[Chem. Pharm. Bull. 23(6)1336—1346(1975)] UDC 547.597.02 : 581.192

Lycopodium Triterpenoids. (8).[1] The Structures of Lycoclavanin, a Triterpenoid-tetraol Possessing Conjugated Ketone Chromophor, and a New Tetraol, Lyclaninol[2]

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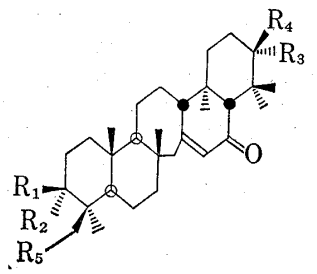
Lycoclavanin, a *Lycopodium* triterpenoid containing four hydroxy-groups and a conjugated ketone, was established as 16-oxoserrat-14-ene-3α,20β,21β,24-tetraol (**7a**) by spectral and chemical means. Correlations of lycoclavanin with 16-oxo-lycoclavanol (**6a**) and with serratenediol (**17a**) were described. A new tetraol occurring in *L. clavatum* was also established as serrat-14-ene-3α,20β,21β,24-tetraol (**23a**).

Lycoclavanin is one of major triterpenoid constituent of *Lycopodium clavatum* isolated in 1962.[4] Lately occurrence of the same compound in *L. complanatum* was confirmed.[5] Since the first isolation of lycoclavanin it had been suggested that the compound was a tetraol with possessing an α,β-unsaturated ketone, though the whole structure was uncertain until our communication[2c] has appeared. A new triterpenoid, lyclaninol, isolated from *L. clavatum*[5] was found to be the corresponding deoxo-compound.[2b] This paper deals the full document of the experiments of their structural elucidations and of their chemical correlations.



1a: $R_1=R_3=OH$, $R_2=R_4=R_5=H$
1b: $R_1=R_3=OAc$, $R_2=R_4=R_5=H$
2a: $R_1=R_4=OH$, $R_2=R_3=R_5=H$
2b: $R_1=R_4=OAc$, $R_2=R_3=R_5=H$
3a: $R_2=R_4=OH$, $R_1=R_3=R_5=H$,
3b: $R_2=R_4=OAc$, $R_1=R_3=R_5=H$
4a: $R_1=R_3=R_5=OH$, $R_2=R_4=H$
4b: $R_1=R_3=R_5=OAc$, $R_2=R_4=H$
5a: $R_1=R_4=R_5=OH$, $R_2=R_3=H$
5b: $R_1=R_4=R_5=OAc$, $R_2=R_3=H$
6a: $R_2=R_4=R_5=OH$, $R_1=R_3=H$
6b: $R_2=R_4=R_5=OAc$, $R_1=R_3=H$

Chart 1

Lycoclavanin

Lycoclavanin is hardly soluble to an usual organic solvent, therefore it is well characterized as its tetraacetate.

The nuclear magnetic resonance (NMR) spectrum of lycoclavanin tetraacetate (**7b**) (Table I) indicated the presence of six C-methyl groups and of four acetyl methyls. Three of four acetoxy-groups are secondary and one is primary, the latter consisting of axial $-CH_2OAc$ as suggested from the chemical shift of the AB quartet at δ 4.09 ppm. Two of three secondary hydroxy-groups constitute α-glycol system as suggested from the positive periodate test of lycoclavanin. The fifth oxygen function in

1) This forms Part XI of "Triterpenoid Chemistry." Part X. Lycopodium Triterpenoids. (7): Y. Tsuda, T. Fujimoto, and K. Kimpara, *Chem. Pharm. Bull.* (Tokyo), **23**, 1290 (1975).
2) A part of this work was presented at the following Symposiums; *a*) 13th Symposium on the Chemistry of Natural Products, Sapporo, Sept., 1969; *b*) 14th Sympodium on the Chemistry of Natural Products, Fukuoka, Oct., 1970; *c*) Preliminary communication of *a*): Y. Tsuda and T. Fujimoto, *Chem. Comm.*, **1970**, 260.
3) Location: *Tsurumaki 5-1-8, Setagaya-ku, Tokyo, 154, Japan*; *a*) Present address: *Takeda Chemical Industries Ltd.*
4) Y. Inubushi, Y. Tsuda, and T. Sano, *Yakugaku Zasshi*, **82**, 1083, 1532 (1962).
5) Y. Tsuda, T. Fujimoto, K. Isobe, T. Sano, and M. Kobayashi, *Yakugaku Zasshi*, **94**, 970 (1974).

lycoclavanin consists of a conjugated ketone chromophor which has the following spectral features, UV: λ_{max} 274 nm (ε 14000), IR: ν_{max} 1665 (s), 1625 (m) cm^{-1}. The NMR signal at δ 5.75 ppm (1H, broad s.) indicated that the double bond is a trisubstituted one. It also showed negative peaks at 350—380 nm in an ORD and a negative maximum at 335 nm ($[\theta]=-1660$) in circular dichroism (CD) spectra. These spectral data are almost identical with those of 16-oxoserratenes (**1**)—(**6**) occurring in the various *Lycopodium* plants thus suggesting that the compound possesses the 16-oxoserrat-14-ene skeleton.[1] The solvent shift experiment for NMR gave further informations (Fig. 1). By continuously changing the solvent from $CDCl_3$ to benzene[6] two methyl groups at the lowest field (δ 1.18 and 1.31 ppm) experienced marked down-field shift. Since they are attributed to 22-dimethyl groups as discussed already, this evidence indicates that neither are carrying acetoxy-group.

Table I. NMR Spectra of the Acetates (δ ppm, 60 MHz. solvent: $CDCl_3$)

Compd.	$-\overset{\vert}{\underset{\vert}{C}}-Me$[a]	$-O-COCH_3$[a]	>CH–OAc	$-CH_2-OAc$[b]	>C=CH–	17-H[c]
7b	0.86(1), 0.91(2), 0.95(1), 1.18(1), 1.31(1)	1.99(1), 2.05(1), 2.09(1), 2.15(1)	4.94(2H)[d] 5.0—5.5 (1H, m)	4.09 (J=12, δ=18 Hz)	5.75[e]	2.45
23b	0.81(1), 0.87(3), 0.95(1), 1.05(1)	1.98(1), 2.05(1), 2.08(1), 2.11(1)	4.99(2H)[d] 5.0—5.5 (1H, m)[f]	4.10 (J=12, δ=18 Hz)	5.37[f,g]	—

a) numbers in parentheses denote number of methyl groups
b) signals appeared as AB quartet of 2H
c) signal appeared as singlet of 1H
d) Protons corresponding to 1H (broad singlet) and to 1H (doublet, J=6 Hz) are overlapped.
e) signals appeared as broad singlet of 1H
f) Signals were overlapped.
g) signal appeared as multiplet

Table II. Type Classification Signals of Acetonides (60 MHz)

Compd.	$O\diagdown\ \diagup Me$ C $O\diagup\ \diagdown Me$	$-CH_2-O$	>CH–O	type
8	1.42(2)	3.66 ABq. J=11, δ_{AB}=23 Hz	4.32 t. J=8 Hz[a]	**B**
	1.36(1) 1.53(1)		3.62 d. J=4.5 Hz 4.0—4.4(1H, m.)[a]	5-membered
9b	1.34(1) 1.51(1)		3.64 d. J=4.5 Hz 4.0—4.4(1H, m.)	5-membered
24	1.42(2)	3.71 ABq. J=11, δ_{AB}=23 Hz	4.35 t. J=8 Hz[a]	**B**
	1.34(1) 1.50(1)		3.76 d. J=5 Hz 4.0—4.4(1H, m.)[a]	5-membered
25b	1.34(1) 1.51(1)		3.75 d. J=5 Hz 4.0—4.4(1H, m.)	5-membered

a) Signals are overlapped.

On application of a forced condition,[7] lycoclavanin formed a mixture of di- and mono-acetonide, **8** and **9a**. Further reaction converted the latter (**9a**) into the former (**8**), and the former slowly decomposed into the latter when heated in methanol. Either compound

6) M. Hashimoto and Y. Tsuda, International Symposium on NMR, Preliminary Report, M-2-13, Tokyo, 1965.
7) Y. Tsuda, T. Sano, A. Morimoto, M. Hatanaka, and Y. Inubushi, *Chem. Pharm. Bull.* (Tokyo), **22**, 2383 (1974).

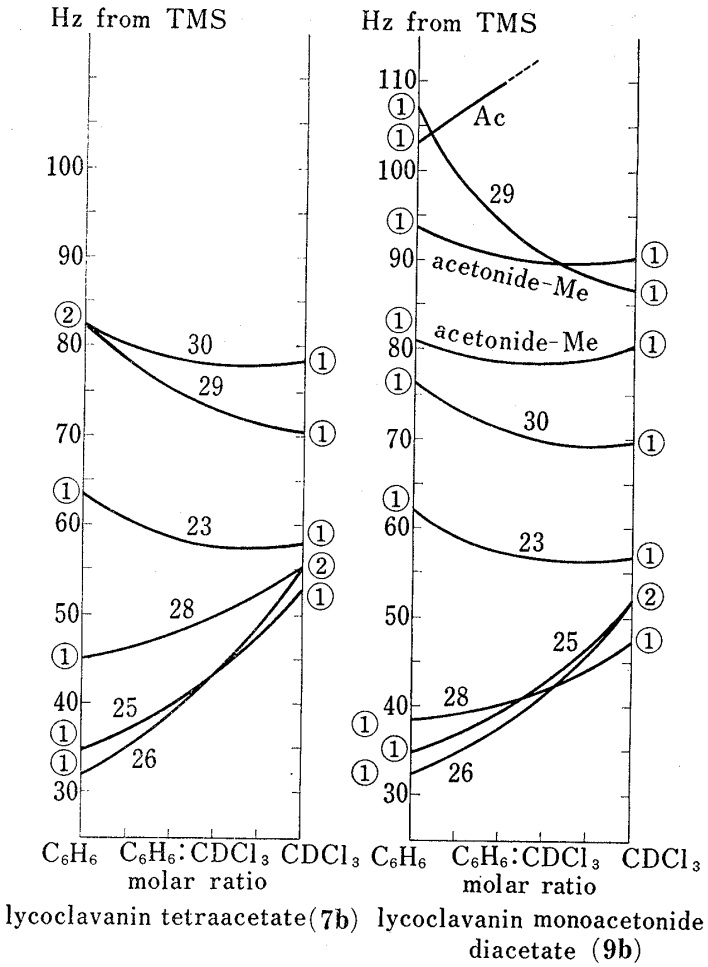


Fig. 1. TH-Effects of Lycoclavanin Derivatives

a) Number in ○ indicates number of methyl groups.
b) Numbers on the curves indicate the position of hydrogens.

regenerated lycoclavanin on acid hydrolysis indicating that no skeletal change took place during these reactions. Acetylation of **9a** with acetic anhydride and pyridine afforded a diacetate (**9b**), whose NMR spectrum exhibited signals indicative of the presence of $-CH_2-$OAc at δ 4.07 (ABq., $\delta_{AB}=18$, $J=$ 12 Hz) and of >CH–OAc at δ 4.95 ppm (1H, broad s.) showing that the O,O-isopropylidene function formed firstly between two secondary hydroxy-groups which must be the afore-mentioned α-glycol system.

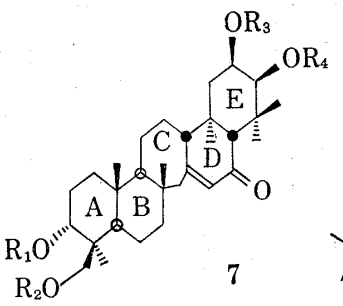
The type classification signals[8] assigned with aid of the solvent shift experiment of the acetonide **8** and **9b** are shown in Table II which disclosed that the O,O-isopropylidene function formed between the primary and a secondary hydroxy-groups are of type **B** (acetonide formed between ax-OH and ax-CH_2–OH) among the four types of acetonide of 1,3-glycol system[8] which therefore must be at ring A as 3α-OH and 4β-CH_2–OH.

Acid hydrolysis of **9b** with acetic acid and tosylation of the resulting diol-diacetate (**7c**) with tosyl chloride and pyridine gave a monotosylate (**7d**) which on slow chromatography over alumina or on heating in pyridine lost *p*-TsOH to furnish a keto-diacetate (**10b**). The formation of the ketonic function was shown by appearance of a new infrared (IR) absorption at 1715 cm^{-1}. Thus the presence of *cis*-1,2-glycol function in lycoclavanin was confirmed, for *trans*-1,2-glycol the product would be an epoxide. Since two of six methyl groups of this compound appeared at fairly low field (δ 1.35), the ketonic function formed should be at the neighbouring carbon, *i.e.*, C-21. The structure **10b** was therefore suggested for the keto-diacetate, which was established by transforming 16-oxol ycoclavanol (**6a**) to the same compound (**10b**).

Chromium trioxide-pyridine oxidation of 16-oxolycoclavanol acetonide (**11**)[1] gave the keto-acetonide (**12**) which on hydrolysis with acetic acid followed by acetylation yielded the keto-diacetate (**10b**) identical with the compound obtained above.

Formation of **10b** from the monotosylate (**7d**) indicates that tosylation on *cis*-20,21-glycol (**7c**) occurred exclusively at 20-hydroxy-group, thus suggesting the equatorial orientation of this group and hence an axial orientation of 21-OH group. Combining this evidence with the fact that 17-H signal of lycoclavanin tetraacetate (**7b**) appeared at δ 2.45 which indicates the axial orientation of 21-OAc, the 20β,21β-configuration of conformation I for lycoclavanin

8) Y. Tsuda, T. Sano, K. Isobe, and M. Miyauchi, *Chem. Pharm. Bull.* (Tokyo), **22**, 2396 (1974).



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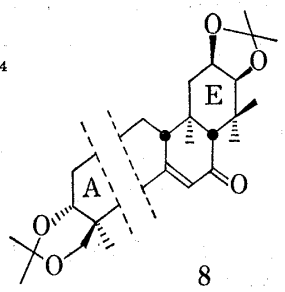

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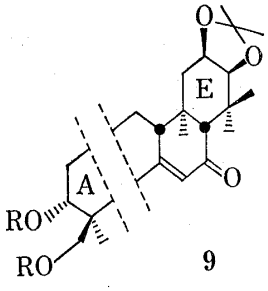

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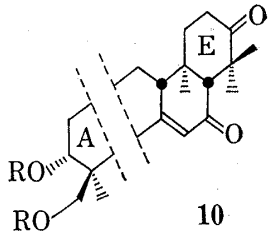
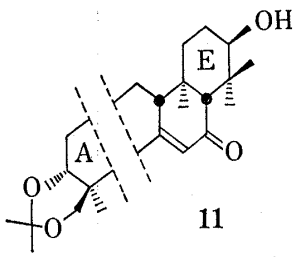


10

9, 10a : R=H
9, 10b : R=Ac

7a : $R_1=R_2=R_3=R_4=H$
7b : $R_1=R_2=R_3=R_4=Ac$
7c : $R_1=R_2=Ac$, $R_3=R_4=H$
7d : $R_1=R_2=Ac$, $R_3=Ts$, $R_4=H$
7e : $R_1=R_2=Ac$, $R_3=R_4=Bz$



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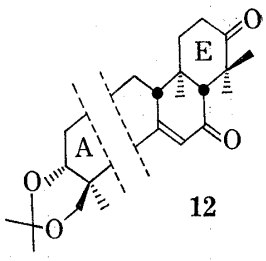
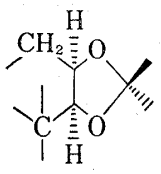


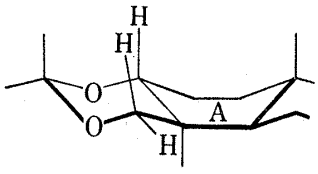
12

Ts=tosyl Bz=benzoyl

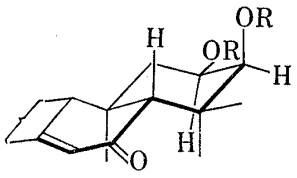
Chart 2



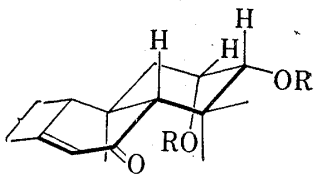
5-membered



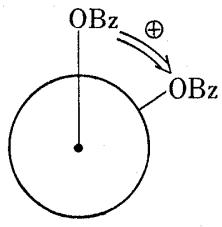
type **B**



I



II



Bz=benzoyl

Chart 3

was concluded. If it has conformation II the signal should appear at δ 2.2 ppm. This conclusion was supported by the positive Cotton effect in CD of the dibenzoate-diacetate (**7e**) prepared by benzoylation of **7c**.

Harada and Nakanishi[9] reported the "dibenzoate chirality rule" which states that the dibenzoate of α-glycol with a clockwise chirality shows a positive Cotton effect at the first extreme around 230 nm, while that with an anti-clockwise chirality shows a negative Cotton effect. The diacetate-dibenzoate (**7e**) showed a positive Cotton effect at 234 nm in its CD spectrum. The $\Delta\varepsilon$ value of **7e** is obviously larger about 27 compared to lycoclavanin tetraacetate (**7b**). Applicating the dibenzoate chirality rule to **7e**, we can conclude that the compound has chair conformation either I or II depending upon the glycol function to be β or α

9) N. Harada and K. Nakanishi, *J. Am. Chem. Soc.*, **91**, 3989 (1969).

orientation, the both of I and II having clockwise chirality, and eliminate any contribution from boat conformations of ring E, for which the *cis*-glycol should have negative chirality regardless to the orientation of the glycol system. Since 21-OAc is pararell to 17-H as mentioned above, the glycol should have the conformation I.

Lycoclavanin is therefore 16-oxoserrat-14-ene-3α,20β,21β,24-tetraol (**7a**).

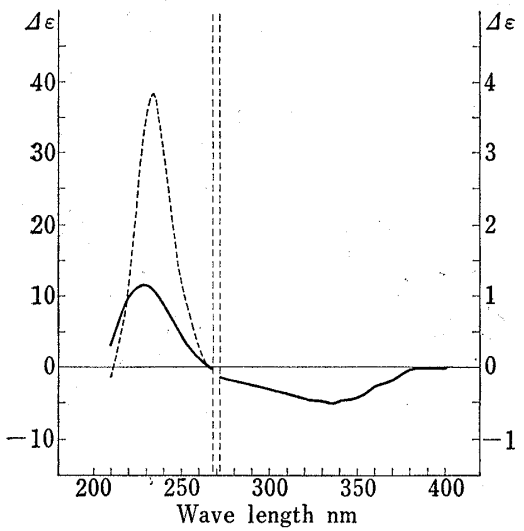


Fig. 2. CD Spectra of Lycoclavanin Tetraacetate and Lycoclavanin Dibenzoate-diacetate (Solvent:Dioxane)

——— : lycoclavanin tetraacetate (**7b**)
····· : lycoclavanin dibenzoate-diacetate (**7e**)

Direct Correlation of Lycoclavanin with Serratenediol

Turning over the process of structural determination of lycoclavanin, it is based upon following chemical correlations: serratenediol ⟷ serratriol ⟷ 16-oxoserratriol ⟷ 16-oxo-lycoclavanol ⟷ lycoclavanin, that is, the goal of long travelling.

This section describes direct correlation of lycoclavanin with serratenediol (**17a**), the most simple representative of serratane group. The procedure herein described will provides an example of general method of correlating poly-oxygenated triterpenoid to the simplest homolog.

For this purpose three of five oxygens in lycoclavanin must be removed and the stereochemistry of remaining two hydroxy-groups should be diminished or inverted. These were accomplished as follows.

Hydrogenation of lycoclavanin tetraacetate (**7b**) over PtO_2 in acetic acid containing a catalytic amount of perchloric acid afforded the deoxo-14β-dihydro derivative (**13b**) as a major product, disappearances of UV absorption and olefinic proton signal in NMR spectrum confirming this transformation (the stereochemistry at C_{14} is proved below). The compound **13b** being isolated by chromatography and fractional crystallizations was hydrolysed and the resulting alcohol (**13a**) was heated with tosyl chloride in pyridine to yield the keto-tosylate (**15a**) which was apparently a detoluenesulfonic acid product of an intermediate ditosylate (**13c**) as mentioned already, an axial 3α-OH group being remained unattacked. Two oxygen functions were thus removed. Removal of 24-OH group was achieved by the method described previously.[10] Treatment of **15a** with *t*-BuOK followed by $NaBH_4$ yielded the secodiol (**16**) in a good yield, meanwhile 21-keto group being reduced to an equatorial alcohol.

For preparation of this compound from serratenediol (**17a**) two hydroxy groups at C_3 and C_{21} must be differenciated. Thus serratenediol 3-acetate (**17b**) was chosen as a starting material. Hydrogenation of **17b** and fractional crystallizations of the product yielded 14β-dihydro derivative (**18a**) in a pure form. The stereochemistry at C_{14} was proved by converting the compound to 14β-serratane (**19**)[11] by usual sequences (see Experimental). This was benzoylated and the resulting 3-acetate-21-benzoate (**18b**) was partially hydrolysed to the 21-benzoate (**18c**). Treatment of **18c** with lead tetraacetate followed by LAH reduction yield a seco-diol which was completely identical with the compound (**16**) obtained from lycoclavanin.

10) Y. Tsuda, K. Isobe, T. Sano, and A. Morimoto, *Chem. Pharm. Bull.*, (Tokyo), **23**, 98 (1975).
11) *a*) Y. Inubushi, Y. Tsuda, T. Sano, T. Konita, S. Suzuki, H. Ageta, and Y. Otake, *Chem. Pharm. Bull.* (Tokyo), **15**, 1153 (1967); *b*) Y. Tsuda, T. Sano, and Y. Inubushi, *Tetrahedron*, **26**, 751 (1970).

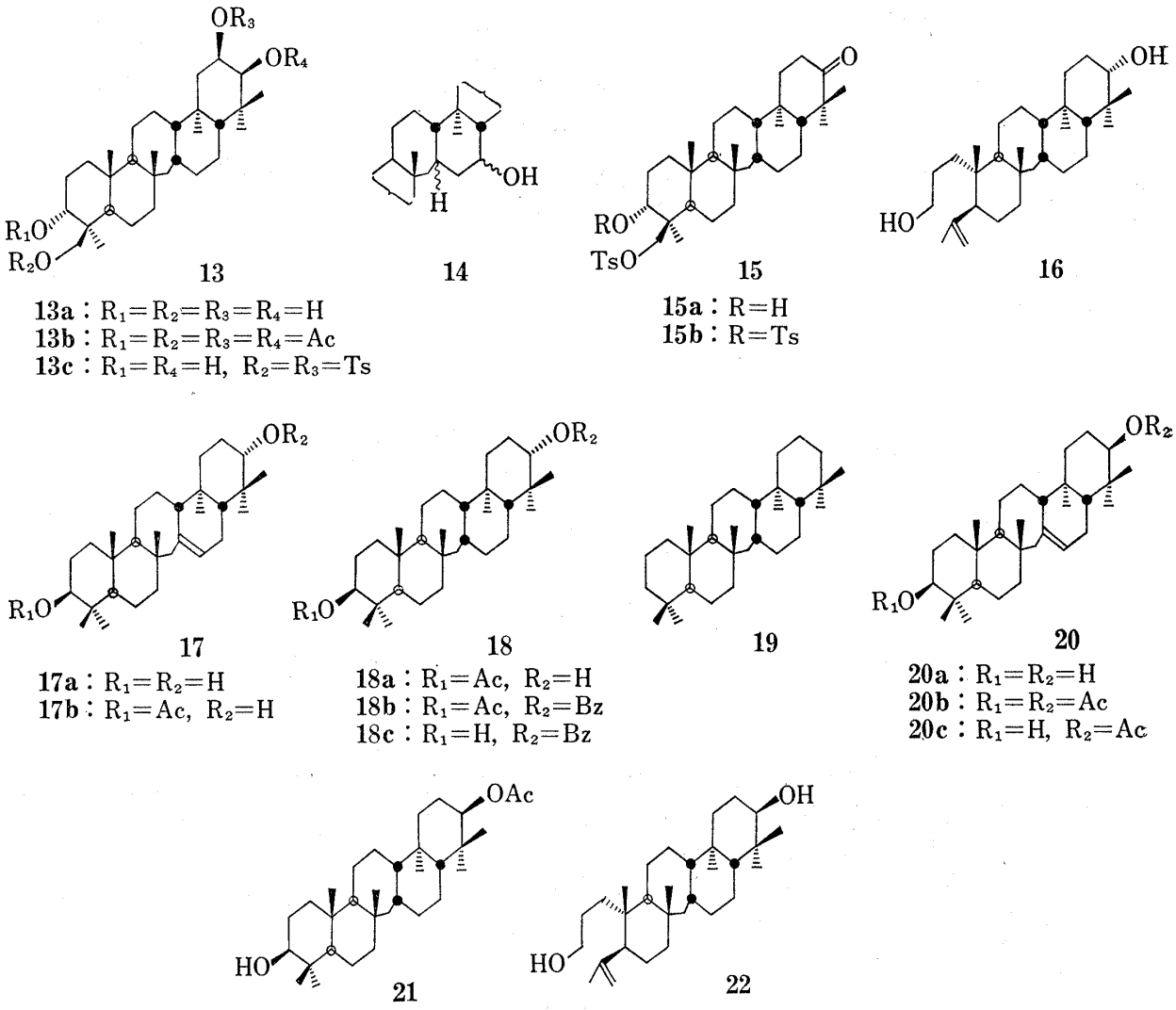


Chart 4

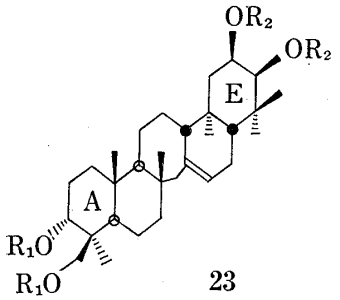
21-Episerratenediol (**20a**) gave likewise the epimeric seco-diol (**22**) which was apparently different from the above seco-diol (**16**) (for details of experiments-see Experimental).

Lyclaninol

During separation of triterpenoids from *L. clavatum* we have isolated a new tetraol, $C_{30}H_{50}O_4$, which was well characterized as a tetraacetate (**23b**), mp 223—226°, and the name lyclaninol was suggested.[2b,5]

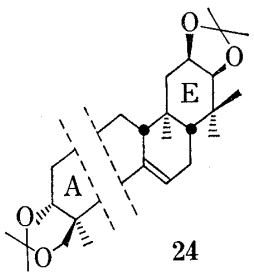
Lyclaninol formed the diacetonide (**24**) under a forced condition,[7] which was easily hydrolysed to the monoacetonide (**25a**) and then to the original tetraol, lyclaninol. These reactions are parallel to those of lycoclavanin. The type classification signals of the diacetonide (**24**) and the monoacetonide-diacetate (**25b**) (Table II) were also similar to those of the corresponding derivatives of lycoclavanin. These and other spectroscopic evidence suggested that lycoclavanin and lyclaninol differ only in the point that the former has a ketone while the latter has not the system, *i.e.*, lyclaninol is the deoxo derivative of lycoclavanin.

Confirming this, the monoacetonide-diacetate (**25b**) was converted to the keto-diacetate (**26**) by the sequence analogous as described in lycoclavanin, whereupon the product was identical with the keto-diacetate previously prepared from lycoclavanol[7] (**27**).

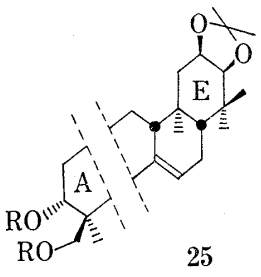


23

23a : $R_1=R_2=H$
23b : $R_1=R_2=Ac$
23c : $R_1=Ac$, $R_2=H$
23d : $R_1=Ac$, $R_2=Bz$

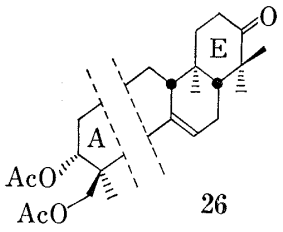


24

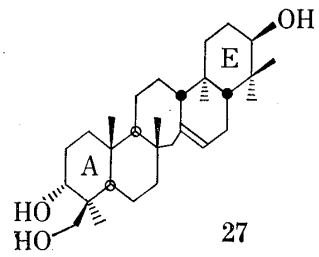


25

25a : R=H
25b : R=Ac



26



27

Bz=benzoyl

Chart 5

Application of the dibenzoate chirality rule to the diacetate-dibenzoate (**23d**) also confirmed the clockwise chirality of the 20,21-glycol system: the first Cotton effect at 238 nm was a positive and the second Cotton effect at 213 nm was a negative one. Hence lyclaninol is serrat-14-ene-3α,20β,21β,24-tetraol (**23a**). This conclusion was established by oxidation of lyclaninol to the corresponding 16-oxo-derivative, lycoclavanin, with the method described in the preceding paper.[1)]

Experimental

Unless otherwise stated, the infrared (IR) spectra were taken in a Nujol mull, and the nuclear magnetic resonance (NMR) spectra were measured in $CDCl_3$ solution by using a 60 MHz machine and the chemical shifts are given in δ ppm referred to the internal tetramethylsilane (TMS). For acetonides proton signals except those listed in Table II (type classification signals) were given in this section. Melting points below 300° were determined on Yanagimoto mp apparatus and those above 300° were taken by an open capillary using Ishii block-heater apparatus, and uncorrected. All organic extracts had been washed with water and dried over K_2CO_3 or Na_2SO_4 before evaporation. Acid-washed alumina was used for column chromatography, and for thin-layer chromatography (TLC) Silica gel G as an absorbent and $CHCl_3$–MeOH as a developing solvent. Acetylations were carried by heating the compound with excess acetic anhydride and pyridine for a few min. and keeping the mixture overnight at room temp, then worked up as usual. Identities were confirmed by IR and TLC comparisons, and by mixed fusion with the authentic specimens when the melting points were below 300°.

Lycoclavanin Tetraacetate (7b)——mp 238—240°, colorless needles from MeOH. ORD ($c=0.4\times10^{-3}$, dioxane) [ϕ] (nm): −1050° (373) (trough). CD ($c=0.4\times10^{-3}$, dioxane) [θ] (nm): +38000 (229) (positive maximum), −1660 (335) (negative maximum). On alkaline hydrolysis it gave **lycoclavanin (7a)**, mp 344—346°, colorless needles from dimethylformamide (DMF).

Lycoclavanin Monoacetonide (9a) and Diacetonide (8)——i) Lycoclavanin (100 mg) and anhy. $CuSO_4$ (1 g) in anhyd. acetone (300 ml) were heated under reflux for a week. An insoluble material was removed by filtration and the filtrate was evaporated. The residue was crystallized from CH_2Cl_2–MeOH to give the monoacetonide **9a** (10 mg), mp 306—309°. IR (KBr) cm^{-1}: 3450 (OH), 1668, 1628 (conj. CO). NMR ($CDCl_3$–pyridine): –$\overset{|}{\underset{|}{C}}$–$CH_3$ and O–CMe_2–O 0.78 (6H), 0.87 (3H), 1.28 (3H), 1.42 (3H), 1.49 (3H), 1.59 (3H), 1.64 (3H). *Anal.* Calcd. for $C_{33}H_{52}O_5$: C, 74.96; H, 9.91. Found: C, 74.78; H, 9.79.

On acetylation it gave the monoacetonide-diacetate **9b**, mp 307—310°, needles from CH_2Cl_2–MeOH.

IR (KBr) cm^{-1}: 1738, 1245 (OAc), 1663, 1628 (conj. CO). NMR: -Ç-CH_3 0.79 (3H), 0.87 (6H), 0.96 (3H), 1.16 (3H), 1.47 (3H); -Ç-CH_2-OAc 4.07 (2H, ABq., J=11, δ_{AB}=18 Hz); >CH-OAc 4.95 (1H, broad s.); >CH=CH- 5.77 (1H, broad s.). *Anal.* Calcd. for $C_{37}H_{56}O_7$: C, 72.51; H, 9.21. Found: C, 72.58; H, 8.93.

ii) Lycoclavanin (1.388 g) and *p*-TsOH (25 mg) in 2,2-dimethoxypropane (20 ml) and DMF (60 ml) were heated under gentle reflux for 2 hr. The mixture was concentrated to a half volume, poured into 5% K_2CO_3 (90 ml), and extracted with CH_2Cl_2. The residue obtained by evaporation of the solvent from the extract was chromatographed over Florisil and the column was eluted with benzene, then with CH_2Cl_2–MeOH, and the eluate was collected in 50 ml fractions. Fractions 1—6 made up the benzene eluate. Fractions 1—3 yielded the diacetonide **8** (0.6 g) which after crystallizations from CH_2Cl_2–MeOH was obtained as colorless prisms, mp 225—227°. IR cm^{-1}: 1668, 1630 (conj. CO). NMR: -Ç-CH_3 0.78 (3H), 0.87 (3H), 1.06 (3H), 1.17 (6H), 1.44 (3H); >C=CH- 5.76 (1H, broad s.). *Anal.* Calcd. for $C_{36}H_{56}O_5$: C, 76.01; H, 9.92. Found: C, 75.84; H, 9.77.

Fractions 8 and 9 yielded the monoacetonide **9a** (0.4 g), needles from CH_2Cl_2–MeOH, identical with the compound obtained above.

iii) Lycoclavanin (1 g) and *p*-TsOH (25 mg) were suspended in a mixture of 2,2-dimethoxypropane (2 ml), acetone (30 ml), and DMF (30 ml) and heated on a water bath for 30 min, whereupon the solution became clear. The mixture was poured into water and worked up as above to give **9a** (0.7 g) and a small **mo**unt of **8**.

iv) The monoacetonide (0.5 g) was allowed to the reaction as described in ii) and worked up. The diacetonide **8** (0.4 g) was isolated.

The compounds **9a** (7 mg) and **8** (10 mg) respectively regenerated, on heating with $CHCl_3$ (4 ml)–MeOH (4 ml)–AcOH (0.5 ml)–H_2O (0.1 ml) for 2 hr, lycoclavanin (**7a**) which was identified as its tetraacetate (**7b**).

The Diol-diacetate (7c)——The compound **9b** (100 mg) in $CHCl_3$ (5 ml)–AcOH (3 ml)–H_2O (5 drops) was heated under reflux for 3 hr. Evaporation of the solvent and crystallization of the residue from CH_2Cl_2-MeOH yielded **7c**, mp 307—310°. IR (KBr) cm^{-1}: 3460 (OH), 1745, 1245 (OAc), 1667, 1623 (conj. CO). NMR (pyridine-d_5): -Ç-CH_3 0.70 (3H), 0.77 (3H), 0.95 (3H), 1.08 (3H), 1.41 (6H); -OCOCH_3 2.01 (3H), 2.17 (3H). *Anal.* Calcd. for $C_{34}H_{52}O_7$: C, 71.29; H, 9.15. Found: C, 71.46; H, 8.91.

The Diacetate-monotosylate (7d)——The compound **7c** (100 mg) and *p*-TsCl (150 mg) in pyridine (4 ml) were heated on a water-bath for 3 hr. The mixture was poured into water and extracted with ether. The residue from the ether extract was dissolved in benzene, passed through a short column of alumina, and the column was eluted with CH_2Cl_2. Crystallizations of CH_2Cl_2 eluate from *n*-hexane–benzene afforded **7d**, mp 202—205°. IR (KBr) cm^{-1}: 3345 (OH), 1740, 1245 (OAc), 1670, 1620 (conj. CO), 1650, 1600, 1178 (OTs).

The Keto-diacetate (10b)——i) The monotosylate **7d** (50 mg) in pyridine (10 ml) was heated under reflux for 6 hr. The solvent was removed in vacuo and the residue was taken up in CH_2Cl_2. Evaporation of the solvent and crystallizations of the residue from CH_2Cl_2–MeOH furnished **10b** as prisms, mp 278—282°. IR (KBr) cm^{-1}: 1738, 1233 (OAc), 1715 (CO), 1665, 1627 (conj. CO). NMR: -Ç-CH_3 0.87 (6H), 0.97 (3H), 1.03 (3H), 1.35 (6H); -OCOCH_3 2.06 (3H), 2.09 (3H); 17-H 2.45 (1H, s.); -Ç-CH_2-OAc 4.08 (2H, ABq., δ_{AB}=18, J=12 Hz); >CH-OAc 4.96 (1H, broad s.), >C=CH- 5.83 (1H, broad s.). *Anal.* Calcd. for $C_{34}H_{50}O_6$: C, 73.61; H, 9.09. Found: C, 73.39; H, 8.87.

ii) The compound **7d** (20 mg) in benzene was chromatographed over alumina. The eluate showed two spots corresponding to **10b** and the starting material (**7d**).

The Keto-diacetate (10b) from O,O-Isopropylidene-16-oxolycoclavanol (11)——The acetonide **11** (45 mg) and CrO_3 (100 mg)–pyridine (1 ml) complex in pyridine (2 ml) were stirred and kept overnight at room temp. The mixture was poured into water, extracted with CH_2Cl_2, and the extract was washed with 5% K_2CO_3 and evaporated to dryness. The residue (**12**) was dissolved in $CHCl_3$ (3 ml)–AcOH (1 ml)–H_2O (5 drops) and heated on a water-bath for 2 hr. Evaporation of the solvent and acetylation of the residue gave a solid which on chromatography in benzene over alumina yielded the keto-diacetate **10b**, mp 278—282°, prisms from CH_2Cl_2–MeOH. This was identical with the keto-diacetate **10b** obtained from lycoclavanin.

The Diacetate-dibenzoate (7e)——The diol-diacetate **7c** (85 mg) and benzoyl chloride (0.25 ml) in pyridine (4 ml) were kept at room temp for 24 hr. The mixture was poured into water and extracted with ether. Chromatography of the extract in benzene over alumina yielded **7e**, mp 166—170°, needles from CH_2Cl_2–MeOH. IR (KBr) cm^{-1}: 1739, 1728 (OAc, OBz), 1675, 1623 (conj. CO). NMR: -Ç-CH_3 0.89 (3H), 0.96 (6H), 1.05 (3H), 1.31 (3H), 1.44 (3H); -OCOCH_3 2.06 (3H), 2.09 (3H); -Ç-CH_2-OAc 4.09 (2H, ABq., δ_{AB}=18, J=11 Hz); >CH-OAc 4.95 (1H, broad s.); >CH-OBz 5.34 (1H, d., J=2 Hz), 5.62 (1H, m.); >C=CH- 5.82 (1H, broad s.); aromatic-H 7.3—8.2 (10H). CD (c=0.4×10^{-3}, dioxane) [θ] (nm): +125900 (234) (positive maximum), −1700 (335) (negative maximum). *Anal.* Calcd. for $C_{48}H_{60}O_9$: C, 73.82; H, 7.74. Found: C, 73.66; H, 7.53.

Catalytic Hydrogenation of Lycoclavanin Tetraacetate (7b)——Lycoclavanin tetraacetate (**7b**; 1 g) in AcOH (50 ml) containing 70% $HClO_4$ (0.5 ml) was hydrogenated over PtO_2 (500 mg) for 7 hr at room temp. The catalyst was removed by filtration and the solvent was condensed to about one-third of the original

volume under reduced pressure. After dilution with water, the mixture was extracted with CH_2Cl_2. Evaporation of the solvent left a gum which was chromatographed in benzene over alumina to give the following fractions: fr. 1 (19 mg), fr. 2 (712 mg), fr. 3 (85 mg), and fr. 4 (186 mg). Crystallizations of fr. 2 from *n*-hexane–ether gave deoxo-14β-dihydrolycoclavanin tetraacetate (**13b**), mp 220—224°, as needles (600 mg). IR (KBr) cm^{-1}: 1740, 1250 (OAc). NMR: –Ç–CH_3 0.80 (3H), 0.86 (3H), 0.91 (3H), 0.93 (3H), 1.03 (6H); –OCOCH_3 1.97 (3H), 2.06 (3H), 2.10 (3H), 2.11 (3H); –Ç–CH_2–OAc 4.08 (2H, ABq., $\delta_{AB}=16$, $J=12$ Hz); >CH–OAc 4.95 (1H) and 5.00 (1H) (overlapped two peaks), 5.30 (1H, m.). *Anal.* Calcd. for $C_{38}H_{60}O_8$: C, 70.77; H, 9.38. Found: C, 70.58; H, 9.15.

Fraction 4 yielded a compound of mp 143—146°, needles from *n*-hexane–acetone, which probably is a 16-alcohol (**14**). IR (KBr) cm^{-1}: 1745, 1250 (OAc). NMR: –Ç–CH_3 0.81 (3H), 0.92 (3H), 0.94 (3H), 1.00 (3H), 1.36 (3H), 1.42 (3H), –OCOCH_3 1.96 (3H), 2.06 (3H), 2.11 (6H), –Ç–CH_2–OAc 4.09 (2H, ABq., $\delta_{AB}=17$, $J=11$ Hz), >CH–OAc 4.33 (1H, m.), 4.97 (2H).

The Keto-tosylate (15a) from Deoxo-14β-dihydro Compound (13)——i) The Tetra-ol (**13a**): The tetra-acetate **13b** (460 mg) was hydrolysed with 7% KOH–MeOH on heating under reflux for 1.5 hr to give **13a** (310 mg), mp>300°.

ii) Tosylation of **13a**: The tetra-ol **13a** (140 mg) and *p*-TsCl (500 mg) in pyridine (12 ml) were kept overnight at room temp and heated on a water-bath for 4 hr. After cooling, the mixture was poured into 5% K_2CO_3, extracted with CH_2Cl_2, and the extract was washed with 3% HCl, water, and evaporated. The residue was chromatographed over alumina and the column was eluted with *n*-hexane–benzene (1: 1), with benzene, with benzene–CH_2Cl_2 (1: 1), with CH_2Cl_2, then with CH_2Cl_2–MeOH. Crystallization of benzene–CH_2Cl_2 eluate from *n*-hexane–ether yielded the keto-tosylate **15a** (71 mg) as needles, mp 148—149°. IR (KBr) cm^{-1}: 1700 (CO), 1600, 1177, 947 (OTs). NMR: –Ç–CH_3 0.63 (3H), 0.85 (3H), 1.01 (3H), 1.05 (9H); Ar–CH_3 2.46 (3H); –Ç–CH_2–OTs 4.00 (2H, ABq., $\delta_{AB}=19$, $J=10$ Hz); >CH–OH 3.69 (1H, broad s.); Ar–H 7.56 (4H, ABq., $\delta_{AB}=27$, $J=9$ Hz); –OH 1.54 (1H, s.).

The *n*-hexane–benzene and benzene eluates were combined and rechromatographed to afford the keto-ditosylate **15b** as an oil, which was not further purified. NMR: –Ç–CH_3 0.61 (3H), 0.73 (3H), 0.83 (3H), 1.06 (9H); Ar–CH_3 2.47 (6H); –Ç–CH_2–OTs 3.93 (2H, ABq., $\delta_{AB}=23$, $J=10$ Hz); >CH–OTs 4.50 (1H, m.); Ar–H 7.56 (8H, ABq., $\delta_{AB}=27$, $J=8$ Hz).

The Seco-diol (16)——The keto-tosylate **15a** (28 mg) and $NaBH_4$ (100 mg) were heated with *t*-BuOK in *t*-BuOH (200 mg of K per 1 ml of the solution) (10 ml) on a water-bath for 1 hr. After cooling excess of $NaBH_4$ and *t*-BuOK was decomposed by AcOH and the mixture extracted with CH_2Cl_2. The residue (22 mg) obtained from the extract was purified by preparative-TLC to give **16**. It formed needles from *n*-hexane–MeOH, and had double mp 188° and 222—224°. IR (KBr) cm^{-1}: 3070 (OH), 1640, 890 (>C=CH_2). NMR; –Ç–CH_3 0.78 (3H), 0.85 (3H), 0.91 (3H), 0.96 (3H), 0.99 (3H); =C–CH_3 1.75 (3H, s.); >CH–OH 3.30 (1H, m.); –CH_2–CH_2–OH 3.61 (2H, t., $J=7$ Hz); >C=CH_2 4.77 (2H, d., $J=9$ Hz). *Anal.* Calcd. for $C_{30}H_{52}O_2$: C, 81.02; H, 11.79. Found: C, 80.77; H, 11.58.

Hydrogenation of Serratenediol 3-Acetate (17b)——Serratenediol 3-acetate (**17b**; 934 mg) in AcOH–cyclohexane (1: 1) (200 ml) was hydrogenated over PtO_2 (502 mg) for 10 hr. Removal of the catalyst and the solvent left a solid which was chromatographed in benzene over alumina. Several crystallizations of the eluate from $CHCl_3$–MeOH afforded 14β-dihydro-compound (**18a**) (310 mg) as needles, mp 277—280°. IR cm^{-1}: 3500 (OH), 1725, 1234 (OAc). NMR: –Ç–CH_3 0.81 (3H), 0.83 (6H), 0.88 (6H), 0.92 (3H), 1.00 (3H); –OCOCH_3 2.07 (3H); >CH–OH 3.24 (1H, m.); >CH–OAc 4.48 (1H, m.). *Anal.* Calcd. for $C_{32}H_{54}O_3$: C, 78.96; H, 11.18. Found: C, 78.92; H, 11.19.

The 3-Acetate-21-benzoate (18b)——The compound **18a** (280 mg) and benzoyl chloride (1 ml) in pyridine (8 ml) were kept overnight at room temp. The mixture was poured into 5% $NaHCO_3$, stirred for 1 hr, and extracted with $CHCl_3$. Evaporation of the solvent left **18b** needles from $CHCl_3$–MeOH, mp 275—277°. IR cm^{-1}: 1700 (OAc, OBz).

The Monobenzoate (18c)——A solution of the 3-acetate-21-benzoate **18b** (270 mg) in $CHCl_3$ (10 ml)–EtOH (8 ml) and c.HCl (5 ml)–$CHCl_3$ (6 ml)–EtOH (6 ml) were combined, and the mixture was heated under reflux for 3 hr. Water was added to the cooled mixture and extracted with $CHCl_3$. Evaporation of the solvent left a solid which was separated by preparative-TLC to the starting material **18b** (114 mg) and the 21-benzoate **18c** (150 mg), mp 243—246°. IR cm^{-1}: 3300 (OH), 1700, 1265 (OBz). NMR: –Ç–CH_3 0.81 (6H), 0.97 (6H), 1.01 (6H), 1.09 (3H); >CH–OH 3.23 (1H, m.); >CH–OBz 4.77 (1H, m.); Ar–H 7.5—8.2 (5H). *Anal.* Calcd. for $C_{37}H_{56}O_3 \cdot 1/2H_2O$: C, 79.66; H, 10.30. Found: C, 79.75; H, 10.36.

The Seco-diol (16)——The compound **18c** (160 mg), $Pb(OAc)_4$ (600 mg), and $CaCO_3$ (5 mg) in abs. benzene (20 ml) were heated under reflux for 15 hr. The mixture was filtered and the filtrate washed with 5% KI, 10% $Na_2S_2O_3$, and water, and evaporated to dryness. The only residue (180 mg) in tetrahydrofuran (THF) (10 ml) was reduced with LAH (180 mg) on heating under reflux for 4 hr. After decomposition of excess hydride by addition of a few drops of water, the mixture was filtered and the filtrate was evaporated

to dryness to give a gum which showed three spots on TLC. Separation of this by preparative-TLC gave the seco-diol **16** (16 mg). It formed needles from cyclohexane–ether, mp 210—220°, which contained cyclohexane as a crystallizing solvent as shown by NMR spectrum. Drying this sample in vacuo overnight gave needles, mp 218—222°, which was identical with the compound obtained from lycoclavanin.

14β-Serratane (19) from the Monobenzoate (18c)——The benzoate **18c** (9 mg) and LAH (20 mg) in THF (5 ml) were heated under reflux for 2 hr, and worked up as usual. The resulting diol was dissolved in pyridine (1 ml) and oxidized with CrO_3 (20 mg)–pyridine (0.2 ml) complex on keeping overnight at room temp. The diketone obtained on working up was heated with anhyd. hydrazine (7 drops) and KOH (400 mg) in triethyeneglycol (4 ml) at 160° for 2 hr. The excess hydrazine was evaporated and the heating at 200—210° was continued for further 3 hr. The mixture was poured into water, extracted with *n*-hexane which was evaporated to dryness. Chromatography of the residue in *n*-hexane over basic alumina gave white needles, mp 165—173°, (lit[11], 184—187°) which showed only one peak identical with 14β-serratane in GC, the peak corresponding to 14α-serratane being negligible.

21-Episerratenediol 21-Monoacetate (20c)——To a warm solution of 21-episerratenediol diacetate (**20b**; 3.30 g) in $CHCl_3$ (10 ml)–EtOH (90 ml) was added c.HCl (14 ml)–EtOH (36 ml) and the mixture was heated under reflux for 30 hr. The mixture was poured into ice-water, neutrallized with $NaHCO_3$, and extracted with $CHCl_3$. Evaporation of the solvent left a gummy residue which was chromatographed in benzene over alumina. The first benzene eluate gave the starting material (2.2 g) and the following eluates gave the 21-acetate **20c** (660 mg) needles from $CHCl_3$–MeOH, mp 240—244°, which was different from 21-episerratenediol 3-monoacetate (mp 315—317°).[12] IR cm^{-1}: 3600 (OH), 1716, 1248 (OAc). NMR: –Ç–CH_3 0.73 (3H), 0.80 (3H), 0.86 (6H), 0.90 (3H), 0.97 (3H), 1.00 (3H); $-OCOCH_3$ 2.11 (3H); >CH–OH 3.19 (1H, m.); >CH–OAc 4.68 (1H, broad s.); >C=CH– 5.35 (1H, m.). *Anal.* Calcd. for $C_{32}H_{52}O_3$: C, 79.28; H, 10.81. Found: C, 79.31; H, 11.00.

Hydrogenation of the 21-Monoacetate (20c)——The monoacetate **20c** (1.1 g) in AcOH–cyclohexane (1 : 1) (100 ml) was hydrogenated over PtO_2 (500 mg) for 15 hr. Removal of the catalyst and the solvent left a solid which was chromatographed in benzene over alumina. Several crystallizations of the eluate from *n*-hexane–benzene yielded the dihydro-compound **21**, mp 272—274°, as needles (270 mg). The compound was negative to tetranitromethane. IR cm^{-1}: 3600 (OH), 1718, 1242 (OAc). NMR: –Ç–CH_3 0.81 (6H), 0.86 (6H), 0.94 (6H), 1.01 (3H); $-OCOCH_3$ 2.10 (3H); >CH–OH 3.25 (1H, m.); >CH–OAc 4.64 (1H, broad s.). *Anal.* Calcd. for $C_{32}H_{54}O_3$: C, 78.96; H, 11.18. Found: C, 78.76; H, 11.13.

The Seco-diol (22)——The compound **20c** (170 mg), $Pb(OAc)_4$ (550 mg), and $CaCO_3$ (5 mg) in abs. benzene (20 ml) were heated under reflux for 14 hr. The mixture was worked up exactly the same as described above. The oily product thus obtained gave three spots on TLC, which was separated by preparative-TLC to yield the epimeric seco-diol (**22**) as an oil (17 mg). IR ($CHCl_3$) cm^{-1}: 3790, 3500 (OH), 892 (>C=CH_2). NMR: –Ç–CH_3 0.80 (3H), 0.88 (6H), 0.96 (6H); =C–CH_3 1.77 (3H), >CH–OH 3.43 (1H, broad s.); $-CH_2OH$ 3.61 (2H, t., J=6 Hz); >C=CH_2 4.77 (2H, d., J=7 Hz).

Lyclaninol Tetraacetate (23b)——Needles from *n*-hexane, mp 223—226°. It was hydrolysed by 5% KOH–MeOH to **lyclaninol** (**23a**), mp 361—363°, needles from $CHCl_3$–MeOH.

Lyclaninol Diacetonide (24)——Lyclaninol (**23a**; 150 mg), *p*-TsOH (5 mg), and 2,2-dimethoxypropane (5 ml) in DMF (20 ml) were heated under gentle reflux for 3 hr. On working up as described in preparation of **8**, the product in benzene was passed through the column of Florisil. The eluate was purified by preparative-TLC to yield the diacetonide **24**, mp 204—206°, needles from MeOH. NMR: –Ç–CH_3 0.62 (3H), 0.81 (3H), 0.93 (3H), 1.02 (6H), 1.12 (3H); >C=CH– 5.38 (1H, m.).

The Monoacetonide (25a)——i) The diacetonide **24** was kept in $CDCl_3$ for 2 hr after measurement of NMR spectrum. The compound recovered from the solution had been hydrolysed. Crystallization of this from MeOH gave the monoacetonide (**25a**) as needles, mp 291—293°.

Acetylation of this yielded the diacetate **25b**, mp 245—247°, needles from MeOH–CH_2Cl_2. IR cm^{-1}: 1740 (OAc). NMR: –Ç–CH_3 0.65 (3H), 0.84 (6H), 0.94 (6H), 1.04 (3H); $-OCOCH_3$ 2.05 (3H), 2.07 (3H): –Ç–CH_2–OAc 4.09 (2H, ABq., δ_{AB}=18, J=12 Hz); >CH–OAc 4.96 (1H, broad s.); >C=CH– 5.40 (1H, m.).

The acetonide-diacetate **25b** (15 mg) and LAH (20 mg) in THF (5 ml) were stirred overnight at room temp. The product obtained on working up was identical with the monoacetonide (**25a**), mp 291—293°.

The Diol-diacetate (23c)——The monoacetonide-diacetate **25b** (102 mg) in $CHCl_3$ (10 ml)–MeOH (12 ml)–H_2O (3 ml)–AcOH (3 ml) was heated under reflux for 3 hr. Evaporation of solvent gave a crystalline residue which in CH_2Cl_2 was passed through a short column of alumina. Crystallization of the eluate from *n*-hexane–acetone gave **23c** as needles, mp 263—266.5°. IR cm^{-1}: 3500, 1740.

Acetylation of this gave lyclaninol tetraacetate (**23b**).

The Keto-diacetate (26)——The diacetate **23c** (30 mg) and *p*-TsCl (45 mg) in pyridine (1 ml) were heated on a water-bath for 7.5 hr. The mixture was poured into water, extracted with $CHCl_3$ and the extract

12) Y. Inubushi, Y. Tsuda, T. Sano, and R. Nakagawa, *Chem. Pharm. Bull.* (Tokyo), **13**, 104 (1965).

was washed with 5% HCl, water, then evaporated. The crystalline residue was chromatographed in benzene over alumina to give the keto-diacetate **26**. It formed prisms from *n*-hexane, mp 222—227°, and formed needles from MeOH, mp 195—197°. The latter was completely identical with the keto-diacetate (**26**)[7] prepared from lycoclavanol.

The Diacetate-dibenzoate (23d)——The diol-diacetate **23c** (30 mg) and benzoyl chloride (0.1 g) in pyridine (1 ml) were kept overnight at room temp. The mixture was poured into 5% Na_2CO_3 solution, stirred for 1 hr, extracted with CH_2Cl_2, and the extract was washed with 5% Na_2CO_3 and evaporated. Chromatography of the residue in benzene over alumina gave **23d** as a gum. CD ($c=0.1\times10^{-2}$, dioxane) [θ] (nm): −13000 (213) (negative maximum), +17300 (238) (positive maximum).

Oxidation of Lyclaninol Tetraacetate (23b) with (Pyridine)$_2$-Chromium Trioxide Complex——The reagent $(C_6H_5N)_2\cdot CrO_3$ was prepared from 1 g of CrO_3 and 10 ml of pyridine according to the method by Dauben, *et al.*[13] washed well with *n*-hexane, and used immediately.

Lyclaninol tetraacetate (**23b**; 190 mg) and the reagent in CH_2Cl_2 (10 ml) were stirred at room temp for 48 hr. The mixture was filtered and the residue washed with CH_2Cl_2. The combined filtrate was chromatographed in benzene over alumina. Elution with benzene gave a gum which was rechromatographed in *n*-hexane–benzene (2: 1) over alumina. First eluate with *n*-hexane–benzene (2: 1) showed several spots on TLC and was discarded. Following elution with benzene gave a solid which on crystallization from *n*-hexane–ether yielded needles (10 ml), mp 234—237°, which was identical with lycoclavanin tetraacetate (**7b**).

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13) W.G. Dauben, M. Lorber, and D.S. Fullerton, *J. Org. Chem.*, **34**, 3587 (1969).