

**Triterpenoid Chemistry. XIII.¹⁾ Lycopodium Triterpenoid. (9).²⁾
The Structure of Lyclavatul, a Novel Triterpenoid of
Bisnoronocerane Type³⁾**

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Lyclavatul, a constituent of *L. clavatum*, was elucidated as a novel type of bisnor-triterpenoid, 3 α ,8 β ,14 α ,21 β -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 3 α ,8 β ,14 α ,21 β -tetrahydroxy-26,27-bisnoronocerane (1a).³⁾ In the preliminary communication (and also in ref. 5) this compound was described with a trivial name clavatul. However, since the name clavatul was already used for a metabolite (2,4-dihydroxy-3,5-dimethylacetophenone) of *Aspergillus clavatus*,⁶⁾ we wish to change it and use hereafter a new name "lyclavatul" for this triterpenoid.

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

Lyclavatul, 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 lyclavatul nor its acetate gave constant elementary analyses to deduce their molecular formulae probably due to the variable solvations. High resolution mass spectrum of the lyclavatul acetate exhibited a highest mass peak at *m/e* 558.393 corresponding to C₃₄H₅₄O₆ (calc. 558.392) together with fragment ions due to successive losses of AcOH at *m/e* 498.373 (498.371 calc. for C₃₂H₅₀O₄), at *m/e* 438.353 (438.350 calc. for C₃₀H₄₆O₂), and at *m/e* 378.328 (378.329 calc. for C₂₈H₄₂). This indicated that lyclavatul has C₂₈-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 (C₃₆H₅₈O₈)⁺ 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 character 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) 0.95(2) 0.98(2)	2.07(4)		4.65(2H) 5.04(2H)
Diacetate (1c)	0.88(2) 0.97(4)	2.04(2)	3.45(2H)	5.05(2H)
Diketo-diacetate (5)	1.12(6)	2.07(2)		5.10(2H)
Triacetate (2)	0.88(2) 0.95(3) 1.03(1)	2.07(3)	3.78(1H)	4.64(2H) 5.33(1H)
Keto-triacetate (3)	0.73(1) 0.87(1) 0.95(4)	2.04(1) 2.09(1) 2.14(1)		4.67(2H) 5.11(1H)
Tetraketone (4)	0.90(2) 1.07(2) 1.20(2)			
Bisnoronocerandione diacetate (7)	0.70(2) 0.92(2) 0.98(2)	2.08(2)		4.60(2H) ^{c)}
Tetraacetate (8b)	0.87(4) 0.97(2)	2.02(4)		4.40(2H) ^{c)} 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 $3\beta,21\alpha$ -acetoxy-26,27-bisnoronocerane-8,14-dione (**7**), an ozonization product of α -onocerin diacetate (**6b**),⁷⁾ yielded the $3\beta,8\beta,14\alpha,21\alpha$ -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.

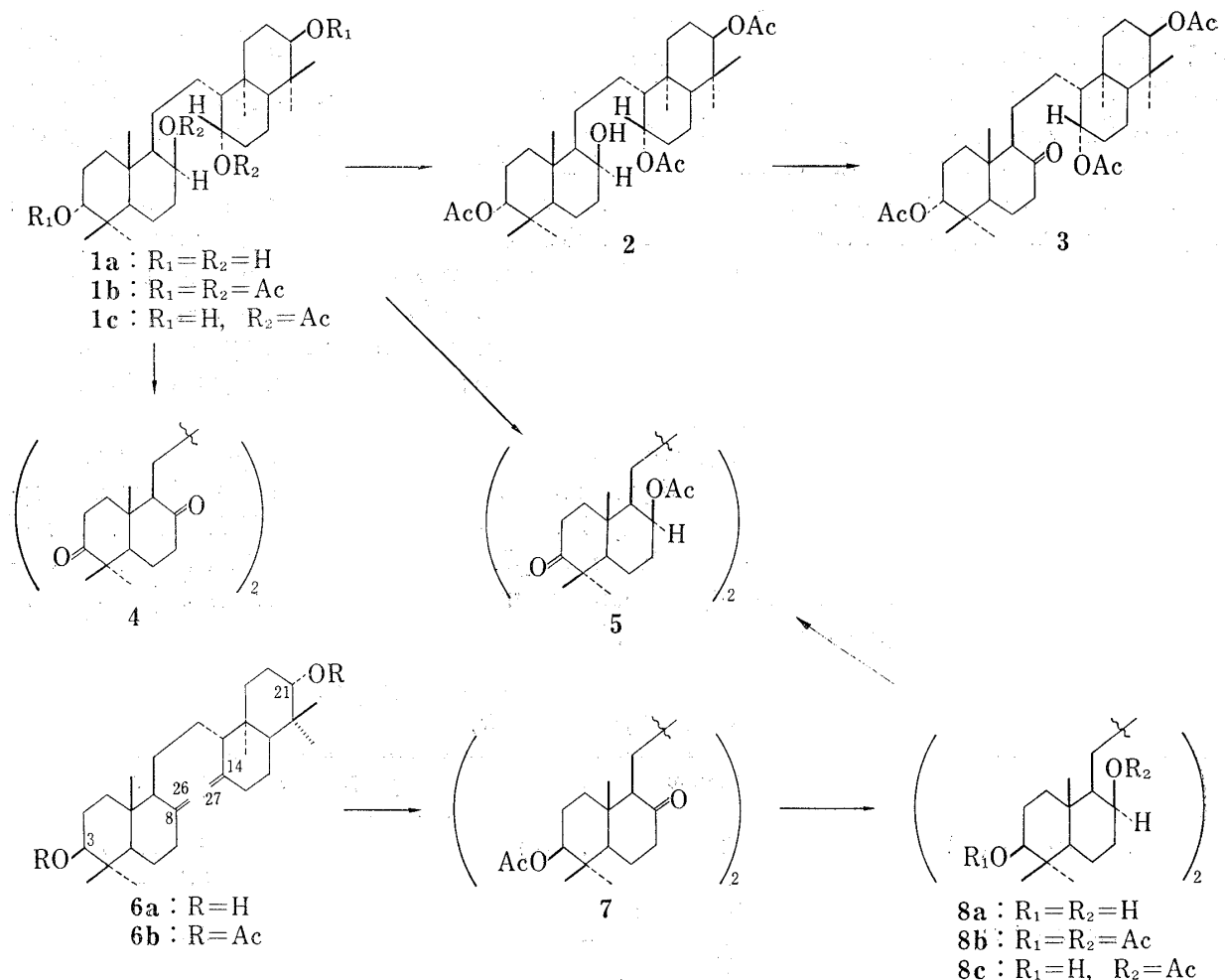


Chart 1

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 $3\beta,21\alpha$ -diol- $8\beta,14\alpha$ -diacetate (**8c**). Jones' oxidation of this gave the 3,21-diketone (**5**) which showed a positive Cotton effect ($[\theta] = +3050$) in circular dichroism (CD) at 280 nm as expected. On the contrary, the 8,14-diketone (**7**) exhibited a strong negative Cotton effect ($[\theta] = -20600$) 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 $3\alpha,8\beta,14\alpha,21\beta$ -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 C₃-C₂₁ and C₈-C₁₄.

Experimental

Unless otherwise stated, the IR spectra were taken in a Nujol mull and the CD spectra are for methanol solutions. The NMR spectra were measured in CDCl₃ 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 CHCl₃-MeOH as a developing solvent. Identities were confirmed by IR, NMR, and TLC comparisons and by mixed fusion with the authentic specimens.

Lyclavatul Tetraacetate (1b)—This formed needles from CH₂Cl₂-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 lyclavatul (1a), mp 277–279°, fine needles from CHCl₃-MeOH. IR (KBr) cm⁻¹: 3320 (OH).

Partial Acetylation of Lyclavatul (1a)—Lyclavatul (1a; 204 mg) in pyridine (6 ml) and Ac₂O (3 ml) was heated on a water bath for 30 min. The mixture was poured into ice-water, extracted with CH₂Cl₂, and the extract was washed with water, dried over K₂CO₃, and evaporated. The oily residue in benzene was chromatographed over alumina (0.8 × 10 cm). Elution with benzene gave a mixture (150 mg) of lyclavatul tetraacetate (1b) and a partially acetylated product. Repeated crystallizations 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 C₃₄H₅₆O₇ - 2 × CH₃COOH; 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 ($c = 0.7 \times 10^{-3}$) [θ] (nm): -12600 (293). MS: highest peak (intensity); 574.3997 (6). Calcd. for C₃₄H₅₄O₇; 574.3870.

Jones' Oxidation of Lyclavatul (1a)—Lyclavatul (1a; 80 mg) in acetone (20 ml) was oxidized with Jones' reagent at 0°. The mixture was diluted with H₂O, extracted with ether, and the extract was washed with water, dried over Na₂SO₄, 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 ($c = 0.2 \times 10^{-3}$) [θ] (nm): -24100 (295).

Partial Hydrolysis of Lyclavatul 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 CH₂Cl₂, and the extract was washed with water, dried over MgSO₄, and evaporated. The crystalline residue in benzene was passed through a short column of alumina. Crystallization of the eluate from acetone-*n*-hexane gave lyclavatul 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 C₃₂H₅₄O₆ - (CH₃COOH + H₂O); 456.3604.

Jones' Oxidation of Lyclavatul 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 Na₂SO₄, 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 diketone-diacetate (5) as prisms, mp 203–206°, which was identical with 8 β ,14 α -diacetoxy-26,27-bisnoronoceran-3,21-dione described below. IR cm⁻¹: 1730 (OAc), 1702 (CO). CD ($c = 0.2 \times 10^{-3}$) [θ] (nm): +3050 (280). MS: highest peak (relative intensity); 470.3370 (4). Calcd. for C₃₂H₅₀O₆ - CH₃COOH; 470.3394.

3 β ,21 α -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 (H₂O), 1730 (OAc), 1705 (CO). CD ($c = 0.14 \times 10^{-3}$) [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).

3 β ,8 β ,14 α ,21 α -Tetrahydroxy-26,27-bisnoronocerane (8a) and Its Tetraacetate (8b)—The diacetoxy-dione 7 (300 mg) in MeOH (15 ml) was treated with NaBH₄ (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 H₂O. The precipitate was crystallized from acetone to give 8a as needles, mp 165–169°. This (200 mg) was acetylated with Ac₂O (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 C₃₆H₅₈O₈ - 2 × CH₃COOH; 498.3707.

8 β ,14 α -Diacetoxy-3 β ,21 α -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 $\text{C}_{32}\text{H}_{54}\text{O}_8-(\text{CH}_3\text{COOH}+\text{CH}_2\text{CO})$; 432.3602.

8 β ,14 α -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.