[Chem. Pharm. Bull. 23(8)1784—1788(1975)]

UDC 547.597.02 : 581.192

Triterpenoid Chemistry. XIII.[1] Lycopodium Triterpenoid. (9).[2] The Structure of Lyclavatol, a Novel Triterpenoid of Bisnoronocerane Type[3]

Takehiro Sano, Takunori Fujimoto, and Yoshisuke Tsuda

Showa College of Pharmaceutical Sciences[4]

(Received December 17, 1974)

Lyclavatol, a constituent of *L. clavatum*, was elucidated as a novel type of bisnor-triterpenoid, 3α,8β,14α,21β-tetrahydroxy-26,27-bisnoronocerane (**1a**).

α-Onocerin (**6a**), a probable biogenetic precursor of triterpenoids of serratane group, is widely distributed in *Lycopodium* plants, and in some particular plants such as *L. clavatum*, *L. sitchense*, *L. obscurum*, *L. casuarinoides*, and *L. inundatum* it occurs as a major triterpenoidal constituent.[5] The fact lead us to the speculation that the plants *Lycopodium* might contain not only the pentacyclic triterpenoids of serratane group but also the tetracyclic derivative related to α-onocerin; this was proved to be the case. During separation of triterpenoids from *L. clavatum* we have isolated a new triterpenoid-alcohol and determined its structure as 3α,8β,14α,21β-tetrahydroxy-26,27-bisnoronocerane (**1a**).[3] In the preliminary communication (and also in ref. 5) this compound was described with a trivial name clavatol. However, since the name clavatol was already used for a metabolite (2,4-dihydroxy-3,5-dimethylaceto-phenone) of *Aspergillus clavatus*,[6] we wish to change it and use hereafter a new name "lyclavatol" for this triterpenoid.

This paper presents details of experiments on the structural elucidation of lyclavatol.

Lyclavatol, mp 277—279°, showed a strong hydroxyl absorption at 3320 cm^{-1} in the infrared (IR) spectrum. In agreement with this it easily formed an acetate, mp 259—260°. Neither lyclavatol nor its acetate gave constant elementary analyses to deduce their molecular formulae probably due to the variable solvations. High resolution mass spectrum of the lyclavatol acetate exhibited a highest mass peak at *m/e* 558.393 corresponding to $C_{34}H_{54}O_6$ (calc. 558.392) together with fragment ions due to successive losses of AcOH at *m/e* 498.373 (498.371 calc. for $C_{32}H_{50}O_4$), at *m/e* 438.353 (438.350 calc. for $C_{30}H_{46}O_2$), and at *m/e* 378.328 (378.329 calc. for $C_{28}H_{42}$). This indicated that lyclavatol has C_{28}-carbon skeleton and at least three hydroxy-groups.

In the nuclear magnetic resonance (NMR) spectrum of the acetate, there appeared three C-Me peaks at δ 0.88, 0.95, and 0.98 ppm, and acetyl-methyls at δ 2.07 ppm as a single peak. The protons geminal to acetoxy-group were observed at δ 4.64 and at δ 5.04 ppm with equal intensity. The intensity ratio of acetyl-methyl and C-Me (tertiary) was 2: 3. These evidence suggested that the number of acetoxy-group should be even. Hence the highest mass peak in the mass spectrum must be attributable to a fragment ion (M^+-AcOH) due to loss of acetic acid, thus suggesting the parent ion $(C_{36}H_{58}O_8)^+$ which was not observed would contain four

1) Part XII: Y. Tsuda, K. Isobe, T. Tanno, and A. Ukai, *Chem. Pharm. Bull.* (Tokyo), **23**, 1775 (1975).
2) Lycopodium Triterpenoid (8): Y. Tsuda, T. Fujimoto, A. Morimoto, and T. Sano, *Chem. Pharm. Bull.* (Tokyo), **23**, 1336 (1975).
3) Preliminary communication: T. Sano, T. Fujimoto, and Y. Tsuda, *Chem. Commun.*, **1970**, 1274.
4) Location: *Tsurumaki 5-1-8, Setagaya-ku, Tokyo, 154, Japan.*
5) Y. Tsuda, T. Fujimoto, K. Isobe, T. Sano, and M. Kobayashi, *Yakugaku Zasshi*, **94**, 990 (1974).
6) H. Hassal and A.R. Todd, *J. Chem. Soc.*, **1947**, 611. We thank to Dr. J.F. Grove, the Chemical Laboratory of the University of Sussex, for this information.

acetoxy-groups. Since lyclavatol does not contain double bond (no olefinic proton signal and negative tetranitromethane test), it was concluded to be a tetracyclic bisnortriterpenoid of a symmetrical structure having four hydroxy and six tertiary C-methyl groups with the formula $C_{28}H_{46}(OH)_4$. The symmetrical charactor of lyclavatol was also supported by the NMR spectra of other derivatives except **2** and **3** shown in Table I.

Attempt to obtain unsymmetrical derivative was accomplished by partial acetylation of lyclavatol, which yielded a triacetate (**2**). Oxidation of this with Jones' reagent gave a keto-triacetate (**3**). Introduction of the third acetyl group would have produced the steric hindrance on the fourth hydroxy-group. The NMR spectra of **2** clearly indicated the presence of six C-Me, three acetyl groups, and one hydroxy group, since the intensity ratio of OAc *vs.* C-Me was 1: 2 and that of >CH–OAc *vs.* >CH–OH was 3: 1, thus confirming the above conclusion on the functionality of lyclavatol.

Combining these evidence with biogenetic consideration which was suggested at the top of this text lyclavatol was assumed to be a derivative of 26,27-bisnoronocerane. This assumption was proved by following correlation of lyclavatol with α-onocerin (**6a**). Oxidation of lyclavatol with Jones' reagent gave a tetra-ketone, $C_{28}H_{42}O_4$, mp 206—208°, which was completely identical with the tetraketone (**4**), prepared from α-onocerin by Barton and Overton.[7] Hence lyclavatol in 3,8,14,21-tetrahydroxy-26,27-bisnoronocerane.

Table I. NMR Spectra of Bisnoronocerane Derivatives
(in $CDCl_3$, δ ppm, 60 MHz)

	C–Me[a]	–OCOMe[a]	>CH–OH[b]	>CH–OAc[b]
Tetraacetate (**1b**)	0.88(2)	2.07(4)		4.65(2H)
	0.95(2)			5.04(2H)
	0.98(2)			
Diacetate (**1c**)	0.88(2)	2.04(2)	3.45(2H)	5.05(2H)
	0.97(4)			
Diketo-diacetate (**5**)	1.12(6)	2.07(2)		5.10(2H)
Triacetate (**2**)	0.88(2)	2.07(3)	3.78(1H)	4.64(2H)
	0.95(3)			5.33(1H)
	1.03(1)			
Keto-triacetate (**3**)	0.73(1)	2.04(1)		4.67(2H)
	0.87(1)	2.09(1)		5.11(1H)
	0.95(4)	2.14(1)		
Tetraketone (**4**)	0.90(2)			
	1.07(2)			
	1.20(2)			
Bisnoronocerandione diacetate (**7**)	0.70(2)	2.08(2)		4.60(2H)[c]
	0.92(2)			
	0.98(2)			
Tetraacetate (**8b**)	0.87(4)	2.02(4)		4.40(2H)[c]
	0.97(2)			5.05(2H)

a) numbers in parentheses denote number of methyl groups
b) signal appears as a broad singlet
c) signal appears as a multiplet

Stereochemistry of all hydroxy-groups in lyclavatol are suggested to be axial from the NMR spectrum of its acetate, which exhibited all protons geminal to acetoxy group as broad singlets. This was proved as follows. Sodium borohydride reduction followed by acetylation of the known 3β,21α-acetoxy-26,27-bisnoronoceran-8,14-dione (**7**), an ozonization product of α-onocerin diacetate (**6b**),[7] yielded the 3β,8β,14α,21α-tetraacetoxy derivative (**8b**), mp 202—204°. Since the hydride reduction at C_8 and C_{14} would proceed from the less hindered face

7) D.H.R. Barton and K.H. Overton, *J. Chem. Soc.*, **1955**, 2639.

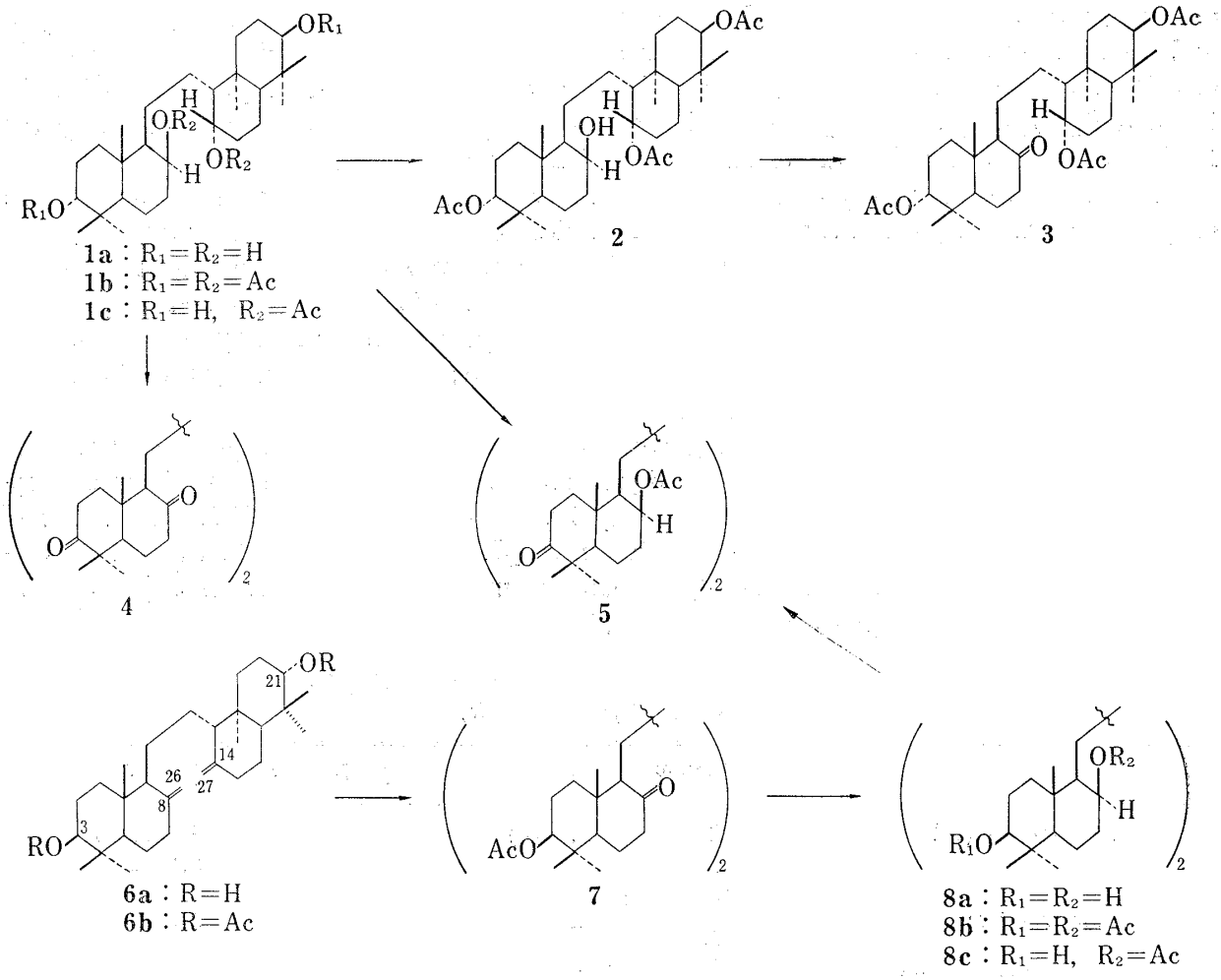

Chart 1

of the molecule, the configurations of newly formed acetoxy-group must be axial as being shown by new appearance of a broad singlet at δ 5.05 ppm in the NMR spectrum of the product. This tetraacetate (**8b**) was different from lyclavatol tetraacetate (**1b**). Comparing the NMR spectra of the both tetraacetate it was revealed that the protons at C_8 (and C_{14}) are almost equal and the others at C_3 (and C_{21}) are different in shapes and in chemical shifts (see Table I); the evidence indicates the both compounds are the same in configurations at C_8 and C_{14} and different in those at C_3 and C_{21}.

Partial hydrolysis of this tetraacetate (**8b**) with 5% methanolic potassium hydroxide effected saponification of less hindered (equatorial) acetoxy-groups thus yielding 3β,21α-diol-8β,14α-diacetate (**8c**). Jones' oxidation of this gave the 3,21-diketone (**5**) which showed a positive Cotton effect ([θ]=+3050) in circular dichroism (CD) at 280 nm as expected. On the contrary, the 8,14-diketone (**7**) exhibited a strong negative Cotton effect ([θ]=−20600) at 293 nm.[8]

Partial hydrolysis of lyclavatol tetraacetate similarly gave a diol-diacetate (**1c**), whose NMR peak at δ 5.05 ppm (broad singlet) indicated that acetoxy-groups at C_3 and C_{21} had been hydrolysed and those at C_8 and C_{14} remained unaffected. Jones' oxidation of this gave the diketone (**5**) which was completely identical with the 3,21-diketone obtained above.

Hence lyclavatol is 3α,8β,14α,21β-tetrahydroxy-26,27-bisnoronocerane (**1a**). This is the first example of the bisnoronocerane derivative occurring in nature. Biogenetically it

8) C. Djerassi and D. Marshall, *Tetrahedron*, **1**, 238 (1957).

would be produced from α-onocerin by oxidative losses of exomethylene groups and by oxidation-reduction at C_3–C_{21} and C_8–C_{14}.

Experimental

Unless otherweise stated, the IR spectra were taken in a Nujol mull and the CD spectra are for methanol solutions. The 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, which are listed in Table I. Melting points were determined on Yanagimoto mp apparatus, and uncorrected. Acid-washed alumina was used for column chromatography, and for thin-layer chromatography (TLC) silica gel G as an adsorbent and $CHCl_3$–MeOH as a developing solvent. Identities were confirmed by IR, NMR, and TLC comparisons and by mixed fusion with the authentic specimens.

Lyclavatol Tetraacetate (1b)——This formed needles from CH_2Cl_2–MeOH, mp 259—260°. IR (KBr) cm^{-1}: 1743, 1245 (OAc). Mass Spectrum (MS) (relative intensity): 558 (4), 498 (55), 456 (15), 438 (65), 423 (10), 396 (7), 378 (85), 363 (50), 335 (8), 296 (11), 276 (12), 262 (19), 248 (59), 235 (29), 202 (65), 188 (74), 176 (74), 175 (72), 173 (82), 119 (100). On saponification it gave lyclavatol (**1a**), mp 277—279°, fine needles from $CHCl_3$–MeOH. IR (KBr) cm^{-1}: 3320 (OH).

Partial Acetylation of Lyclavatol (1a)——Lyclavatol (**1a**; 204 mg) in pyridine (6 ml) and Ac_2O (3 ml) was heated on a water bath for 30 min. The mixture was poured into ice-water, extracted with CH_2Cl_2, and the extract was washed with water, dried over K_2CO_3, and evaporated. The oily residue in benzene was chromatographed over alumina (0.8×10 cm). Elution with benzene gave a mixture (150 mg) of lyclavatol tetraacetate (**1b**) and a partially acetylated product. Repeated crystalliziatons of the latter from acetone–*n*-hexane afforded the triacetate (**2**) as prisms, mp 210—213°. IR cm^{-1}: 3550 (OH), 1730, 1720 (OAc). MS: highest peak (relative intensity); 456.3634 (20). Calcd. for $C_{34}H_{56}O_7$ $-2\times CH_3COOH$; 456.3603.

Jones' Oxidation of the Triacetate (2)——The triacetate **2** (25 mg) in acetone (5 ml) was oxidized with Jones' reagent at 0° for 30 min. The product was crystallized from *n*-hexane to give the monoketone (**3**) as needles (17 mg), mp 165—169°. IR cm^{-1}: 1740, 1720 (OAc, CO). CD ($c=0.7\times10^{-3}$) [θ] (nm): −12600 (293). MS: highest peak (intensity); 574.3997 (6). Calcd. for $C_{34}H_{54}O_7$; 574.3870.

Jones' Oxidation of Lyclavatol (1a)——Lyclavatol (**1a**; 80 mg) in acetone (20 ml) was oxidized with Jones' reagent at 0°. The mixture was diluted with H_2O, extracted with ether, and the extract was washed with water, dried over Na_2SO_4, and evaporated. The crystalline residue in benzene was passed through a column of alumina to give the tetraketone (**4**) (30 mg) (prisms from MeOH), mp 206—208°. This was identical with 26,27-bisnoronoceran-3,8,14,21-tetraone (**4**) described below. CD ($c=0.2\times10^{-3}$) [θ] (nm): −24100 (295).

Partial Hydrolysis of Lyclavatol Tetraacetate (1b)——The acetate **1b** (175 mg) in 5% methanolic KOH (10 ml) was heated under reflux for 3 hr. The mixture was diluted with water, extracted with CH_2Cl_2, and the extract was washed with water, dried over $MgSO_4$, and evaporated. The crystalline residue in benzene was passed through a short column of alumina. Crystallization of the eluate from acetone–*n*-hexane gave lyclavatol diacetate (**1c**) as needles (140 mg), mp 256—259°. IR cm^{-1}: 3610, 3480 (OH), 1735 (OAc). MS: highest peak (relative intensity); 456.3638 (7). Calcd. for $C_{32}H_{54}O_6-(CH_3COOH+H_2O)$; 456.3604.

Jones' Oxidation of Lyclavatol Diacetate (1c)——The diacetate **1c** (80 mg) in acetone (10 ml) was oxidized with Jones' reagent under stirring at 10°. After addition of the slight excess reagent the mixture was stirred for further 10 min, diluted with water, and extracted with ether. The extract was washed with water, dried over Na_2SO_4, and evaporated to give a residue which was passed in benzene through a short column of alumina. Crystallization of eluate from *n*-hexane gave a diketo-diacetate (**5**) as prisms, mp 203—206°, which was identical with 8β,14α-diacetoxy-26,27-bisnoronoceran-3,21-dione described below. IR cm^{-1}: 1730 (OAc), 1702 (CO). CD ($c=0.2\times10^{-3}$) [θ] (nm): +3050 (280). MS: highest peak (relative intensity); 470.3370 (4). Calcd. for $C_{32}H_{50}O_6-CH_3COOH$; 470.3394.

3β,21α-Diacetoxy-26,27-bisnoronoceran-8,14-dione (7)——This was prepared by ozonolysis of α-onocerin diacetate (**6a**) according to Barton and Overton and had mp 118—120° (lit[7] mp 120—125°). IR cm^{-1}: 3540 (H_2O), 1730 (OAc), 1705 (CO). CD ($c=0.14\times10^{-3}$) [θ] (nm): −20600 (293).

26,27-Bisnoronoceran-3,8,14,21-tetraone (4)——This was prepared from **7** by hydrolysis followed by Jones' oxidation and had mp 204—206° (lit[7] mp 196—198°). IR cm^{-1}: 1700 (CO).

3β,8β,14α,21α-Tetrahydroxy-26,27-bisnoronocerane (8a) and Its Tetraacetate (8b)——The diacetoxy-dione **7** (300 mg) in MeOH(15 ml)was treated with $NaBH_4$ (345 mg) at room temp for 3 hr. The mixture was then heated with 5% NaOH (5 ml) on a water-bath for 1 hr and diluted with H_2O. The precipitate was crystallized from acetone to give **8a** as needles, mp 165—169°. This (200 mg) was acetylated with Ac_2O (3 ml) and pyridine (6 ml) by heating on a water-bath. Crystallization of the product from *n*-pentane yielded the tetraacetate (**8b**) as prisms (160 mg), mp 202—204°. IR cm^{-1}: 1740 (OAc). MS: highest peak (relative intensity); 498.3699 (12). Calcd. for $C_{36}H_{58}O_8$ $-2\times CH_3COOH$; 498.3707.

8β,14α-Diacetoxy-3β,21α-dihydroxy-26,27-bisnoronocerane (8c)——The tetraacetate **8b** (120 mg) in 5% KOH–MeOH (10 ml) was heated under reflux for 30 min. The product was chromatographed in benzene

over alumina. Elution with benzene and benzene–CH_2Cl_2 (1 : 1) gave the diol-diacetate (**8c**) (90 mg), mp 190—192°, needles from acetone–*n*-hexane. MS: highest peak (relative intensity); 432.3575 (**10**). Calcd. for $C_{32}H_{54}O_6-(CH_3COOH+CH_2CO)$; 432.3602.

8β,14α-Diacetoxy-26,27-bisnoronoceran-3,21-dione (5)——The diol-diacetate **8c** (22 mg) in acetone was oxidized with Jones' reagent at 0°. On working up the diketo-diacetate (**5**) mp 204—207°, (prisms from acetone–*n*-hexane), was obtained.