Triterpenoids of Hoelen (fuling), Sclerotia of *Poria cocos* Wolf. II.1)  
3β-Hydroxylanosta-7,9(11),24-trien-21-oic Acid

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The new triterpene carboxylic acid from 'fuling' was characterized as 3β-hydroxy-
lanosta-7,9(11),24-trien-21-oic acid (Ia) by the physical properties of the derivatives and
by the derivation of Ia into dihydroagnosterol (VIa).

'Fuling' (茯苓) is the naturally occurring sclerotium of *Poria cocos* (Schw.) Wolf. (syn.  
Pachyma Hoelen Rumph.) (Polyporaceae) and has been frequently prescribed in Chinese
medicine as a diuretic and for palpitation. In the previous paper  
the triterpenoid fraction of the fungus has been extensively analyzed and the presence of a new triterpene carboxylic
acid (Ia) besides the known acids, pachymic acid(3,4) (IIa) and tumulosic acid(5) (IIb), in
the sclerotia imported from Korea, was reported. This paper deals with the identification of the
acid with 3β-hydroxylanosta-7,9(11),24-trien-21-oic acid (Ia).

![Chart 1](image)

The ether extract of the fungus, after removal of the hexane soluble part, was derived
into the mixture of the methyl esters of the acidic substances by the treatment with diazo-
methane and separated into the components by passing through an alumina column. Methyl
pachymate(3,4) (IIc), the methyl ester of the new acid (Ib), mp 142—142.5°, [x]_D^2 +42° (CHCl₃),
and methyl tumulosate(5) (IId) were thus separated in the respective yields of 0.10, 0.008,
and 0.004%.

2) Location: Kamiyogawa-1-chome, Setagaya-ku, Tokyo.  
(1958).  
1954, 3713.
The molecular formula of the methyl ester (Ib) was established as $C_{31}H_{46}O_2$ from analyses and mass spectra of Ib and the derivatives. The presence of a secondary hydroxyl group in Ib, shown by the infrared spectrum (IR) of Ib, was confirmed by the formation of the monooctoate (Ic) and the ketone (Id). From the IR absorption (1705 cm$^{-1}$) of Id, the secondary hydroxyl group in Ib must be in a six-membered ring. The nuclear magnetic resonance spectra (NMR) of Ib, Ic, and Id show that Ib possesses five methyls$^6$ and one isopropylidene group ($\tau$ 8.33 (3H), 8.43 (3H), 4.94 (1H)). The presence of the latter group was confirmed by the preparation of the dihydro derivative (Ie) by catalytic hydrogenation of Ib. NMR of Ie shows no peaks due to the isopropylidene group and, instead, the presence of seven methyl groups. Besides these groups, NMR spectra of these compounds reveal the presence of two olefinic protons (ca. 4.7 and 4.5 $\tau$) and they are assigned as those in trisubstituted double bonds by IR absorption (ca. 810 cm$^{-1}$). The ultraviolet (UV) absorptions of Ib—Ie show a characteristic triplet at 236, 243, and 252 m$\mu$ ($\log \varepsilon$, 4.1, 4.2, 4.0), indicating the presence of a conjugated heteroannular diene system and being consistent with those of lanostane-7,9(11)-diene series.$^{10}$

The methyl ester (Ib) was resistant to hydrolysis. The parent acid, mp 257—259$^9$, $[\alpha]_D +105^\circ$ (CHCl$_3$), was recovered by the hydrolysis under a forcing condition, i.e., by boiling Ib with alkali in diethylene glycol.

Because of the coexistence of the acid with pachymic acid (IIa) and tumulosic acid (IIb) in the fungus and from the comparison of the properties with the known fungal triterpenic carboxylic acids of lanostane series.$^9$ IIa is supposed to be a $C_{30}$ lanostane derivative with a carboxyl group at C-20 and a hydroxyl group at C-3. Reduction of the ketone (Id) with sodium borohydride afforded the original ester (Ib), which showed the signal of 3-H proton at 6.78 $\tau$, indicating the presence of 3$\beta$-hydroxy.

\[ \text{ROH}_2C \rightarrow \text{TsOH}_2C \]

\[ \text{LiAlH}_4 \rightarrow \text{POO}_2 \]

\[ \text{Ib} \]

\[ \text{IVa : } R = H \]
\[ \text{b : } R = \text{CH}_3 \text{CO} \]

\[ \text{V} \]

\[ \text{VIa : } R = H \]
\[ \text{b : } R = \text{CH}_3 \text{CO} \]

\[ \text{Chart 2} \]

$^6$ One of the methyl signals generally appears at a higher field (ca. 9.42 $\tau$) and is assigned as 18-methyl group in lanostane-7,9(11)-diene series by the comparison with those in Ib—Ie, IVa, IVb, VIa, VIIb, methyl dehydrotumulosate;$^7$ methyl polypropenonate C;$^8$ 3-oxolanostane-7,9(11),24-trien-26-oic acid.$^9$

$^7$ Unpublished data of our laboratory.

$^8$ Private communication from Prof. H. Inouye, Kyoto University.


These observations lead to the assumption that the acid (Ia) must be 3β-hydroxyylanost-7,9(11),24-trien-21-oic acid (Ia). The corresponding 8,24-dienoic acid (IIIa), designated trameotenolic acid, has been isolated from Fomes hartigii (Fr.) ALLESCHE (=Phellinus hartigii ALLESCHE. et SCHNABL.) IMASEKI,11 Trametes orodata (WULF.) Fr. (=Gloeopilum odoratum (WULF. ex Fr.) IMASEKI),12 and Lenzites trabea PERS. ex Fr. (=Gloeopilum trabeum (PERS. ex Fr.) Murr.)13 and the 3-oxo-8,24-dienoic acid (IIIb), designated pinicolic acid A, from Polyporus pinscola Fr. (=Fomitopsis pinscola (Fr.) KARST.).14 The tetracyclic triterpenoids of lanost-8-ene series from Basidiomycetes have been isolated, in general, accompanying a small amount of the corresponding 7,9(11)-dienes and the separation of the mixture has so far been unsuccessful even in gas and thin-layer chromatography. However once they are obtained in pure state, they show different physical properties. The comparison of the methyl ester acetate (Ic) and the ketone (Id) with the authentic samples of methyl acetyltrimetanol (IIIc) and methyl pinicolate A (IIIId) respectively showed some similarities but clear difference in IR absorptions and retention times in gas chromatography. Since any authentic samples of 7,9(11)-diene series for direct comparison and the starting materials for the preparation of these compounds were not available, the direct correlation of Ib with agnosteryl (V) was carried out as follows (Chart 2). The methyl ester (Ib) was converted with lithium aluminum hydride into the diol (IVA),15 which was partially tosylated and then treated with lithium aluminum hydride to afford agnosteryl (V). For direct comparison V was derived to the dihydro compound (VIA) and the acetate (VIB), both of which showed the identity with the specimens16–18 prepared from lanoster in every respects.

On the basis of these facts, the structure of the new acid has been elucidated as 3β-hydroxyylanost-7,9(11),24-trien-21-oic acid (Ia). Although lanosta-7,9(11)-diene derivatives frequently appear in nature as the contaminants in the corresponding 8-ene derivatives especially in higher fungi, there have been a few example of thier isolation in a pure state.9,15,19

Experimental19

Methyl 3β-Hydroxyylanost-7,9(11),24-trien-21-oate (Ib) — Thepowdered sclerotia of Poria cocos (5 kg) were extracted with ether and the extract was concentrated to ca. 1 liter, from which crude pachymycin acid (IIa) (3.5 g) separated out. After methylation and purification the methyl ester, mp 182–183°C, [α]D +38.9° (ε=0.88) (lit.9 mp 185–187°C, [α]D +41.5°), was identified with the authentic sample of methyl pachymycine (IIc). The ethereal mother liquor was evaporated to dryness, washed with hexane, and methylated with CH3N2 in ether. The reaction products (15.3 g), the mixture of methyl esters, were chromatographed on

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20) Melting points were determined in a Yanagimoto melting point apparatus and are not corrected; the rotations were measured in CHC13 with an Applied Electric Lab. Automatic Polarimeter Model MP-11; the UV spectra were taken in EtOH solution on a Hitachi EPU-2A Spectrophotometer; the IR spectra were measured on a Nihon Bunko DS-301 Spectrophotometer in KBr discs; the NMR spectra were run in CDC13 solution on a Varian Associates (100 Mc) and recorded in v values with TMS as the internal standard; and the mass spectra were determined on a Hitachi RMU-6D mass spectrometer with direct inlet system. At the each stage of the separation and the purification thin-layer and gas-liquid chromatography was adopted for monitoring the purity of the specimen. For thin-layer chromatography silica-gel G or H were used and the detection was carried out by heating on a hot plate after spraying 20% H2SO4 or vanillin-phosphoric acid. Gas chromatography was carried out on 1.8% SE-30 or 1.3% OV-17 column at 250°C on a Hitachi F-6 gauge chromatograph. Unless otherwise specified alumina (Woelm, neutral) and silica-gel (Mallinkrodt) were used for column chromatography.
an alumina column (Wako, 500 g). Elution with benzene-ether (95:5) afforded further amount (1.6 g) of IIc, methyl toulualosate (IId) (0.2 g), mp 162–163°C (from MeOH), \([\delta_2^3 = 24.1^{\circ} (c = 0.79) (\text{lit}.)\] mp 165–167°C, \([\delta_2^2 + 23.6^{\circ}]\), which was identified with the authentic sample, and the methyl ester (Ib) of the new acid (0.4 g), methyl 3β-hydroxyestranosta-7,9(11),24-trien-21-oate (Ib), as colorless needles of mp 142–145°C (from MeOH), \([\delta_2^2 + 42^{\circ} (c = 1.0)]\). Anal. Calcd. for C₃₆H₅₄O₄: C, 79.43; H, 10.32. Found: C, 79.26; H, 10.11. UV \(\lambda_{\max} \mu \mu \mu (\text{log} e): 237 (4.08), 244 (4.15), 252 (3.97). IR \(\nu_{\max} \text{cm}^{-1}: 3310 (\text{OH}), 1725 (\text{C=O}), 810 (\text{C=C(CH)}).\) NMR: 9.42 (3H, s), 9.19 (6H, s), 9.05 (3H, s), 9.02 (3H, s), 8.43 (6H, s), 8.33 (3H, s), 6.78 (1H, m), 6.36 (3H, s), 4.94 (1H, m), 4.72 (1H, m), 4.53 (1H, m). 

**Methyl 3β-Acetoxyestranosta-7,9(11),24-trien-21-oate (Ic)**—Acetylation of Ib (131 mg) with Ac₂O (1 ml) in pyridine (1 ml) at room temperature overnight gave Ic as colorless needles (117 mg) of mp 144–145°C (from MeOH), \([\delta_2^2 + 53.7^{\circ} (c = 0.7)]\). Anal. Calcd. for C₃₆H₅₂O₈: C, 77.06; H, 10.11. Found: C, 77.01; H, 9.86. Mass Spectrum \(m/e, 510 (M^+, 100), 450 (24), 435 (25), 353 (57), 314 (45), 313 (38), 254 (26), 253 (83), 241 (26), 240 (44), 225 (38).\) UV \(\lambda_{\max} \mu \mu \mu (\text{log} e): 237 (4.12), 244 (4.12), 253 (4.01).\) IR \(\nu_{\max} \text{cm}^{-1}: 1725, 1720 (\text{C=O}), 1245 (\text{ester}), 810 (\text{C=C(CH)}).\) NMR: 9.42 (3H, s), 9.13 (6H, s), 9.05 (3H, s), 9.02 (3H, s), 8.44 (3H, s), 8.34 (3H, s), 7.97 (3H, s), 6.36 (3H, s), 5.51 (1H, m), 4.95 (1H, m), 4.71 (1H, m), 4.56 (1H, m).

**Methyl 3-Oxolanosta-7,9(11),24-trien-21-oate (Id)**—Ib (200 mg) was dissolved in pyridine (2 ml) and allowed to stand with Cr₂O₃-pyridine (200 mg in 2 ml) at room temperature overnight. The reaction product was extracted with ether and chromatographed through an alumina column. The elution with ether, followed by crystallization from MeOH, afforded Id as colorless needles (11 mg) of mp 140–142°C. UV \(\lambda_{\max} \mu \mu \mu (\text{log} e): 237 (4.01), 244 (4.08), 253 (3.92).\) IR \(\nu_{\max} \text{cm}^{-1}: 3400 (\text{OH}), 1720 (\text{C=O}), 808 (\text{C=C(CH)}).\) NMR: 9.42 (3H, s), 9.17 (6H, d, \(J = 6 \text{cps}\)), 9.14 (6H, s), 9.05 (3H, s), 9.03 (3H, s), 6.82 (1H, m), 6.38 (3H, s), 4.74 (1H, m), 4.60 (1H, m).

**3β-Hydroxyestranosta-7,9(11),24-trien-21-oic Acid (Ia)**—Ib (38 mg) was heated under reflux with KOH (1 g) in diethylene glycol (10 ml) for 4 hr. The mixture was poured into water, acidified, and extracted with ether. The acidic fraction was chromatographed on a column of alumina (1 g) and eluted with ether to give Ia as colorless needles (3 mg) of mp 257–258°C (from ether-isopropanol), \([\delta_2^2 + 103^{\circ} (c = 0.1)].\) UV \(\lambda_{\max} \mu \mu \mu (\text{log} e): 236 (4.18), 243 (4.22), 252.5 (4.05).\) IR \(\nu_{\max} \text{cm}^{-1}: 3400 (\text{OH}), 1725, 1700 (\text{C=O}), 805 (\text{C=C(CH)}).\)

Methylation of Ia with CH₃ONa afforded the starting material (Ib).

**Reduction of Methyl 3-Oxolanosta-7,9(11),24-trien-21-oate (Id) by Sodium Borohydride**—Id (43 mg) in dioxane (5 ml) was added with stirring at room temperature to NaBH₄ (20 mg) in water (2 ml) and dioxane (2 ml). After 1 hr the excess amount of NaBH₄ was decomposed by the addition of AcOH and the product was extracted with CHCl₃ and chromatographed on alumina. The elution with ether followed by recrystallization from MeOH afforded Ib as colorless needles (34 mg) of mp 141–142°C. The identity was established by a mixed fusion, IR, TLC, and GLC.

**Lonrostia-7(11),24-trien-3β,21-diol (IVA)**—Ib (160 mg) in ether (anhyd., 100 ml) was added dropwise with stirring at room temperature to LiAlH₄ (400 mg) suspended in ether (anhyd., 20 ml) and the mixture was refluxed for 1 hr. The excess amount of LiAlH₄ was destroyed by wet ether. After acidification with dil. HCl the mixture was extracted with ether, washed with water, and dried over Na₂SO₄. The residue after the evaporation of the solvent was purified by chromatography through an alumina column to give IVa as colorless needles (91 mg) of mp 196–198°C (from MeOH), \([\delta_2^2 + 68^{\circ} (c = 0.40) (\text{lit}.)\] mp 194–196°C, \([\delta_2^2 + 72^{\circ}]\). UV \(\lambda_{\max} \mu \mu \mu (\text{log} e): 237 (4.00), 244 (4.08), 252 (3.91).\) IR \(\nu_{\max} \text{cm}^{-1}: 3300 (\text{OH}), 810 (\text{C=C(CH)}).\) NMR: 9.41 (3H, s), 9.11 (3H, s), 9.10 (3H, s), 9.02 (3H, s), 8.99 (3H, s), 8.39 (3H, s), 8.32 (3H, s), 6.76 (1H, m), 6.38 (2H, m), 4.88 (1H, m), 4.68 (1H, m), 4.52 (1H, m). Acetylation of IVa gave 3β,21-diacetoxylstanosta-7,9(11),24-triene (IVb), mp 119–120°C (lit.) mp 122°C.

**Conversion of Lonrostia-7(11),24-trien-3β,21-diol (IVA) into Dihydroaegnostest (Lonrostia-7(9)(11)-dien-3β-ol) (IVA)**—The reaction condition for the partial Tosylation at C-21 of IVA was examined by TLC of the etheral extract of the reaction products using the SiH₂₄₄₄ plates employing hexane-AcOEt (7:3) as the solvent. The fraction showing higher Rf value than IVA, after extraction from the plate, revealed the characteristic IR absorptions of tosylate \(\nu_{\max} \text{cm}^{-1}: 3400 (\text{OH}), 1600, 1363, 1175, 825 (\text{p-Ts-O}), 810 (\text{C=C(CH)}).\) IVA (76 mg) was tosylated with p-toluenesulfonyl chloride (200 mg) in pyridine (1.5 ml) for 30 min at room temperature and the reaction mixture was poured into water and extracted with ether. The etheral extract (53 mg), showing the presence of the monotosylate as the main component, was dissolved in tetrahydrofuran (anhyd., 20 ml) and added with LiAlH₄ (200 mg). The mixture was kept standing at room temperature for 2 days and the excess amount of LiAlH₄ was destroyed by the addition of wet ether. After acidification with dil. HCl the reaction product was extracted with ether. The extract was
washed with water, dried over Na₂SO₄, and chromatographed through an alumina column (50 g). The fraction eluted with ether–benzene (5:95) was further separated by TLC into two fractions using the plate of SiHF₆ employing hexane–AcOEt (7:3) as the developer. One of the two was identified with the starting material (IVA) (19 mg). The other (5 mg), mp 148–151⁰, showing the same Rf value with lanosterol in TLC and being assumed to be impure agnostosterol (V), was dissolved in EtOH (5 ml) and hydrogenated in the presence of Pt–catalyst. Recrystallization of the product from MeOH gave colorless needles (3.1 mg) of mp 155–156⁰. UV λmax cm⁻¹ (log ε): 237 (4.08), 244 (4.15), 253 (3.98). IR νmax cm⁻¹: 3290 (OH), 810 (νC=CH–). The identity with the authentic sample of dihydroagnostosterol (VIIa) was established by a mixed fusion, TLC, and GLC.

Acetylation of the product (0.9 mg) with Ac₂O (0.2 ml) in pyridine (0.2 ml) gave the acetate, colorless needles (0.7 mg) of mp 168–170⁰. IR νmax cm⁻¹: 1720 (C=O), 1248 (ester), 813 (νC=CH–). The identity with the authentic sample of dihydroagnosteryl acetate (VIIb) was confirmed by a mixed fusion, TLC, and GLC.

Preparation of Dihydroagnostosterol (VIIa) —— Dehydrogenation of dihydrolanosteryl acetate with selenium dioxide followed by the purification through an alumina column and recrystallization from MeOH gave dihydroagnosteryl acetate (VIIb), mp 168–169⁰, [α]D +92⁰ (c = 0.96) (lit.17) mp 167–168⁰, [α]D +88⁰. UV λmax μ (log ε): 236 (4.16), 244 (4.23), 252 (4.05). IR νmax cm⁻¹: 1720 (C=O), 1248 (ester), 813 (νC=CH–). NMR: 9.44–9.06 (24H), 7.97 (3H, s), 5.49 (1H, m), 4.77 (1H, m), 4.55 (1H, m). VIIb was hydrolyzed by 5% KOH–EtOH at room temperature to give dihydroagnostosterol (VIIa), mp 156–157⁰ (from MeOH), [α]D +71.8⁰ (c = 0.98) (lit.18) mp 158–159⁰, [α]D +67.5⁰. UV λmax μ (log ε): 236 (4.17), 244 (4.23), 251 (4.07). IR νmax cm⁻¹: 3290 (OH), 810 (νC=CH–). NMR: 9.44–9.01 (24H), 6.78 (1H, m), 4.70 (1H, m), 4.55 (1H, m).

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