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Saponin and Sapogenol. III.[1,2] Seeds Sapogenols of *Thea sinensis* L. (3). Theasapogenol E and Minor Sapogenols

Itiro Yosioka, Tadashi Nishimura, Akiko Matsuda, and Isao Kitagawa

Faculty of Pharmaceutical Sciences, Osaka University[3]

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1) The structure of theasapogenol E, one of seeds sapogenols of *Thea sinensis* L. has been established as 3β,16α, 21β, 22α, 28-pentahydroxy-23-oxo-olean-12-ene (III). In conjunction with the study on alcoholic alkaline treatment of theasapogenol E leading the isomerization and/or the Meerwein–Ponndorf type reduction of the aldehyde function of III, it has been suggested that camelliagenin D (X) might be an artefact sapogenol. 2) The other two minor seeds sapogenols designated as theasapogenols D and C have been revealed identical with dihydropriverogenin A (=camelliagenin A) (IV) and camelliagenin C (V) respectively. Furthermore, the evidence supporting 22α-hydroxy (equatorial) of dihydropriverogenin A (IV) has been presented, which has led the revision of the previous structures of dihydropriverogenin A (e), priverogenin A (h) and priverogenin B (i). 3) On the basis of NMR examination, the conformation of some acetonide derivatives of dihydropriverogenin A (IV), barringtogenol C (I), and theasapogenol A (II) has been discussed.

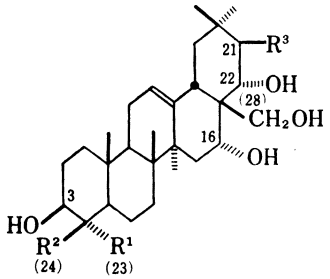
In previous reports, we described the full details of the investigation on the structures of barringtogenol C(I)[1] and theasapogenol A(II),[2] which are sapogenol constituents of *Thea sinensis* L. seeds (Theaceae). As mentioned in Part I,[1] the additional three other sapogenols, designated theasapogenol E(III), dihydropriverogenin A(IV)(=camelliagenin A, theasapogenol D) and camelliagenin C(V) (=theasapogenol C), have also been isolated from the same plant source. The present paper deals with the chemical evidences related to these sapogenols which are consistent with the proposed structures respectively.

Theasapogenol E

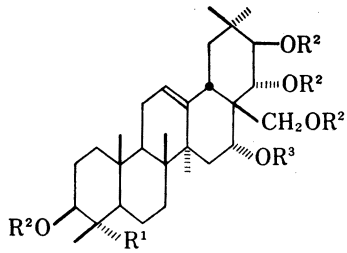
Theasapogenol E(III),[4] $C_{30}H_{48}O_6$, mp 260—263°; $[\alpha]_D$+34° (pyridine), possesses the aldehyde function in addition to the double bond and hydroxyl group as revealed by its infrared (IR) spectrum (KBr): 3560, 3380, 2680, 1725, 1635 cm^{-1}. On mild acetylation using acetic anhydride and pyridine at room temperature, it afforded a tetraacetate(VI), $C_{30}H_{44}O_2(OCOCH_3)_4$, mp 184—187°, while the acetylation of III with acetic anhydride and *p*-toluenesulfonic acid furnished a pentaacetate(VII), $C_{30}H_{43}O(OCOCH_3)_5$, (amorphous), which has no free hydroxyl.

The nuclear magnetic resonance (NMR) examination (Table I) of these acetates in comparison with barringtogenol C tetraacetate(VIII)[1] and theasapogenol A pentaacetate(IX)[2] evidently suggests that theasapogenol E lacks the C-23 carbinol in theasapogenol A and possesses an aldehydic function instead. Thus, the signals in the lower region of VI ascribable to hydrogens at C-12, C-16, C-21, C-22, and C-28 are quite resembled to those of IX, whereas the equatorial[5] aldehydic hydrogens of VI and VII are observed as singlets at 0.74 and 0.71 τ

1) Part I: I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **18**, 1610 (1970).
2) Part II: I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **18**, 1621 (1970).
3) Location: *Toneyama, Toyonaka, Osaka.*
4) I. Yosioka, A. Matsuda, T. Nishimura, and I. Kitagawa, *Chem. Ind.* (London), **1966**, 2202 (Preliminary report on the structure).
5) *a*) T.J. King and J.P. Yardley, *J. Chem. Soc.*, **1961**, 4308; *b*) W.R. Chan, C. Willis, M.P. Cava, and R.P. Stein, *Chem. & Ind.*, **1963**, 495; *c*) R.A. Laidlaw and J.W.W. Morgan, *J. Chem. Soc.*, **1963**, 644.



I : $R^1=CH_3$, $R^2=CH_3$, $R^3=OH$ barringtogenol C
II : $R^1=CH_2OH$, $R^2=CH_3$, $R^3=OH$ theasapogenol A
III : $R^1=CHO$, $R^2=CH_3$, $R^3=OH$ theasapogenol E
IV : $R^1=CH_3$, $R^2=CH_3$, $R^3=H$ dihydropriverogenin A (=camelliagenin A) (=theasapogenol D)
V : $R^1=CH_2OH$, $R^2=CH_3$, $R^3=H$ camelliagenin C (=theasapogenol C)
X : $R^1=CH_3$, $R^2=CHO$, $R^3=OH$ camelliagenin D
XI : $R^1=CH_3$, $R^2=CH_2OH$, $R^3=OH$ protoaescigenin



VI : $R^1=CHO$, $R^2=Ac$, $R^3=H$
VII : $R^1=CHO$, $R^2=Ac$, $R^3=Ac$
VIII : $R^1=CH_3$, $R^2=Ac$, $R^3=H$
IX : $R^1=CH_2OAc$, $R^2=Ac$, $R^3=H$

Chart 1

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

Compound	C-3H	C-16H	C-21H	C-22H
VIII[1)]	5.45 (t.l.)	5.77 (m)	4.40, 4.54 (2H, ABq, 10)	
IX[2)]	5.20 (q, 9.6 & 6.1)	5.81 (m)	4.48, 4.59 (2H, ABq, 10)	
VI	5.00 (t.l.)	5.84 (m)	4.50, 4.62 (2H, ABq, 10)	
VII	5.01 (t.l.)		4.68 (3H, m)	
XIV[d)]	5.50 (t.l.)	5.76 (m)	—	4.69 (q, 6 & 12)
XVII[d)]	5.50 (t.l.)	5.91 (m $W^h/_2=8$)	—	5.78 (t, 3)
XVIII	5.50 (t.l.)	5.15 (m)	—	6.01 (q, 5 & 13)
XXI	5.50 (t.l.)	4.63 (m)	—	6.04 (t, 9)
XXII	5.42 (t.l.)	4.50 (m)	—	4.80 (m)
XXIII	5.50 (t.l.)	4.33 (m)	—	6.07 (q, 8 & 10)
XXIV	5.51 (t.l.)	5.02 (m)	—	—
XXVI[d)]	5.50 (t.l.)	5.91 (m)	—	4.67 (t.l., $W^h/_2<10$)

Compound	C-28H	C-12H[b)]	Lowest CH_3[c)]	Others
VIII[1)]	6.30 (2H, br. s.)	4.60	8.53	
IX[2)]	6.33 (2H, br. s.)	4.63	8.56	6.09, 6.23 (2H, ABq, 11.6): $C_{(23)}H_2OAc$
VI	6.35 (2H, br. s.)	4.63	8.53	0.74 (1H, s): $C_{(23)}HO$
VII	6.26 (2H, br. s.)	4.68	8.66	0.71 (1H, s): $C_{(23)}HO$
XIV[d)]	6.23, 6.34 (2H, ABq, 11)	4.68	8.57	
XVII[d)]	6.21, 6.32 (2H, ABq, 11)	4.70	8.74	
XVIII	6.40, 6.64 (2H, ABq, 11)	4.73	*ca.* 8.57	
XXI	5.82, 6.18 (2H, ABq, 11)	4.63	8.67	
XXII	6.64, 6.92 (2H, ABq, 12)	4.50	8.68	
XXIII	6.34, 6.67 (2H, ABq, 11)	4.71	8.66	
XXIV	5.86, 6.10 (2H, ABq, 10)	4.54	8.69	
XXVI[d)]	5.99, 6.20 (2H, ABq, 12)	4.67	8.65	

a) br.s.=broad singlet, d=doublet, m=multiplet, q=quartet, s=singlet, t=triplet, t.l.=triplet like
b) observed as multiplet
c) assigned to $C_{(27)}H_3$, which is allylic; Some signals (*eg.* in VIII, IX, VI, XIV—) are appearing as low as *ca.* 8.5—8.6 τ due to the deshielding effect caused by $C_{(16)}$-αOH.
d) measured at 100 MHz

respectively. Moreover, the lower field appearance of C-3α hydrogens of VI and VII (at 5.00 and 5.01 τ) as compared with those of VIII and IX is in agreement with the location of the aldehyde function in the vicinity. Accordingly, the structure expressed as 3β, 16α, 21β, 22α, 28-pentahydroxy-23-oxo-olean-12-ene(III), is advanced reasonably for theasapogenol E, which has been verified by converting theasapogenol E to theasapogenol A(II) *via* $LiAlH_4$ reduction and to barringtogeol C(I) through Huang-Minlon reduction.[6]

As mentioned briefly in Part I,[1] at the early stage of the investigation on tea seeds sapogenols, we have noticed on TLC that the intensive alkaline treatment of theasapogenol E unexpectedly results in the formation of theasapogenol A in considerably good yield. Therefore, the further study along this line has been performed and the findings are summarized below.

At first, theasapogenol E(III) was treated at reflux under several different alkaline conditions as given in Table II monitoring the reaction progress by thin-layer chromatography (TLC). Under the mild reaction condition (*i.e.* refluxing one hour in 2%K_2CO_3-MeOH-5%KOH-MeOH), the product was revealed to consist of camelliagenin D(X)[7] in addition to recovered theasapogenol E. On the other hand, the more forced condition brought out the formation of theasapogenol A(II) predominantly. For instance, the refluxing for 2 hours in 10% KOH-EtOH converted theasapogenol E completely to yield theasapogenol A (major) and protoaescigenin(XI).[8] It has therefore been clarified that the alkaline treatment of theasapogenol E results in: i) the epimerization of C-23 aldehyde function and ii) the reduction of the aldehyde function to the corresponding primary carbinol.[9]

TABLE II

Alkali	Solvent	Concentration of alkali (%)
K_2CO_3	MeOH	2; 4
KOH	MeOH	2.5; 5; 10
KOH	EtOH	2.5; 5; 10

As for the reaction mechanism concerned in the epimerization of the aldehyde function, the reverse aldolization followed by the aldol type recyclization is inferred as illustrated in Chart 2. Since an identical reaction product (a mixture of III and X) was obtained by refluxing one hour in 2%K_2CO_3-MeOH starting either from theasapogenol E or camelliagenin D, it is assumed that the equilibrium (III⇆(a)⇆X) is readily reached even under such a mild condition.[10]

Further, the above mechanistic consideration (Chart 2) suggests the possible formation of an aldehydic compound possessing C-3α hydroxyl function (as (b)) and/or another aldehyde expressed as (d). Yet, the examinations attempted so far have not led the isolation of such compounds.

6) After the structure of theasapogenol E was established as III, it was noticed that the pentaacetate (VII) was identical with the intermediary compound (XXVII in ref. 2) prepared on the conversion of theasapogenol A (II) to barringtogenol C (I), so that the identification of both was done by IR spectral comparison in CCl_4.

7) S. Ito and T. Ogino, *Tetrahedron Letters*, **1967**, 1127.

8) I. Yosioka, T. Nishimura, A. Matsuda, K. Imai, and I. Kitagawa, *Tetrahedron Letters*, **1967**, 637 and literatures cited therein.

9) Since small amount of the acidic component was also detected in the reaction mixture, the Cannizzaro type reaction could not be excluded. However, the major paths are regarded to be these two as based on the product analysis.

10) That the equilibrium mixture contained richer amount of III than X is presumably ascribed to the less favored structure of X in view of the sterical congestion (increased 1,3-diaxial interaction between CH_3 at C-10 and CHO at C-4 in X).

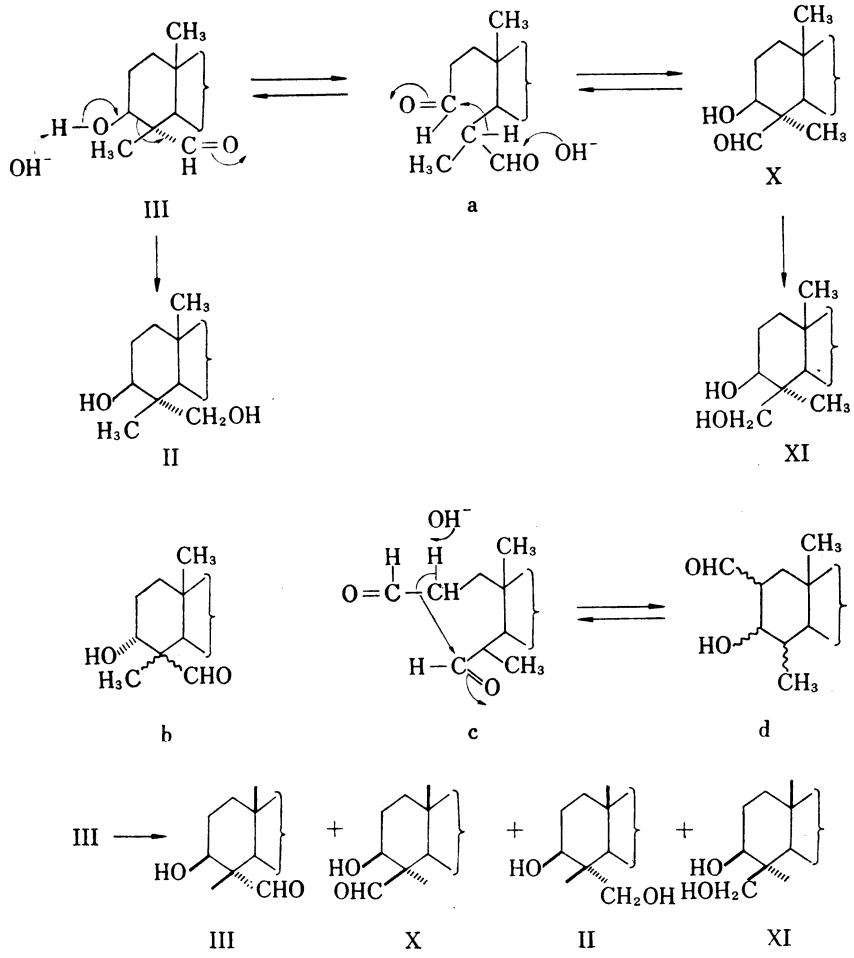
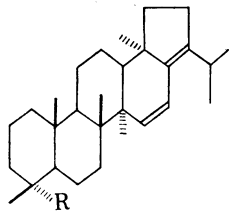


Chart 2

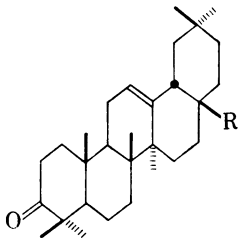
As is apparent from the sequel, the isomerization (III⇆X) is also expected to occur in the acidic medium. In fact, it has been disclosed on TLC that the acid treatment similar as for the hydrolysis of saponin caused the isomerization of III and X each other. Consequently, although the further experiment (*e.g.* the enzymatic hydrolysis of saponin) is needed, camelliagenin D (X), which was isolated in minor quantity from seeds of *Thea sinensis* L. and *Camellia sasanqua* Thunb. by Ito and Ogino[7] and by us[11] and is the only sapogenol possessing an oxygenated axial function at C-4 among the sapogenols, seems to be an artefact sapogenol (probably derived secondarily from the abundant theasapogenol E during the hydrolysis of saponin) on the above mentioned basis.

The occurrence of Meerwein–Ponndorf type reaction is presumed for the reduction of aldehyde function to the corresponding primary alcohol under the forced alkaline condition. It follows on the whole that the above-mentioned isomerization giving a mixture of III and X takes place in the beginning and then the reduction of each aldehydic function follows affording theasapogenol A (II) and protoaescigenin (XI) respectively. The presumption is supported by the fact that the alkaline reduction proceeded in MeOH, EtOH, and iso-PrOH, while not in *tert*-BuOH which lacks α-hydrogen. Further, the more abundant yield of II as compared

11) I. Yosioka, A. Matsuda, R. Takeda, and I. Kitagawa, to be published.

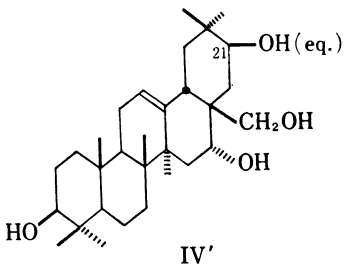


XII : R=CHO
XIIa : R=$COOCH_3$
XIIb : R=CH_2OH



XIII : R=CHO
XIIIa : R=CH_2OH

Chart 3



IV'

Chart 4

with XI is inferred due to the rich content of III in the initial equilibrium along with the probably more favored reactivity of the equatorial aldehyde in III than the axial one in X.

Although the reduction of ketonic function in the alcoholic alkaline medium has been reported,[12] since the present case seems to be unprecedented,[13] the additional examination was performed using two aldehydic triterpenoids at hand. The one (XII), which is a derivative of leucotylic acid[14] and possesses an equatorial aldehyde at C-4 but lacking a hydroxyl at C-3, was treated with alcoholic alkali. It has been found that the aimed reduction giving XIIb needs a severe condition (reflux 13 hours, in 10%KOH–EtOH) in this case. The less reactivity towards the reduction has been more distinct for the second example (XIII), having an aldehyde at C-17, which needs 20 hours to complete the reduction to furnish XIIIa under the same alkaline medium. These observations are assumed due to the absence of the C-3 hydroxyl in XII and to the different sterical environment causing less reactivity of the aldehyde function in XIII. Especially as noticed in comparison with the reactivity of III and XII, to clarify the probable participation of the hydroxyl at C-3 during the reduction of aldehyde function, further investigation is necessitated.

Dihydropriverogenin A(=Camelliagenin A, Theasapogenol D) (IV)[15]

Dihydropriverogenin A (=theasapogenol D) (IV),[17] $C_{30}H_{50}O_4$, mp 273—275°, $[\alpha]_D$+30° (ethanol), possesses the hydroxyl function and double bond as disclosed by its infrared (IR) absorption bands(KBr) at 3401 and 1629 cm^{-1}. It gave a triacetate (XIV), $C_{30}H_{47}O(OCOCH_3)_3$, mp 198—200°, on acetylation under ordinary condition. The NMR inspection (Table I) of the triacetate (XIV) in conjunction with barringtogenol C triacetate (VIII)[1] has led the assumption that dihydropriverogenin A (=theasapogenol D) has a structure lacking one hydroxyl group in ring E(either at C-21 or C-22) of barringtogenol C(I). Namely, the NMR

12) *a*) G.H. Hargreaves and L.N. Owen, *J. Chem. Soc.*, **1947**, 750; *b*) D.C. Kleinfelter, *J. Org. Chem.*, **32**, 840 (1967); *c*) S. Uyeo, K. Ueda, and Y. Yamamoto, *Yakugaku Zasshi*, **86**, 1172 (1966) and the literatures cited therein.

13) Recently Vystrčil and Buděšínský reported an interesting paper dealing with an intramolecular hydride transfer reaction in which an aldehyde function is reduced by an intramolecular primary alcoholic function under basic condition (*Tetrahedron Letters*, **1968**, 4173).

14) I. Yosioka, T. Nakanishi, and E. Tsuda, *Tetrahedron Letters*, **1966**, 607.

15) I. Yosioka, T. Nishimura, N. Watani, and I. Kitagawa, *Tetrahedron Letters*, **1967**, 5343 (Preliminary report on the structure and identity of theasapogenol D with dihydropriverogenin A and camelliagenin A, leading the revision of the structure of dihydropriverogenin A proposed by Tschesche, *et al.*[16]).

16) R. Tschesche, B.T. Tjoa, and G. Wulff, *Ann.*, **696**, 160 (1966).

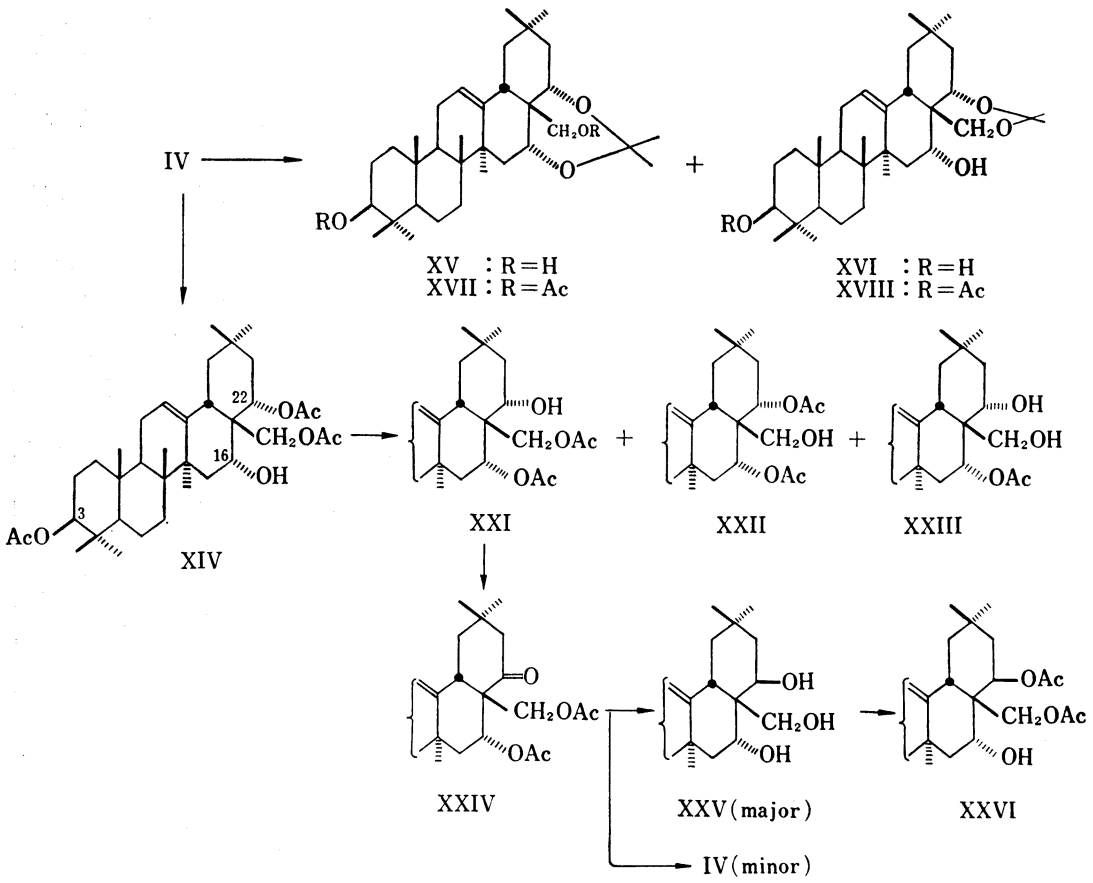
17) As is mentioned later, since the identity of theasapogenol D with camelliagenin A and dihydropriverogenin A has been established, the study using the sapogenol isolated from tea seeds in our laboratory is headed by this expression for the discussion here.

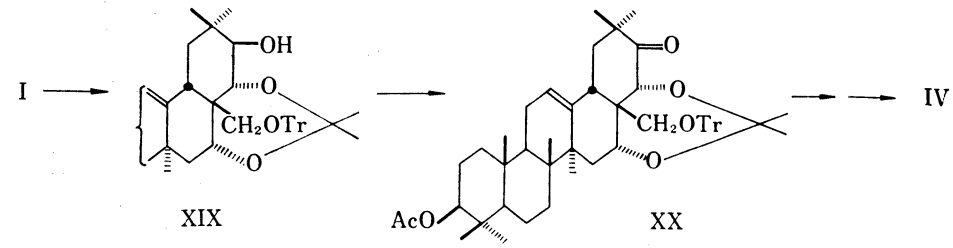
spectrum of the triacetate lacks the AB quartet signal due to C-21,22 glycol in VIII while it shows a one-proton quartet at 4.69 τ (J=6 & 12 Hz), which is assignable to an axial hydrogen at C-21 or C-22 bearing an equatorial acetoxyl function. Accordingly, two plausible structures (IV and IV') are presented for dihydropriverogenin A (=theasapogenol D). To verify IV, two isomeric monoacetonides, $C_{33}H_{54}O_4$, XV, mp 256—260° and XVI, mp 272—275°, were prepared with acetone and anhydrous cupric sulfate and the NMR data of their respective acetyl derivatives, XVII, $C_{33}H_{52}O_2(OCOCH_3)_2$, mp 186—188°, and XVIII, $C_{33}H_{53}O_3(OCOCH_3)$, mp 226—228°, were examined. The significant feature in the spectra is that a signal ascribable to C-16βH of XVIII is seen in the deshielded region (at 5.15 τ). The fact is in good accordance with the acetonide formulation in XVIII between C-28 and C-22 hydroxyls (derived from IV) rather than C-28 and C-21 hydroxyls (from IV') as has been discussed for jegosapogenin[18] and the sapogenols of *Pittosporum tobira* Ait.[19] before and the further discussion on this subject is given later. The structure expressed as 3β,16α,22α,28-tetrahydroxy-olean-12-ene (IV) is now preferred for dihydropriverogenin A (=theasapogenol D), which is coincided with the structure of camelliagenin A proposed by Murakami, *et al.*[20] and Ito, *et al.*[21] independently. The direct comparison (mixed mp, IR, and TLC) of dihydropriverogenin A (=theasapogenol D) with camelliagenin A proved the identity of both samples.

Next, the conversion of barringtogenol C (I)[1] to dihydropriverogenin A (=theasapogenol D) has been attempted. Thus, 3-O-acetyl-28-O-trityl-16,22-O-monoacetonide(XIX) was prepared from I via successive treatment with trityl chloride–pyridine and acetone–anhydrous cupric sulfate followed by partial acetylation. The chromic anhydride-pyridine oxidation of the monoacetyl-monotrityl-acetonide (XIX), furnished a ketone (XX), $C_{52}H_{65}O_4(OCOCH_3)$, mp 262—263°, which was subjected to Huang-Minlon reduction to remove the carbonyl function at C-21. However, the ketone (XX) was unaffected by the treatment probably because of its sparing solubility and the sterically hindered location of the carbonyl as deduced by the Dreiding model inspection. The direct reduction of the carbonyl of XX was therefore abandoned. The acetonide bonding of XX was then removed under a mild acidic treatment and the product was subjected to Huang-Minlong reduction followed by detritylation. The final product was proved identical with dihydropriverogenin A (=theasapogenol D), although the yield was extremely low. Since the epimerization of C-22 and/or isomerization (through enolization) giving 22-CO, 21-OH compound can not be excluded, the derivation from I to IV described above does not necessarily offer the difinite proof of the location and configuration[22] of hydroxyl at C-22 of dihydropriverogenin A (=theasapogenol D). However, it follows that the carbon skeleton in addition to the disposition of the other functions of dihydropriverogenin A (=theasapogenol D) (IV) has been established.

On the other hand, during the course of the study on sapogenols of several *Primulaceous* plants,[23] we have found that the major sapogenols are primulagenin A and dihydropriverogenin A. The structure of the latter has initially been proposed by Tschesche and co-workers[16] as (e) carrying axial C-22βOH. The important reason of their deduction was that $NaBH_4$ reduction of the C-22 ketone derivative(f) gave mainly an epimer, for which they assigned the structure (g) having the equatorial hydroxyl (*i.e.* 22α-OH) and consequently original dihydropriverogenin A was given the structure (e). However, since theasapogenol D (=camellia-

18) T. Hayashi, C. Koshiro, T. Adachi, I. Yosioka, and I. Kitagawa, *Tetrahedron Letters*, **1967**, 2353.
19) I. Yosioka, I. Kitagawa, T. Hino, A. Matsuda, and Y. Nakagawa, *Chem. Pharm. Bull.* (Tokyo), **16**, 195 (1968).
20) H. Itokawa, N. Sawada, and T. Murakami, *Chem. Pharm. Bull.* (Tokyo), **17**, 474 (1969); *idem*, *Tetrahedron Letters*, **1967**, 597.
21) S. Ito, M. Kodama and M. Konoike, *Tetrahedron Letters*, **1967**, 591.
22) As was discussed in our preliminary report,[15] the stable equatorial OH is more likely during the procedure from XX to IV (*i.e.* 22α-OH provided that the position of the hydroxyl is at C-22).
23) *a*) I. Kitagawa, A. Matsuda, T. Nishimura, S. Hirai, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **15**, 1435 (1967); *b*) I. Kitagawa, A. Matsuda, and I. Yosioka, *Tetrahedron Letters*, **1968**, 5377.


IV
RO
CH₂OR
XV : R=H
XVII : R=Ac
XVI : R=H
XVIII : R=Ac
AcO
CH₂OAc
OAc
OH
XIV
XXI
XXII
XXIII
CH₂OH
XXIV
XXV(major)
XXVI
IV(minor)


I
CH₂OTr
XIX
XX
IV

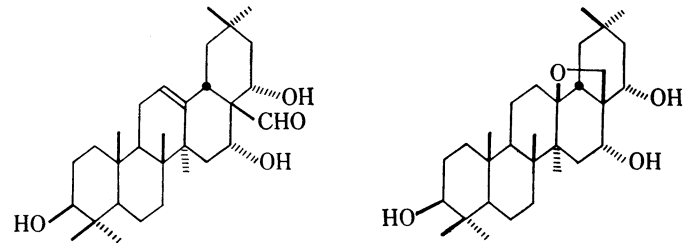

CHO
HO
XXVII : priverogenin A
XXVIII : priverogenin B

Chart 5

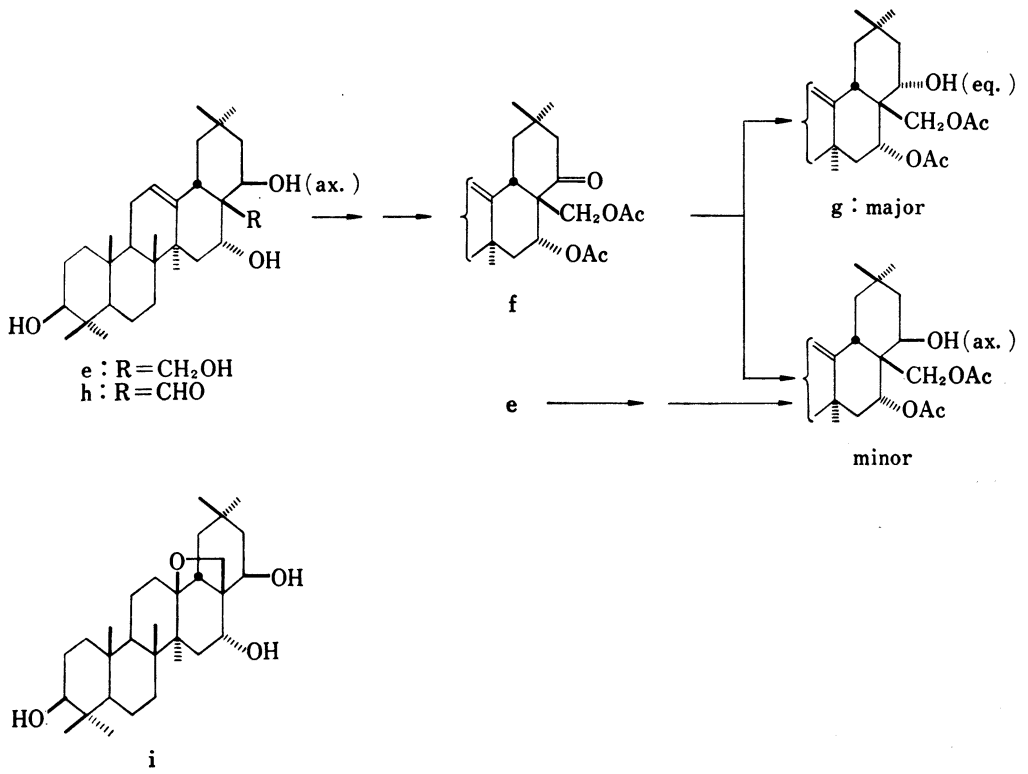


Chart 6. Proposed Structures by Previous Workers[16]

genin A) (IV) has been found identical in all respects (mixed mp, IR, TLC) with dihydropriverogenin A which was obtained from *Primulaceous* plants in our laboratory,[23] it seemed worthwhile to shed light on the ambiguity. Thus, by passing through neutral alumina column in benzene solution, the triacetate (XIV) afforded two isomeric acetyl-migrated products, $C_{30}H_{47}O(OCOCH_3)_3$, XXI, mp 186—189°, and XXII, mp 233—238°, along with a diacetate (XXIII), $C_{30}H_{48}O_2(OCOCH_3)_2$, mp 246—249°, which presumably was formed *via* acetyl migration followed by deacetylation.[24] The structural assignment of these products has been based on the NMR examination (Table I). Interestingly, it has been noticed that the acetyl group attached at 28-O migrates with facility to 22-O or *vice versa* even during the isolation procedure by preparative TLC. The deshielded signals of XXI, XXII, and XXIII, at 4.33—4.63 τ due to C-16βH demonstrate the acetylated feature of the hydroxyl at C-16. Oxidation of XXI with the Kiliani reagent gave a ketone (XXIV), $C_{30}H_{45}O(OCOCH_3)_3$, mp 196—199°, which on $NaBH_4$ reduction followed by alkaline treatment furnished dihydropriverogenin A(=theasapogenol D) (IV) (as minor) and an epimer (XXV) (major) as was described by the previous workers.[16] On acetylation with acetic anhydride and pyridine, the epimer (XXV) gave a triacetate (XXVI), $C_{30}H_{47}O(OCOCH_3)_3$, mp 241.5—242.5°, whose NMR spectrum was examined (Table I). A multiplet[25] ascribable to C-22H at 4.67τ with half-band width of less than 10 Hz supports to assign the axial acetoxyl at C-22 in XXVI, whereas a quar-

24) Acetyl migration of the acetyl derivatives of barringtogenol C(I) has been discussed in ref. 1). See also the literatures cited therein.

25) Due to the overlapping by the signal of C-12H, the precise coupling constant of the signal is obscure.

tet at 4.69 τ (J=6 & 12 Hz) due to C-22H of XIV reasons the equatorial acetoxyl at C-22 as mentioned above. In addition, the signal assignable to C-16βH of XXVI appears at 15 Hz higher field than that of XIV, which probably is ascribed to the absence of anisotropic effect caused by C-22 equatorial oxygen function (in peri disposition) in case of XIV.

The combined evidence described above is in favor of C-22αOH (equatorial) configuration (as IV) in dihydropriverogenin A (=theasapogenol D, camelliagenin A) rather than β-OH (axial) (as e). It has become evident that $NaBH_4$ reduction of the ketone (XXIV) gives rise to the axial hydroxyl product (as XXV) as the major contrary to the presentation by previous workers.[16] Furthermore, the structures of priverogenins A and B, which had previously been proposed as (h) and (i)[16] in connection with dihydropriverogenin A, should be replaced by XXVII and XXVIII respectively. After our proposal,[15] Tschesche and co-workers also have reached the same conclusion.[26]

Camelliagenin C (=Theasapogenol C)

One of the minor sapogenols isolated from tea seeds tentatively named theasapogenol C, $C_{30}H_{50}O_5$, mp 264—266°, IR (KBr) cm^{-1}: 3387 (hydroxyl), 1631 (double bond), has been confirmed identical with camelliagenin C (V)[20,21] by direct comparison (mixed mp, IR, and TLC).

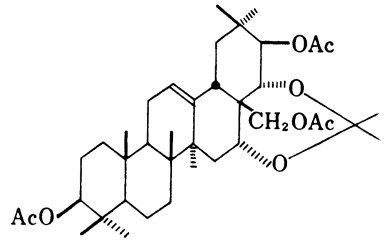
Comment on the Conformation of the Acetonides

In the NMR spectra of most acyl derivatives of sapogenols described in this and previous papers,[1,2] C-16βH gives a multiplet with half-band width of *ca.* 10 Hz. However, 16,20-O-isopropylidene derivatives such as 3,21,28-tri-O-acetyl-barringtogenol C monoacetonide (XXIX)[1] and 21,28-di-O-acetyl-theasapogenol A diacetonide(XXX)[2] show one-proton triplet (J=7 Hz) due to C-16βH at 6.28 and 6.31 τ respectively. Based on the Karplus equation,[27] it is presumed that D ring conformation of XXIX and XXX is in a twist form, in which the dihedral angle between C-15βH and C-16βH is *ca.* 30°. The perspective presentation of D/E ring in XXIX and XXX is most likely given as (j), where the 1,3-dioxane ring appears to be also in a twist form. The large *trans*-diaxial coupling constants (J=8—9)[1,2] between C-21αH and C-22βH of XXIX and XXX also corroborate the presentation.

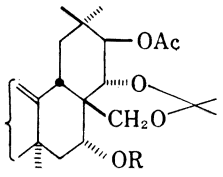
On the other hand, the NMR spectrum of 3,28-di-O-acetyl-dihydropriverogenin A monoacetonide (XVII), which lacks an acetoxyl function at C-21, indicates a multiplet due to C-16βH at 5.91 τ with half-band width of 8 Hz and a triplet (J=3 Hz) assigned to C-22βH at 5.73 τ. These coupling patterns are in good agreement with the expression (k), in which the dioxane ring is in a chair form while E ring is in a twist conformation and consequently C-22βH is bisectional against the methylene hydrogens at C-21. Although the conformation (type k) seems to be more favored as compared with type (j), if XXIX and XXX were in conformation (k), the severe bowsprit-flagpole interaction between C-21βOAc and C-18βH would compel D/E ring conformation of XXIX and XXX to type (j).

The multiplet signals due to C-16βH of 22,28-O-isopropylidene derivatives such as 3,21-di-O-acetyl-barringtogenol C monoacetonide(XXXI),[1] 21-O-acetyl-theasapogenol A diacetonide(XXXII)[2] and 3-O-acetyl-dihydropriverogenin A monoacetonide(XVIII) are appearing at 5.15 τ which is considerably in the deshielded position as compared with the ordinary value (*ca.* 5.7—5.8 τ).[1,2] The feature is distinct in case of XXXIII(C-16βH at 4.16 τ),[1] where C-16αOH is acetylated. These findings are well explained by the perspective formula (m), in which all of D/E ring and the 1,3-dioxane ring are in the most stable chair conformation and consequently C-16βH suffers the anisotropic effect of the C-22 oxygen function (in peri disposition). As mentioned above, the discussion has already briefly been introduced in the structural elucidation of jegosapogenin[18] and others.[19] Furthermore,

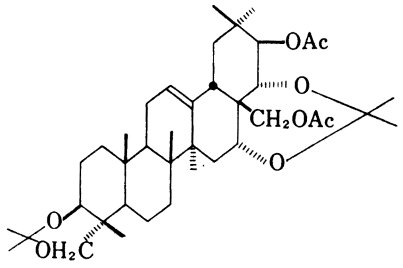
26) R. Tschesche, B.T. Tjoa, and G. Wulff, *Tetrahedron Letters*, **1968**, 183.
27) M. Karplus, *J. Am. Chem. Soc.*, **85**, 2870 (1963).



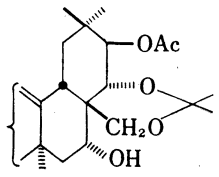
XXIX



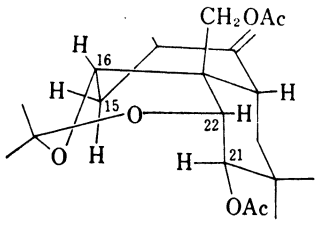
XXXI : R=H
XXXIII : R=Ac



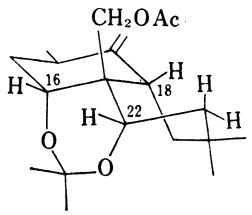
XXX



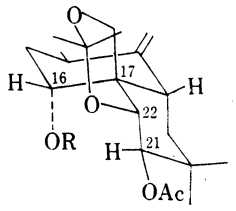
XXXII



j



k



m : R=H or Ac

Chart 7

the less yield of 16,22-O-isopropylidene derivatives as compared with that of 22,28-O-isopropylidene derivatives in the acetonide preparation of barringtogenol C (I)[1] and theasapogenol A (II),[2] is ascribed to the less favored conformation of type (j) in comparison with type (m).

Finally, the genuineness of sapogenols (I, II, III, IV, V) isolated in our laboratory from tea seeds saponin hydrolysate is mentioned. Tschesche and co-workers have pointed out[28] that the oleanane sapogenols having Δ^{12}-17-CH_2OH moiety obtainable by acid hydrolysis of saponin might be in the 13,28-epoxy form (as in XXVIII) in saponin constitution. Since sapogenols I—V possess the similar part-structure, the soil bacterial hydrolysis[29] has been applied for tea seeds saponin to clarify the subject. The results[30] obtained thus far support the genuineness of barringtogenol C(I), theasapogenol A(II), and theasapogenol E(III), which will be detailed in our future paper.

28) R. Tschesche, F. Inchanrrondo, and G. Wulff, *Ann.*, **680**, 107 (1964).
29) *a*) I. Yosioka, M. Fujio, M. Osamura, and I. Kitagawa, *Tetrahedron Letters*, **1966**, 6303; *b*) I. Yosioka, K. Imai, and I. Kitagawa, *Tetrahedron Letters*, **1967**, 2577.
30) *a*) I. Yosioka, A. Matsuda, M. Fujio, T. Nishimura, and I. Kitagawa, The 24th Grand Assembly of the Pharmaceutical Society of Japan, Abstract Papers Kyoto, 1967, 4, p. 464; *b*) I. Yosioka, A. Matsuda, and I. Kitagawa, The 88th Annual Meeting of the Pharmaceutical Society of Japan, Abstract Papers, Tokyo, 1968, 4, p. 256.

Experimental[31]

Theasapogenol E (III)——Silicic acid column chromatography of the crude sapogenol mixture obtained from seeds saponin of *Thea sinensis* L. as described in Part I,[1] gave theasapogenol E as one of the major sapogenols (*ca.* 200 mg from 3 g of the sapogenol mixture). Analytical sample of theasapogenol E was obtained by TLC separation (Camag D-5) followed by recrystallization with benzene–EtOH and aq. EtOH as colorless needles of melting at 260—263°, $[\alpha]_D$ +34° (c=0.6, pyridine). *Anal.* Calcd. for $C_{30}H_{48}O_6$: C, 71.39; H, 9.59. Found: C, 71.01; H, 9.61. IR ν_{max}^{KBr} cm^{-1}: 3560, 3380 (OH), 2680, 1727 (CHO), 1635 (C=C).

3,21,22,28-Tetra-O-acetyl-theasapogenol E (VI)——Ordinary treatment of theasapogenol E (III) (50 mg) with pyridine (2.5 ml) and Ac_2O (1 ml) by keeping four days at room temperature yielded a crude product, which was purified by preparative TLC ($CHCl_3$–AcOEt (5:1)) followed by recrystallization with aq. MeOH to give the tetraacetate (VI), mp 187—188°. *Anal.* Calcd. for $C_{38}H_{56}O_{10}\frac{1}{2}H_2O$: C, 66.93; H, 8.43. Found: C, 67.11; H, 8.31. IR ν_{max}^{KBr} cm^{-1}: 3600, 3416 (OH), 2691, 1736, 1248 (CHO and $OCOCH_3$).

3,16,21,22,28-Penta-O-acetyl-theasapogenol E (VII)——To a suspension of theasapogenol E (III) (150 mg) in Ac_2O (6 ml) was added p-TsOH·H_2O (catalytic amount) and the total mixture was stirred at room temp. for 8 hr. The mixture turned to a clear solution after 2 hr. The crude product (216 mg) obtained by usual work-up was purified by silica gel column giving VII (116 mg). Analytical sample of VII (colorless, amorphous) was prepared by further purification with preparative TLC (Camag D-5, $CHCl_3$–AcOEt (10:2)). All the attempts for crystallization were without success. *Anal.* Calcd. for $C_{40}H_{58}O_{11}$: C, 67.20; H, 8.18. Found: C, 67.10; H, 8.26. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2720, 1748, 1246, 1227 (CHO and $OCOCH_3$).

$LiAlH_4$ Reduction of Theasapogenol E (III) Yielding Theasapogenol A (II)——A solution of theasapogenol E (III) (30 mg) in dioxane (8 ml) was added with $LiAlH_4$ (100 mg) and refluxed for 3 hr. The reaction mixture was then treated with aq. ether and dil.H_2SO_4 successively followed by recrystallization twice with aq. EtOH to yield a product of mp 295—300°, which was proved identical with theasapogenol A (II)[2] by mixed mp, IR (KBr), and TLC.

Huang-Minlon Reduction of Theasapogenol E (III) Yielding Barringtogenol C (I)——A solution of theasapogenol E (III) (25 mg) in EtOH (2.7 ml) and diethylene glycol (6.7 ml) containing 80% hydrazine hydrate (2.5 ml) was refluxed 2 hr in an oil bath (temp. 150°). After adding KOH (0.5 g), the reaction mixture was heated by raising the bath temp. gradually to 220—230° with a downward condenser to distill off the low boiling component. Then the mixture was heated further 4 hr in the oil bath (temp. 230°) and poured into ice–water. The collected precipitate was repeatedly recrystallized with aq. EtOH to give the reaction product (mp 279—283.5°), which was identical with barringtogenol C (I)[1] by mixed mp, IR (KBr), and TLC.

Isomerization of Aldehyde Function of Theasapogenol E (III)——*a*) With Alkali: A mixture of theasapogenol E (III) (200 mg) in MeOH (20 ml) and water (1 ml) containing KOH (1 g) was refluxed one hour. The mixture was then poured into ice–water, acidified with dil.H_2SO_4 and the product (180 mg) was purified by silicic acid column eluting with $CHCl_3$–MeOH (97:3). Product from earlier eluate (30 mg, 15%, mp 225—230°, recrystallized with MeOH) was identical with authentic camelliagenin D (X)[7] (IR (KBr) and TLC), while the later eluate gave recovered theasapogenol E (102 mg) (50%).

b) With Acid (TLC Scale): Theasapogenol E (III) (10 mg) was treated with 4 ml of HCl–MeOH solution (conc. HCl 1.2 ml, H_2O 3 ml, MeOH 15 ml; identical acidity with the condition used for acid hydrolysis of saponin[1]) by refluxing 10 hr. TLC (Camag D-5; $CHCl_3$–MeOH (10:1)) revealed that the major spot coincided with theasapogenol E and the second major with camelliagenin D.

Reduction of Aldehyde Function of Theasapogenol E (III) Giving Theasapogenol A (II) and Protoaescigenin (XI)——A mixture of theasapogenol E (III) (500 mg) in MeOH (50 ml) and water (3 ml) containing KOH (5 g) was refluxed 8 hr. Methanol was removed while the addition of water and the resulting precipitate was collected by filtration giving a neutral product (300 mg). After acidification with dil.HCl, the filtrate was extracted with ether giving an acidic product (57 mg). The neutral product (300 mg) was chromatographed on silicic acid (100 mg) column eluting with $CHCl_3$–MeOH mixture successively. From $CHCl_3$–MeOH (98:2—97:3) eluate, an unidentified product (16 mg, 3% yield; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1720 (sh.), 1690, 1650, 1625) having higher *Rf* value than camelliagenin D (X) was obtained, whereas the subsequent

31) Melting points were taken on the Yanagimoto Micromelting-point Apparatus (a hot-stage 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). TLC plates were made with silica gel (Camag D-5 or Merck G) and developed by spraying 1%Ce $(SO_4)_2$/10% H_2SO_4 solution followed by heating unless specified otherwise. For the column chromatography, the following adsorbents were used: silica gel (Merck, 0.05—0.2 mm), silicic acid (Mallinckrodt), and alumina (Woelm neutral, Merck standard, Sumitomo).

fraction eluted with $CHCl_3$–MeOH (97:3) afforded theasapogenol E (17 mg, 3.3%). The eluate with $CHCl_3$–MeOH (97:3—95:5) gave protoaescigenin (XI) (11 mg, 2.1%, identified with the authentic sample[8] by IR (KBr) and TLC). Finally the eluate with $CHCl_3$–MeOH (93:7) gave pure theasapogenol A (II) (170 mg, 34%) and $CHCl_3$–MeOH (90:10) eluate gave crude theasapogenol A (49 mg) contaminated with a more polar component. Although the acidic portion (57 mg) was shown by TLC (Camag D-5, $CHCl_3$–MeOH (10:1)) to consist of five components, only one of them was affected by CH_2N_2 treatment. Due to the shortage of the material, the further examination was abandoned.

In another experiment, the treatment of theasapogenol E with 10% KOH–EtOH by refluxing 2.5 hr afforded protoaescigenin in 5.6% yield.

$LiAlH_4$ Reduction of Methyl Leucotylidienate (XIIa) affording XIIb——To a solution of methyl leucotylidienate (XIIa)[14] (250 mg) in tetrahydrofuran (50 ml) was added $LiAlH_4$ (1 g) and the total mixture was refluxed for 5 hr. After working up in a usual manner, the colorless crystalline product (single spot on TLC, 215 mg) was recrystallized with $CHCl_3$–MeOH to give the analytical sample of XIIb, mp 160—161°, $[\alpha]_D$ +94°(c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{48}O$: C, 84.84; H, 11.39. Found: C, 84.86; H, 11.14. NMR ($CDCl_3$)τ: 9.24 (3H, s), 9.15 (6H, s), 9.10 (3H, s), 8.86 (3H, s) (–C(CH$_3$)–CH$_3$×5), 9.05, 9.03 (3H each, d, J=7 Hz) (–CH(C**H**$_3$)$_2$), 6.90, 6.58 (2H, ABq., J=11 Hz) (–C**H**$_2$OH), 4.41, 3.82 (2H, ABq., J=10 Hz) (–C**H**=C**H**–).

Oxidation of XIIb giving the Aldehyde (XII)——A mixture of pyridine (8 ml) solution of XIIb (170 mg) and CrO_3–pyridine complex (CrO_3 240 mg, pyridine 5 ml) was stirred at room temp. for 3.5 hr. The mixture was then poured into water, extracted with ether and worked up in a usual manner giving a crude product (157 mg). Purification of the product through silicic acid column chromatography followed by recrystallization with EtOH gave the crystalline product (XII) (48 mg) of mp 157—158° (from $CHCl_3$–MeOH). *Anal.* Calcd. for $C_{30}H_{46}O$: C, 85.24; H, 10.97. Found: C, 84.87; C, 11.08. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2700, 1725 (CHO), 1647 (C=C). NMR ($CDCl_3$) τ: 9.14 (6H), 9.10 (6H), 8.96 (6H), 8.83 (3H) (totally seven methyls), 4.45, 3.84 (2H, ABq. J=10 Hz) (–C**H**=C**H**–), 0.78 (1H, s) (C**H**O). Mass Spectrum m/e: 422 (M^+, 10%), 407 (51%), 187 (100%).

Reduction of Aldehyde Function of XII giving XIIb——A solution of XII (20 mg) in 10% KOH–EtOH (8 ml) was refluxed while monitoring by TLC. After 13 hr reflux the starting aldehyde was consumed, and the product was poured into dil. H_2SO_4 and extracted with ether. Working up in a usual manner followed by silicic acid column chromatography and recrystallization with MeOH, the colorless needles of mp 160—162° were obtained. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3630, 3450 (OH), 1625 (C=C). The product was proved identical with XIIb by TLC, mixed mp, and IR.

Oxidation of Erythrodiol furnishing Keto-aldehyde (XIII)——A solution of erythrodiol (100 mg) in pyridine (3 ml) was treated with CrO_3–pyridine complex (CrO_3 300 mg, pyridine 5 ml) by stirring at room temp. for 3 hr. After usual work up the crude product (77 mg) was chromatographed on silicic acid column giving the keto-aldehyde (XIII) (40 mg), mp. 144° (from $CHCl_3$–MeOH,) $[\alpha]_D$+97° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{46}O_2$: C, 82.13; H, 10.57. Found: C, 82.15; H, 10.43. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2730, 1727, 1705 (CHO, CO). Mass Spectrum m/e: 438 (M^+, 13%), 232 (41%), 203 (100%).

Reduction of Aldehyde Function of XIII affording XIIIa——After refluxing 5 hr, the mixture (XIII, 20 mg in 10% KOH–EtOH, 2 ml) was poured into dil. H_2SO_4 and extracted with ether. The extract was then worked up in a usual manner to give a crude product ,which was then separated by TLC affording XIIIa (major, 7.2 mg) and the recovered keto-aldehyde XIII (4.6 mg). The analytical sample of XIIIa (recryst. from aq. MeOH) showed mp 156—159°. *Anal.* Calcd. for $C_{30}H_{48}O_2$: C, 81.76; H, 10.98. Found: C, 81.35; H, 11.14. IR $\nu_{max}^{KBr}$$cm^{-1}$: 3500 (OH), 1705 (CO).

Dihydropriverogenin A (=Theasapogenol D) (IV)——Alumina column chromatography of the crude sapogenol mixture[1] followed by repeated recrystallization with aq. MeOH gave analytical sample of theasapogenol D (IV), mp 273—275°, $[\alpha]_D$+30° (c=0.7, EtOH). *Anal.* Calcd. for $C_{30}H_{50}O_4$: C, 75.90; H, 10.62. Found: C, 75.98; H, 10.54. IR ν_{max}^{KBr} cm^{-1}: 3401 (OH), 1629 (C=C). Theasapogenol D thus obtained was identified (mixed mp, IR (KBr), and TLC) with camelliagenin A[20] supplied by Prof. T. Murakami and with dihydropriverogenin A[16] supplied by Prof. R. Tschesche.

3,22,28-Tri-O-acetyl-dihydropriverogenin A (XIV)——A solution of dihydropriverogenin A (IV) (100 mg) in pyridine (5 ml)–Ac_2O (3 ml) mixture was let stand one overnight at 25°. The product obtained after usual work-up was passed through a short alumina column (Woelm, neutral) with the aid of benzene to decolorize and recrystallized with n-hexane and then with aq. EtOH giving XIV, mp 198—200°, $[\alpha]_D$+17° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{36}H_{56}O_7$: C, 71.96; H, 9.40. Found: C, 72.17; H, 9.55. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600, 3530, 3460 (OH), 1740, 1735, 1245 ($OCOCH_3$).

Acetonide Derivatives, XV and XVI, from Dihydropriverogenin A (IV)——To a solution of dihydropriverogenin A (IV) (300 mg) in dry acetone (50 ml) was added anhydrous cupric sulfate (1.5 g) and the total mixture was stirred at room temp. for 5 hr and filtered. Concentration of the filtrate gave colorless solid (334 mg). The preparative TLC separation of the product (100 mg) using Alumina G (Merck) and $CHCl_3$ furnished XV (64 mg) and XVI (31 mg). Analytical sample of XV was obtained by passing through a short column (Woelm, neutral, grade I) to decolorize with the aid of ether followed by recrystallization with benzene–n-hexane yielding colorless crystals of XV (59 mg), mp 256—260°. *Anal.* Calcd. for $C_{33}H_{54}O_4$: C, 76.99; H, 10.57.

Found: C, 76.71; H, 10.36. Analytical sample of XVI was prepared by repeated recrystallization with ether–*n*-hexane giving colorless needles of melting at 272—275° (in a sealed tube). *Anal.* Calcd. for $C_{33}H_{54}O_4$: C, 76.99; H, 10.57. Found: C, 77.14; H, 10.75.

Acetylation of XV and XVI, Yielding XVII and XVIII——a) Acetylation of XV (25 mg) with pyridine–Ac_2O (2.5—1.5 ml) mixture by keeping at 25° for 15.5 hr followed by ordinary treatment gave a crude product (27 mg), which was once dissolved in ether to remove insoluble portion and crystallized from aq. MeOH to give colorless leaflets (22 mg) (XVII). Analytical sample of XVII was prepared by further recrystallization with aq. MeOH. Colorless leaflets, mp 186—188°. *Anal.* Calcd. for $C_{37}H_{58}O_6$: C, 74.21; H, 9.76. Found: C, 74.47; H, 9.68. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1736, 1244, 1230 ($OCOCH_3$), no hydroxyl.

b) The crude product obtained by treatment of XVI (62 mg) with pyridine (2.5 ml) and Ac_2O (1.5 ml) as above was recrystallized repeatedly with acetone–*n*-hexane to give colorless needles (XVIII) (32 mg), mp 226—228°. *Anal.* Calcd. for $C_{35}H_{56}O_5$: C, 75.49; H, 10.14. Found: C, 75.75; H, 10.22. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600, 3480 (OH), 1730, 1247 ($OCOCH_3$).

Conversion of Barringtogenol C(I) to Dihydropriverogenin A(IV)——a) Tritylation followed by Acetonide Formation Yielding XIX: A solution of barringtogenol C (2 g), TrCl (3 g) in anhydrous pyridine (50 ml) was refluxed 5 hr. The residue obtained by evaporation of pyridine in vacuo was dissolved in $CHCl_3$ and filtered to remove small amount of insoluble portion. The $CHCl_3$ solution was washed with water and evaporated *in vacuo* giving a residue, which was taken with ether. The ether solution was washed with water and evaporated giving a crude product, which was chromatographed on alumina column (Merck 150 g). Benzene eluate gave triphenylcarbinol, while the subsequent $CHCl_3$ elution gave a mixture of ditrityl derivative (probably 3,28-di-O-trityl-barringtogenol C) and 28-O-trityl derivative and then gave 28-O-trityl-barringtogenol C (1.48 g) (single spot on TLC). Recrystallization with benzene–EtOH–*n*-hexane mixture furnished analytical sample of 28-O-trityl-barringtogenol C (cubic crystals), mp 181—184° (unsharp, softens around 120° and becomes amorphous[32]). $[\alpha]_D$ −16° (c=1.03, $CHCl_3$). *Anal.* Calcd. for $C_{49}H_{64}O_5$: C, 80.29; H, 8.80. Found: C, 80.46; H, 8.94.

A mixture of 28-O-trityl-barringtogenol C (200 mg), anhydrous $CuSO_4$ (2 g) in dry acetone (25 ml) was stirred at room temp. 25 hr and filtered. The filtrate was evaporated to give a crude product, which on TLC (Camag D-5, $CHCl_3$–MeOH (10:1)) was disclosed to be contaminated with small amount of starting material and purified by preparative TLC. Acetylation of 28-O-trityl-16,22-O-monoacetonide (66 mg, single spot on TLC) thus obtained with Ac_2O (1.5 ml)–pyridine (2.5 ml) mixture at 25° overnight followed by usual work-up afforded a crude product. TLC (Camag D-5, $CHCl_3$) of the product showed two spots. The major with lower *Rf* value was collected by preparative TLC to give 3-O-acetyl-28-O-trityl-16,22-O-monoacetonide (XIX) (amorphous, 47 mg, single spot on TLC). The minor with higher *Rf* value is presumed to be diacetyl derivative.

b) Oxidation of XIX Giving XX: To a solution of XIX (47 mg) in pyridine (3 ml) was added CrO_3–pyridine complex (CrO_3 100 mg, pyridine 3 ml) and the total mixture was kept stirring 6 hr at room temp., and let stand at 29° two days. Usual work-up of the reaction mixture gave a crude product (33 mg), which was purified by repeated preparative TLC giving XX (9.0 mg, single spot on TLC). Analytical sample of XX, mp 262—263°, was obtained by recrystallization with acetone–MeOH. *Anal.* Calcd. for $C_{54}H_{68}O_6$: C, 79.76; H, 8.43. Found: C, 79.78; H, 8.54.

c) Deacetonidation of XX followed by Huang-Minlon Reduction affording Dihydropriverogenin A (IV): A solution of XX (*ca.* 10 mg) in acetone (2 ml), dioxane (1.5 ml), and 20% HCl–MeOH (0.5 ml), was let stand 10 min at room temp. and poured into water. The precipitate was collected by ether extraction to give the deacetonidation product. The product was then dissolved in EtOH (1 ml), triethylene glycol (5 ml), and 80% hydrazine hydrate (1 ml), refluxed 3 hr in an oil bath (temp. 130—135°), and added with KOH (0.3 g) and refluxed further 15 min. The bath temp. was then raised gradually to 230° with a downward condenser to remove low boiling component and finally the reaction mixture was heated in the oil bath (temp. 230—240°) 4 hr, poured into water, and extracted with ether. The crude product thus obtained was refluxed in 5% HCl–MeOH 10 min, evaporated, added with water, and extracted with ether. The extracted product was revealed on TLC to contain two component. The one with lower *Rf* value was collected by TLC and recrystallized with aq. MeOH to furnish crystals, identical with dihydropriverogenin A (IV) by mixed mp, IR (KBr) and TLC.

Acetyl Migration of XIV affording XXI, XXII, and XXIII——XIV (450 mg) was adsorbed on alumina column (Woelm, neutral, grade I, 15 g) with the aid of benzene and the column was let stand at room temp. for 13 hr and developed with $CHCl_3$–MeOH (1:1) to elute out all the components. The crude eluate was then chromatographed on silica gel (30 g) developing with i)$CHCl_3$, ii)$CHCl_3$–MeOH (100:0.3) and iii)$CHCl_3$–MeOH (100:1) successively. Combined fraction from i) and early part of ii) was further purified by preparative TLC (Camag D-5, $CHCl_3$–AcOEt (9:1)) to afford XIV (less than 10 mg, identified by TLC) and XXIII (colorless needles 23 mg, after crystallization from ether–*n*-hexane). The latter was recrystallized with aq. MeOH to give the analytical sample of XXII, mp 233—238° (sintering from 221°). *Anal.* Calcd.

32) Using different combinations of solvent mixtures, the crystals obtained showed the similar property.

for $C_{35}H_{56}O_7$: C, 71.96; H, 9.40. Found: C, 72.08; H, 9.42. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3500 (OH), 1730, 1248 ($OCOCH_3$).

Recrystallization with ether–*n*-hexane of the eluate from later part of ii) gave colorless needles of XXI (281 mg, major product), which was recrystallized again with the same solvent mixture to give the analytical sample of XXI, mp 186—189°. *Anal.* Calcd. for $C_{35}H_{56}O_7$: C, 71.96; H, 9.40. Found: C, 71.71; H, 9.35. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600, 3510 (OH), 1738, 1243 ($OCOCH_3$).

Recrystallization with aq. MeOH of the fractions from iii) gave colorless needles of XXIII (26 mg), which was again recrystallized with the same solvent mixture to give the analytical sample of XXIII, mp 246—249°. *Anal.* Calcd. for $C_{34}H_{54}O_6$: C, 73.08; H, 9.74. Found: C, 72.99; H, 9.78. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (OH), 1720, 1252 ($OCOCH_3$).

Oxidation of XXI giving XXIV——Under ice-cooling a solution of XXI (175 mg) in acetone (15 ml) was treated with Kiliani reagent (0.3 ml) for 10 min and poured into ice-water. The precipitate was collected and crystallized from aq. MeOH to afford coloress crystals of XXIV (159 mg). Analytical sample of XXIV was obtained by further recrystallization wit aq. MeOH as colorless needles, mp 196—199°. *Anal.* Calcd. for $C_{36}H_{54}O_7$: C, 72.21; H, 9.09. Found: C, 72.02; H, 9.11. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1745, 1245—1225 (br.) ($OCOCH_3$), 1705 (CO).

Conversion of XXIV to 22-Epimeric Acetate (XXVI)——A solution of XXIV (37 mg) in MeOH (3 ml) was treated with $NaBH_4$ (100 mg) by stirring for 3 hr at room temp. followed by acidification with AcOH. The total mixture was then added with 10% KOH–MeOH to make pH>11, refluxed for one hour, concentrated to remove MeOH, and diluted with water. The precipitated product was collected (36 mg), and crystallized from aq. MeOH furnishing colorless needles, which showed 2 spots on TLC (Camag D-5, $CHCl_3$–MeOH (10:1), Rf=0.53 and 0.47). TLC separation of the crystals gave dihydropriverogenin A (IV) (11.3 mg, having higher Rf value), and its 22-epimer (XXV) (18.2 mg). The latter was then acetylated with pyridine (2 ml)–Ac_2O (1 ml) mixture in a usual manner, and the product was crystallized from acetone–*n*-hexane to give crystals (15 mg) of XXVI. Analytical sample of XXVI was prepared by recrystallization with MeOH as colorless needles, mp 241.5—242.5°. *Anal.* Calcd. for $C_{36}H_{56}O_7$: C, 71.96; H, 9.40. Found: C, 71.93; H, 9.23. IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1728, 1270 (sh.), 1248 ($OCOCH_3$).

Camelliagenin C (=Theasapogenol C) (V)——Analytical sample of theasapogenol C was obtained by TLC separation ($CHCl_3$–MeOH (10:1)), followed by AcOEt–MeOH recrystallization twice, giving colorless needles of mp 264—266°. $[\alpha]_D$+32° (c=1.31, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3378 (OH), 1631 (C=C). *Anal.* Calcd. for $C_{30}H_{50}O_5$: C, 73.43; H, 10.27. Found: C, 73.21; H, 10.16. Theasapogenol C thus obtained was identified (mixed mp, IR (KBr), and TLC) with camelliagenin C supplied by Prof. T. Murakami.[20]

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