followed by successive chromatographic purification with alumina (Merck) column, SiO_2 (Merck) column, and TLC (Merck, SiO_2), afforded 85 mg of the diacetyl derivative of 28-O-trityl-barringtogenol C (XVIIIa). NMR ($CDCl_3$, 100 MHz) τ: 9.68 (3H, s), 9.19 (9H, s), 9.12, 9.08, 8.71 (3H each, s) (totally seven methyls), 8.04, 7.95 (3H each, s, two acetoxyls), 7.31, 6.97 (2H, AB quartet, $J=10$ Hz, $-C_{(28)}\mathbf{H}_2OTr$), 6.10, 4.76 (2H, AB quartet, $J=10$ Hz, $>C_{(21)}\mathbf{H}-C_{(22)}\mathbf{H}<$), 5.71 (1H, m, $>C_{(16)}\mathbf{H}OH$), 5.61 (1H, t-like, $>C_{(3)}\mathbf{H}OAc$), 4.87 (1H, m, $=C_{(12)}\mathbf{H}-$). The NMR data agrees with the formulation of 28-O-trityl-barringtogenol C diacetate (XVIIIa).

2) Oxidation followed by Huang-Minlon Reduction and Detritylation: A solution of the diacetate (XVIIIa) (106 mg) in pyridine (3 ml) was treated with CrO_3–pyridine complex (CrO_3 105 mg, pyridine 2 ml) at room temperature two overnights, and worked up in a usual manner. The crude product (75 mg) was purified by TLC (SiO_2) to give 16-ketone derivative (XIX) (36 mg), whose structure was substantiated by the NMR analysis. NMR ($CDCl_3$, 100 MHz) τ: 9.85 (3H, s), 9.17, 9.11 (6H each, s), 8.90, 8.87 (3H each, s) (totally seven methyls), 7.98, 7.83 (3H each, s, two acetoxyls), 6.89, 6.41 (2H, AB quartet, $J=9$ Hz, $-C_{(28)}\mathbf{H}_2OTr$), 6.76 (2H, m, $-C_{(15)}\mathbf{H}_2-C_{(16)}O-$), 5.72, 4.88 (2H, AB quartet, $J=10$ Hz, $>C_{(21)}\mathbf{H}-C_{(22)}\mathbf{H}<$),[34] 5.57 (1H, t-like, $>C_{(3)}\mathbf{H}OAc$), 4.76 (1H, m, $=C_{(12)}\mathbf{H}-$). A mixture of the 16-ketone derivative (XIX) (51 mg), ethanol (1.5 ml), triethylene glycol (5 ml) and 80% hydrazine hydrate (2 ml) was refluxed 2 hr in an oil bath (temp. 150—160°). After adding KOH (0.55 g), the mixture was heated 30 min with a downward condenser while the bath temperature was gradually raised up to 230°, and the mixture was heated for further 4 hr in the oil bath (temp. 230—240°). The reaction product obtained after the usual working-up was then treated with 3.5% HCl (3 ml) and MeOH (3 ml) by refluxing 10 min, poured into water and extracted with ether (crude product 32 mg). The product was then purified by TLC (SiO_2) giving pure compound (10 mg). One recrystallization with aq. MeOH furnished colorless needles identical with 16-desoxy-barringtogenol C (IX) by mixed mp, IR (KBr), and TLC.

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34) Confirmed by the decoupling experiment, thus establishing the selective oxidation being effected at $C_{(16)}$-OH.