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STRUCTURES OF TOHOGENOL AND TOHOGENINOL
TRITERPENOIDS OF *LYCOPODIUM SERRATUM*

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Abstract— Dehydration of tohogenol diacetate (**2**) and tohogeninol triacetate (**9**), triterpenoids of *Lycopodium serratum*, gave serratenediol diacetate (**4**) and serratriol triacetate (**10**) in a quantitative yield respectively. The structures of tohogenol and tohogeninol were established as **1** and **8**, and their boat conformations in ring D were also suggested.

THE isolation and structures of tohogenol and tohogeninol were reported in preliminary communications.[1,2] In this report we wish to present a full detail.

Tohogenol (**1**), $C_{30}H_{52}O_3 \cdot \frac{1}{2}H_2O$, m.p. 253–256°, was isolated from neutral constituents of *Lycopodium serratum* as a diacetate (**2**), $C_{34}H_{56}O_5$, m.p. 305–306°. The mol wt of the diacetate was determined by osmometric method. The mass spectra of tohogenol and its diacetate did not afford the molecular ion peaks but the highest peaks were *m/e* 442 and *m/e* 526, corresponding to M-18, respectively. The fragmentation patterns were essentially identical with those of serratenediol (**3**) and its diacetate[3] (**4**). The NMR spectrum of **2** showed the presence of seven C-Me groups at δ 0·84 (9H), 0·87 (6H), 0·93 (3H), and 0·96 (3H), and also of two acetyl methyls at δ 2·04 (6H). A broad multiplet at δ 4·45 (2H) attributable to geminal protons to acetoxyl groups indicated the presence of two secondary acetoxyl groups. The absence of a vinylic proton signal together with the fact that the compound gave negative tetranitromethane test indicates that the compound is saturated.

Oxidation of tohogenol with chromium trioxide-pyridine complex afforded a hydroxy-diketone (**5**), $C_{30}H_{48}O_3$. Reduction of **5** with sodium and n-propanol regenerated tohogenol (**2**). The IR spectrum of **5** showed a strong hydroxyl band† at 3484 cm^{-1}, together with carbonyl bands at 1704 and 1684 cm^{-1}. Thus, the function of two of three O atoms in tohogenol was defined as the secondary OH group. The third O atom, which resisted to acetylation and oxidation, is established to provide a tertiary OH group, since the NMR spectrum of **5** did not exhibit any signal below δ 2·8.

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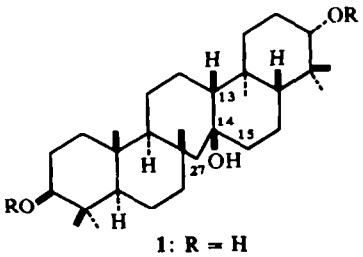
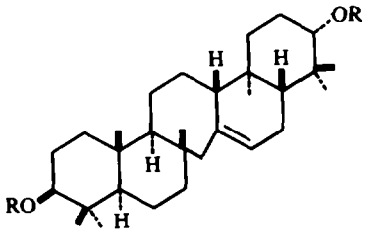
† The IR spectrum of **2** in Nujol mull did not exhibit any appreciable absorption in the hydroxyl region, but in hexachlorobutadiene absorptions at 3359 and $3436_{(w)}$ cm^{-1} were clearly observed.

The hydroxy-diketone (**5**), when heated under reflux in 3% alcoholic hydrochloric acid, was dehydrated to give quantitatively serratenedione[3] (**6**). Similarly, dehydration of **2**, followed by reacetylation of the product, yielded serratenediol diacetate[3] (**4**). Therefore, position of the tertiary OH group is C_{14} of serratane skeleton.

The β-configuration of C_{14}-OH group was established by the fact that dehydration of **2** with thionyl chloride in pyridine formed serratenediol diacetate (**4**) as a sole product. If the configuration of C_{14}-OH group were α, dehydration would produce not only serratenediol diacetate (Δ^{14-15}) but also isoserratenediol diacetate[3] (Δ^{13-14}), since trans-elimination towards C_{13} is possible. However, no isoserratenediol diacetate was found in its reaction mixture as confirmed by TLC. Thus, the C/D ring junction must be *cis* and the configuration of C_{14}-OH group is β. Therefore tohogenol is represented as 3β,14β,21α-trihydroxyserratane (**1**).



1: R = H
2: R = Ac



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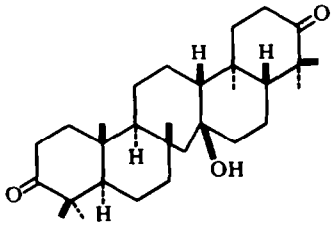
4: R = Ac



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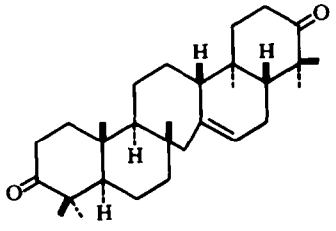

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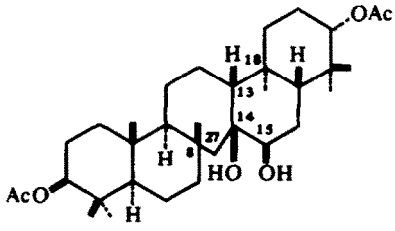
It is noteworthy that dehydration of **2** did not occur towards C_{27} but occurred only towards C_{15}. As has been shown,[4] the most of C/D-*cis* 14β-serratane derivatives adopt boat conformations in ring D. For example, in 3β,21α-diacetoxy-14β,15β-dihydroxyserratane (**7**) the chief driving forces preventing ring D to adopt chair conformation are non-bonded interactions between C_{18}-Me and C_{27}-methylene and between C_{14}-hydroxyl and C_8-Me groups. We consider that the ring D of tohogenol is also in a boat conformation (**1a**), where 14β-OH and 15α-hydrogen are diaxially arranged in a preferred anti-coplanar geometry for trans-elimination, but the arrangement of 14β-OH and 27α-hydrogen are both in equatorial orientations to ring C and unfavoured for trans-elimination. If the ring D were in a chair form (**1b**), elimination should occur more easily towards C_{27} rather than toward C_{15}, for the configuration of 14β-OH is axial to ring C but equatorial to ring D.

Tohogeninol (**8**), $C_{30}H_{52}O_4$, m.p. 311–314°, was isolated as its triacetate (**9**), $C_{36}H_{58}O_6$, m.p. 256–258°. The NMR spectrum of the acetate (**9**) indicated the presence of six C-Me groups at δ 0·87 (9H), 0·91 (3H), and 0·98 (6H), and of three acetyl methyls



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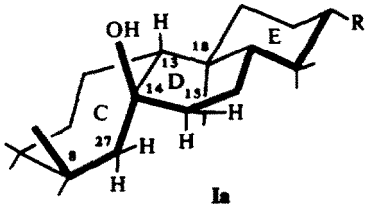
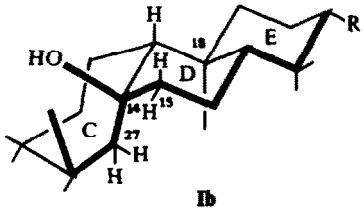
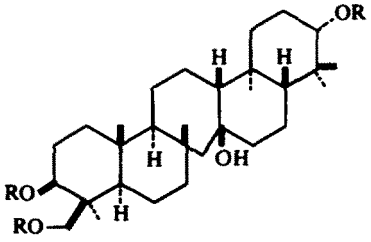


Ia

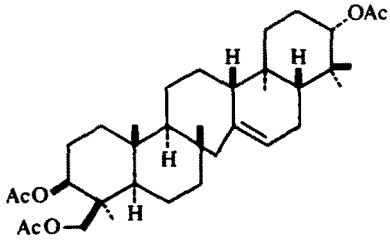


Ib

at 2·05 (9H). Moreover, a AB quartet at 4·34 (2H, δ_{AB} = 18 Hz, J = 12 Hz) and a broad multiplet at 4·57 (2H), attributable to geminal protons to acetoxyl groups, suggested the presence of one primary and two secondary acetoxyl groups. The IR spectrum of **9** in Nujol mull exhibited a strong OH absorption at 3497 cm^{-1}, besides the ester absorption at 1724 and 1250 cm^{-1}. Thus, the fourth O atom in tohogeninol provides a tertiary OH group.



8: R = H
9: R = Ac



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The absence of a double bond in tohogeninol was shown by the absence of a vinylic proton signal in the NMR spectrum of **9** and by its negative test to tetranitromethane. Treatment of the triacetate (**9**) with 3% alcoholic hydrochloric acid and reacetylation of the product, as described in tohogenol, afforded, in excellent yield, an anhydro compound, which is completely identical with serratriol triacetate[5] (**10**). Hence, by analogy with tohogenol, the structure (**8**) was assigned to tohogeninol.

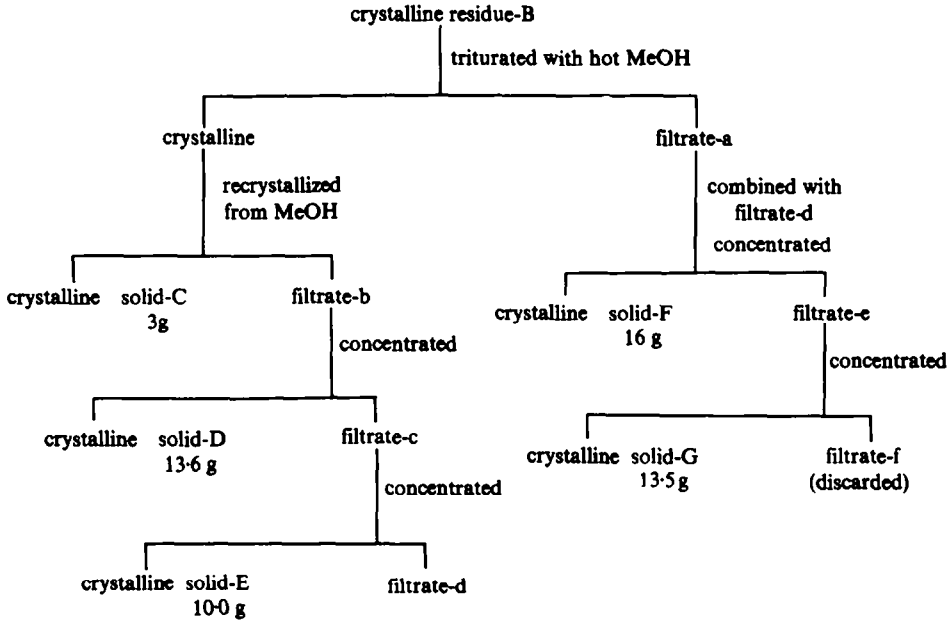
EXPERIMENTAL

Unless otherwise stated m.p's were determined on a Yanagimoto m.p. apparatus, NMR spectra were measured in $CDCl_3$ by a Varian A-60 machine, IR spectra were taken on Nujol mull. Identities were confirmed by IR and by TLC comparisons. Alumina used for chromatography was washed with 2·5% AcOH and reactivated.

Isolation of minor triterpenoids of Lycopodium serratum

Tohogenol diacetate (2). A non-alkaloidal portion (800 g) of methanolic extract *Lycopodium serratum*,[6] was treated with 5% KOH-MeOH (8 L) under reflux for 7 hr. After standing overnight, the resulting ppt was washed with water, and dried to afford a brown crystalline residue-A (140 g). The filtrate was concentrated to a 1/4 volume, diluted with water (ca. 5 L), and the ppt was collected on a filter with aid of Celite, which was extracted with $CHCl_3$-MeOH (1:1) using a Soxhlet apparatus. Evaporation of solvent left a crystalline residue-B (82 g), which was fractionally crystallized from MeOH to give the following fractions (Chart 1).

Chart 1



Acetylation of the crystalline solids-C and D with pyridine and Ac_2O gave serratenediol diacetate (4), 1·2 g and 12 g (prisms from $CHCl_3$-MeOH) respectively, m.p. and mixed m.p. 336–337° (open capillary).

Acetylation of the crystalline solid-E with pyridine (40 ml) and Ac_2O (20 ml) gave a crystalline solid (8 g), which was a mixture of tohogenol diacetate and serratenediol diacetate (ratio, ca. 1:1), shown by TLC.

The crystalline solid-F (16 g) was treated with pyridine (50 ml) and Ac_2O (25 ml) overnight at room temp. The resulting ppt was washed with MeOH to give crude *tohogenol diacetate* (2) (13·8 g), which contained a small amount of serratenediol diacetate, shown by TLC.

Similarly, the crystalline solid-G (13·5 g) was treated with pyridine (40 ml) and Ac_2O (25 ml). The resulting ppt was crude tohogenol diacetate (2; 5·1 g). A part of crude tohogenol diacetate was crystallized several times from $CHCl_3$-MeOH to afford pure specimen as prisms, m.p. 305–306° (open capillary), $[\alpha]_D$ +28° (c = 1·6, $CHCl_3$); NMR (δ): 0·84 (9H), 0·87 (6H), 0·93 (3H), 0·96 (3H), 2·04 (6H), 4·5 (2H); IR: 1721, 1250 cm^{-1} (OAc). (Found: C, 74·84; H, 10·24, MW. 569, $C_{34}H_{56}O_5$ requires: C, 74·95; H, 10·36%, MW. 544·7).

Tohogenol (1). A soln of 2 (600 mg) and KOH (2 g) in MeOH (20 ml) and dioxan (30 ml) was heated under reflux for 2 hr. After cooling, the mixture was diluted with water and the resulting ppt was washed with water. The crude diol (510 mg) was crystallized from acetone to give *tohogenol* (1) as prisms, m.p. 253–256°. (Found: C, 76·61; H, 11·41, $C_{30}H_{52}O_3 \cdot \frac{1}{2}H_2O$, requires: C, 76·77; H, 11·38%).

Serratriol triacetate (10) *and tohogeninol triacetate* (9). The crystalline residue-A (140 g) was acetylated with pyridine (400 ml) and Ac_2O (200 ml) under reflux for 3 hr. After standing overnight at room temp, the resulting ppt was washed with MeOH to give crude serratenediol diacetate (ca. 100 g). The filtrate and washings were combined and evaporated *in vacuo* to give a brown gum (40 g) which was treated with n-hexane (400 ml) under reflux for 1 hr, and separated into a n-hexane-insoluble part (25 g) and n-hexane-

soluble part (14 g). The insoluble part (25 g) was dissolved in n-hexane-benzene (1 : 1) and chromatographed over alumina (200 g) in the following manner.

Fraction	Solvent (vol.)	(g)	
1	benzene (300 ml)	crystalline solid	4·5 g
2–11	benzene (3 L)	crystalline solid	16 g
12–14	benzene (1 L)	brown oil	3 g
15–16	$CHCl_3$ (1 L)	brown oil	

Fraction 1 was crystallized from $CHCl_3$-MeOH to give serratenediol diacetate (**4**). Fraction 2–11 was further chromatographed in benzene over SiO_2 (200 g) in the following manner.

Fraction	Solvent (vol.)	(g)	
1	benzene (200 ml)	crystalline solid	1·2 g
2–11	benzene (2·4 L)	crystalline solid	12 g
12–13	benzene (400 ml)	crystalline solid	0·5 g
14–15	$CHCl_3$ (1 L)	brown oil	1·7 g

Fraction 1 gave serratenediol diacetate (**4**). Fraction 2–11 was crystallized from n-hexane-benzene to give *serratriol triacetate* (**10**; 5·3 g), as needles, m.p. 245–247°, $[\alpha]_D$ +9° (c = 1·9, $CHCl_3$); IR: 1730, 1245 cm^{-1} (OAc); NMR (δ): 0·70 (3H), 0·85 (9H), 0·90 (3H), 1·00 (3H), 2·03 (3H), 2·05 (3H), 4·24 (2H, ABq. δ_{AB} = 19 Hz, J = 12 Hz), 4·51 (2H, m.), and 5·35 (1H, m).). (Found: C, 74·06; H, 9·89, $C_{36}H_{56}O_6$ requires: C, 73·93; H, 9·65%). Fraction 12–13 was further chromatographed in benzene over alumina (5 g). (i) Elution with benzene gave serratriol triacetate (0·2 g). (ii) Further elution with benzene and crystallization from $CHCl_3$-MeOH gave *tohogeninol triacetate* (**9**; 60 mg) as prisms, m.p. 256–258°, $[\alpha]_D$ +11·8° (c = 1·01, $CHCl_3$). (Found: C, 70·98; H, 9·61. $C_{36}H_{58}O_7$ requires: C, 71·15; H, 9·90%).

Serratriol.[5] Serratriol triacetate (**10**; 100 mg) and KOH (2 g) in dioxan (5 ml)-MeOH (10 ml) were heated under reflux for 2 hr. The ppt was washed with water, and crystallized from pyridine to give *serratriol*, m.p. 335–336° (open capillary). (Found: C, 78·43; H, 11·26. $C_{30}H_{50}O_3$ requires: C, 78·55; H, 10·99%).

Tohogeninol (**8**). The triacetate (**9**; 30 mg) in dioxan (1 ml) was hydrolyzed with 5% KOH-MeOH (5 ml) under reflux for 30 min. The resulting triol (15 mg) was purified by sublimation at 240° and 10^{-4} mm to give **8**, m.p. 311–313°. (Found: C, 76·35; H, 10·47. $C_{30}H_{52}O_4$ requires: C, 75·58; H, 11·00%).

21-Episerratenediol diacetate.[1] The mother liquors (17·8 g) from **4** and **2** of the crystalline solids-**D**, **F**, **G**, and n-hexane-soluble part from a crystalline residue-**A** (see above) were combined and chromatographed over alumina (400 g), in the following manner.

Fraction (each 300 ml)	Solvent	(g)	
1–3	benzene-n-hexane (1 : 1)	oil	20 g
4–16	benzene-n-hexane (1 : 1)	oil	2·2 g
17–20	benzene	crystalline solid	1·6 g
21–22	$CHCl_3$	oil	3·0 g

Fraction (1–3) was dissolved in n-hexane and kept overnight at room temp. The resulting needles were collected and crystallized from n-hexane to give crude 21-episerratenediol diacetate (3·5 g), m.p. 209–217°. Further crystallizations from n-hexane furnished pure *21-episerratenediol diacetate*,[1] as fine needles, m.p. 225–229°, $[\alpha]_D$ −29° (c = 2·06, $CHCl_3$). (Found: C, 77·64; H, 10·61. $C_{34}H_{54}O_4$ requires: C, 77·52; H, 10·33%). Fraction (17–20) gave crude tohogenol diacetate (**2**).

21-Episerratenediol.[1] 21-Episerratenediol diacetate (510 mg) in 2·5% KOH-MeOH (50 ml) was heated under reflux for 1·5 hr. After cooling, the mixture was diluted with water, and the ppt was crystallized from $CHCl_3$-MeOH to give *the diol* as needles, m.p. 289–290°. (Found: C, 81·15; H, 11·13. $C_{30}H_{50}O_2$, requires: C, 81·39; H, 11·38%).

The hydroxy-diketone (**5**). Tohogenol (**1**; 200 mg) was treated with pyridine (3 ml)-CrO_3 (0·2 g) complex overnight at room temp. The mixture was poured into ice-water and extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$, and evaporated to dryness to give a residue which was passed in benzene through a column of alumina to give **5** (120 mg) (prisms from MeOH), m.p. 235–237°. $[\phi]_{314\,m\mu}$ +3850°, $[\phi]_{305\,m\mu}$ +3500°, $[\phi]_{271\,m\mu}$ −1220°; IR: 3484 (OH), 1704 and 1684 cm^{-1} (CO). (Found: C, 79·09; H, 10·41, $C_{30}H_{48}O_3$ requires: C, 78·89; H, 10·59%).

To a boiling soln of the hydroxy-diketone (60 mg) in n-PrOH (20 ml) 2 g of Na was added in several portions, and the heating was continued for further 2 hr. Being worked up as usual, tohogenol (1), m.p. and mixed m.p. 253–256°, was isolated. Acetylation of this gave the diacetate (2), m.p. and mixed m.p. 305–307°.

Dehydration of the hydroxy-diketone (5). The hydroxy-diketone (5; 100 mg) in 2·5% HCl-EtOH (60 ml) was heated under reflux on a water-bath for 30 min, then neutralized with 10%$NaHCO_3$ soln. After removal of EtOH under reduced press, the mixture was diluted with water and extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$, and evaporated to give a crystalline residue (96 mg), which was crystallized from MeOH to give serratenedione[3] (6), m.p. and mixed m.p. 207–209°. Identity was further confirmed by comparisons of NMR and ORD spectra.

Dehydration of tohogenol diacetate (2) *by hydrochloric acid.* Tohogenol diacetate (2; 106 mg) was similarly treated with 3% HCl-EtOH (20 ml) as described above. The resulting product was acetylated with pyridine (2 ml) and Ac_2O (1 ml). The mixture was poured into water, and extracted with $CHCl_3$. The extract was washed with dil HCl, water, dried over $MgSO_4$, and evaporated. Crystallization of the residue from $CHCl_3$-MeOH gave 4 (85 mg), m.p. and mixed m.p. 336–338° (open capillary).

Dehydration of tohogenol diacetate (2) *by thionyl chloride.* A soln of 2 (245 mg) in pyridine (10 ml) was treated with $SOCl_2$ (0·5 ml) at room temp for 2 hr. The mixture was poured into ice-water, extracted with CH_2Cl_2. The extract was washed with dil HCl and water, dried over $MgSO_4$, and evaporated to dryness. Crystallization of a colorless crystalline residue from MeOH-$CHCl_3$ gave 4 (215 mg), m.p. and mixed m.p. 336–338° (open capillary). The NMR and TLC of the product did not show the presence of isoserratenediol diacetate.

Dehydration of tohogeninol triacetate (9). A soln of 9 (20 mg) in 3% HCl-EtOH (15 ml) was heated under reflux for 30 min. The mixture was poured into water and extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$, and evaporated to give a residue which was acetylated with pyridine (1 ml) and Ac_2O (0·5 ml). The product was crystallized from n-hexane-benzene to give needles, m.p. 247–249°, identical with 10, confirmed by mixed m.p. and IR spectra.

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