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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-hydroxylanosta-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[1] 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).

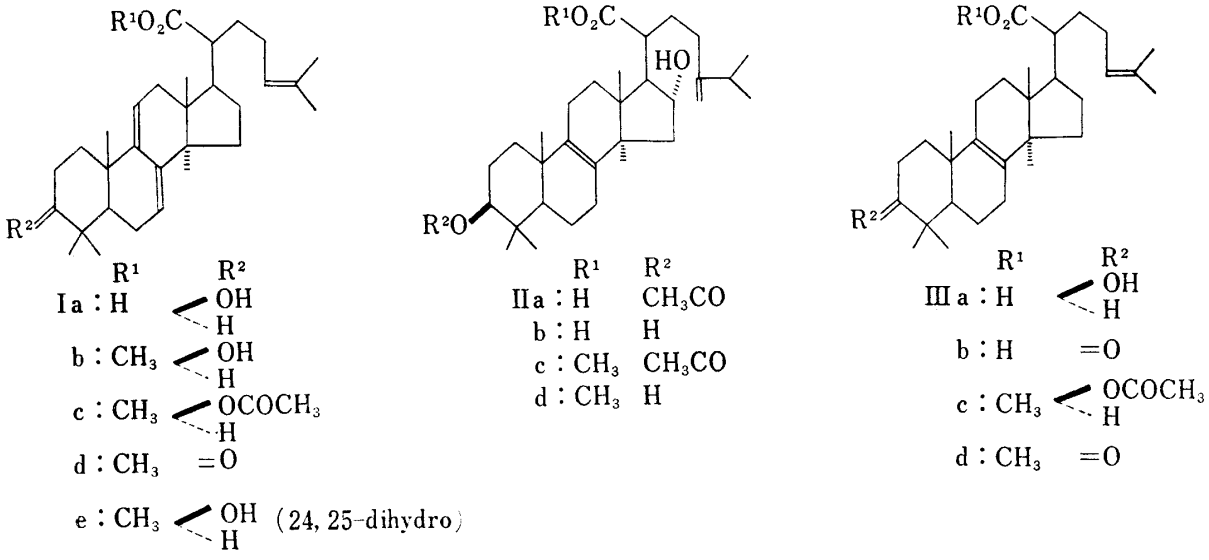

Chart 1

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 diazomethane 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°, $[\alpha]_D$+42° ($CHCl_3$), and methyl tumulosate[5] (IId) were thus separated in the respective yields of 0.10, 0.008, and 0.004%.

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The molecular formula of the methyl ester (Ib) was established as $C_{31}H_{48}O_3$ 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 monoacetate (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 posseses five methyls[6] and one isopropylidene group (τ 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 τ) 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 mμ (log ε, 4.1, 4.2, 4.0), indicating the presence of a conjugated heteroannular diene system and being consistent with those of lanosta-7,9(11)-diene series.[10]

The methyl ester (Ib) was resistant to hydrolysis. The parent acid, mp 257—259°, $[\alpha]_D$ +105° ($CHCl_3$), 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 triterpene carboxylic acids of lanostane series,[10] Ia 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 τ, indicating the presence of 3β-hydroxyl.

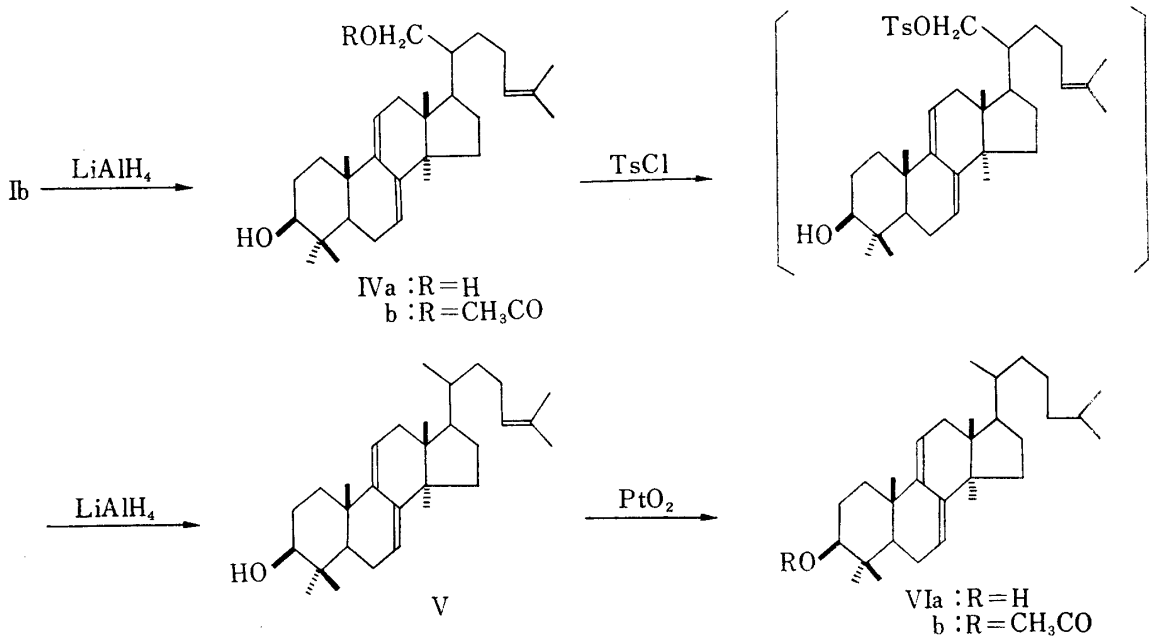

Chart 2

6) One of the methyl signals generally appears at a higher field (*ca.* 9.42 τ) and is assigned as 18-methyl group in lanosta-7,9(11)-diene series by the comparison with those in Ib-Ie, IVa, IVb, VIa, VIb, methyl dehydrotumulosate,[7] methyl polyporenate C,[8] 3-oxolanosta-7,9(11),24-trien-26-oic acid.[9]
7) Unpublished data of our laboratory.
8) Private communication from Prof. H. Inouye, Kyoto University.
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These observations lead to the assumption that the acid (Ia) must be 3β-hydroxylanosta-7,9(11),24-trien-21-oic acid (Ia). The corresponding 8,24-dienoic acid (IIIa), designated trametenolic acid, has been isolated from *Fomes hartigii* (Fr.) Allescher (=*Phellinus hartigii* (Allesch. et Schnabl.) Imazeki),[11] *Trametes odorata* (Wulf.) Fr. (=*Gloeophyllum odoratum* (Wulf. ex Fr.) Imazeki),[12] and *Lenzites trabea* Pers. ex Fr. (=*Gloeophyllum trabeum* (Pers. ex Fr.) Murr.)[13] and the 3-oxo-8,24-dienoic acid (IIIb), designated pinicolic acid A, from *Polyporus pinicola* Fr. (=*Fomitopsis pinicola* (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 acetyltrametenolate (IIIc) and methyl pinicolate A (IIId) 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 agnosterol (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 agnosterol (V). For direct comparison V was derived to the dihydro compound (VIa) and the acetate (VIb), both of which showed the identity with the specimens[16–18] prepared from lanosterol in every respects.

On the basis of these facts, the structure of the new acid has been elucidated as 3β-hydroxylanosta-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]

Experimental[20]

Methyl 3β-Hydroxylanosta-7,9(11),24-trien-21-oate (Ib)——The powderd sclerotia of *Poria cocos* (5 kg) were extracted with ether and the extract was concentrated to *ca.* 1 liter, from which crude pachymic acid (IIa) (3.5 g) separated out. After methylation and purification the methyl ester, mp 182—183°, $[\alpha]_D^{26}$ +38.9° (c=0.88) (lit.[4] mp 185—187°, $[\alpha]_D^{14}$ +41.5°), was identified with the authentic sample of methyl pachymate (IIc). The ethereal mother liquor was evaporated to dryness, washed with hexane, and methylated with CH_2N_2 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 $CHCl_3$ 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 $CDCl_3$ solution on a Varian Associates (100 Mc) and recorded in τ 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% H_2SO_4 or vanillin-phosphoric acid. Gas chromatography was carried out on 1.8% SE-30 or 1.3% OV-17 column at 250° on a Hitachi F6-D gas chromatograph. Unless otherwise specified alumina (Woelm, neutral) and silica-gel (Mallinckrodt) 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 tumulosate (IId) (0.2 g), mp 162—163° (from MeOH), $[\alpha]_D^{26}$ +24.1° (c=0.79) (lit.[4] mp 165—167°, $[\alpha]_D^{14}$ +23.6°), which was identified with the authentic sample, and the methyl ester (Ib) of the new acid (0.4 g), methyl 3β-hydroxylanosta-7,9(11),24-trien-21-oate (Ib), as colorless needles of mp 142—142.5° (from MeOH), $[\alpha]_D^{24}$ +42° (c=1.0). *Anal.* Calcd. for $C_{31}H_{48}O_3$: C, 79.43; H, 10.32. Found: C, 79.26; H, 10.11. UV λ_{max} mμ (log ε): 237 (4.08), 244 (4.15), 252 (3.97). IR ν_{max} cm^{-1}: 3310 (OH), 1725 (C=O), 810 (>C=CH–). NMR: 9.42 (3H, s), 9.13 (6H, s), 9.05 (3H, s), 9.02 (3H ,s), 8.43 (3H, 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-Acetoxylanosta-7,9(11),24-trien-21-oate (Ic)——Acetylation of Ib (131 mg) with Ac_2O (1 ml) in pyridine (1 ml) at room temperature overnight gave Ic as colorless needles (117 mg) of mp 144—145° (from MeOH), $[\alpha]_D^{24}$ +53.7° (c=0.7). *Anal.* Calcd. for $C_{32}H_{50}O_4$: 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 λ_{max} mμ (log ε): 237 (4.12), 244 (4.18), 253 (4.01). IR ν_{max} cm^{-1}: 1725, 1720 (C=O), 1245 (ester), 810 (>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 CrO_3-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 recrystallization from MeOH, afforded Id as colorless needles (140 mg) of mp 155—156°, $[\alpha]_D^{19}$ +28.8° (c=0.6). *Anal.* Calcd. for $C_{31}H_{46}O_3$: C, 79.78; H, 9.94. Found: C, 79.23; H, 9.88. Mass Spectrum *m/e*, 466 (M^+, 100), 451 (14), 311 (24), 309 (48), 295 (29), 270 (48), 269 (48), 257 (20), 256 (18), 244 (19). UV λ_{max} mμ (log ε): 237 (4.17), 244 (4.24), 253 (4.06), 290 (1.7). IR ν_{max} cm^{-1}: 1723, 1705 (C=O), 810 (>C=CH–). NMR: 9.36 (3H, s), 9.11 (3H, s), 8.91 (3H, s), 8.87 (3H, s), 8.81 (3H, s), 8.42 (3H, s), 8.32 (3H, s), 6.35 (3H, s), 4.96 (1H, m), 4.65 (1H, m), 4.50 (1H, m).

Methyl 3β-Hydroxylanosta-7,9(11)-dien-21-oate (Ie)——Ib (16 mg) in EtOH (15 ml) was hydrogenated in the presence of Pt-catalyst. Recrystallization from MeOH gave Ie as colorless neeldes (11 mg) of mp 140—142°. UV λ_{max} mμ (log ε): 237 (4.01), 244 (4.08), 253 (3.92). IR ν_{max} cm^{-1}: 3400 (OH), 1720 (C=O), 808 (>C=CH–). NMR: 9.42 (3H, s), 9.17 (6H, d, J=6 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-Hydroxylanosta-7,9(11),24-trien-21-oic Acid (Ia)——Ib (36 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—259° (from ether-isopropanol), $[\alpha]_D^{21}$ +105° (c=0.1). UV λ_{max} mμ (log ε): 236 (4.18), 243 (4.22), 252.5 (4.05). IR ν_{max} cm^{-1}: 3400 (OH), 1725, 1700 (C=O), 805 (>C=CH–).

Methylation of Ia with CH_2N_2 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 $NaBH_4$ (20 mg) in water (2 ml) and dioxane (2 ml). After 1 hr the excess amount of $NaBH_4$ was decomposed by the addition of AcOH and the product was extracted with $CHCl_3$ and chromatographed on alumina. The elution with ether followed by recrystallization from MeOH afforded Ib as colorless needles (34 mg) of mp 141—142°. The identity was established by a mixed fusion, IR, TLC, and GLC.

Lanosta-7,9(11),24-trien-3β,21-diol (IVa)——Ib (160 mg) in ether (anhyd., 100 ml) was added dropwise with stirring at room temperature into $LiAlH_4$ (400 mg) suspended in ether (anhyd., 20 ml) and the mixture was refluxed for 1 hr. The excess amount of $LiAlH_4$ was destroyed by wet ether. After acidification with dil. HCl the mixture was extracted with ether, washed with water, and dried over Na_2SO_4. 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° (from MeOH), $[\alpha]_D^{28}$ +68° (c=0.40) (lit.[15] mp 194—196°, $[\alpha]_D$ +72°). UV λ_{max} mμ (log ε): 237 (4.00), 244 (4.08), 252 (3.91). IR ν_{max} cm^{-1}: 3300 (OH), 810 (>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-diacetoxylanosta-7,9 (11),24-triene (IVb), mp 119—120° (lit.[15] mp 122°).

Conversion of Lanosta-7,9(11),24-trien-3β,21-diol (IVa) into Dihydroagnosterol (Lanosta-7,9(11)-dien-3β-ol) (VIa)——The reaction condition for the partial tosylation at C-21 of IVa was examined by TLC of the ethereal extract of the reaction products using the $SiHF_{254}$ 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 tosylates (ν_{max} cm^{-1}: 3400 (OH), 1600, 1363, 1175, 825 (*p*-Ts-O), 810 (>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 ethereal extract (53 mg), showing the presence of the monotosylate as the main component, was dissolved in tetrahydrofuran (anhyd., 20 ml) and added with $LiAlH_4$ (200 mg). The mixture was kept standing at room temperature for 2 days and the excess amount of $LiAlH_4$ 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 Na_2SO_4, 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 $SiHF_{254}$ 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—151°, showing the same *Rf* value with lanosterol in TLC and being assumed to be inpure agnosterol (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—156°. UV λ_{max} cm^{-1} (log ε): 237 (4.08), 244 (4.15), 253 (3.98). IR ν_{max} cm^{-1}: 3290 (OH), 810 (>C=CH–). The identity with the authentic sample of dihydroagnosterol (VIa) was established by a mixed fusion, TLC, and GLC.

Acetylation of the product (0.9 mg) with Ac_2O (0.2 ml) in pyridine (0.2 ml) gave the acetate, colorless needles (0.7 mg) of mp 168—170°. IR ν_{max} cm^{-1}: 1720 (C=O), 1248 (ester), 813 (>C=CH–). The identity with the authentic sample of dihydroagnosteryl acetate (VIb) was confirmed by a mixed fusion, TLC, and GLC.

Preparation of Dihydroagnosterol (VIa)——Dehydrogenation of dihydrolanosteryl acetate with selenium dioxide[16] followed by the purification through an alumina column and recrystallization from MeOH gave dihydroagnosteryl acetate (VIb), mp 168—169°, $[\alpha]_D^{22}$ +92° (c=0.96) (lit.[17] mp 167—168°, $[\alpha]_D$ +88°). UV λ_{max} mμ (log ε): 236 (4.16), 244 (4.23), 252 (4.05). IR ν_{max} cm^{-1}: 1720 (C=O), 1248 (ester), 813 (>C=CH–). NMR: 9.44—9.06 (24H), 7.97 (3H, s), 5.49 (1H, m), 4.77 (1H, m), 4.55 (1H, m).

VIb was hydrolysed by 5% KOH–EtOH at room temperature to give dihydroagnosterol (VIa), mp 156—157° (from MeOH), $[\alpha]_D^{22}$ +71.8° (c=0.98) (lit.[18] mp 158—159°, $[\alpha]_D$ +67.5°). UV λ_{max} mμ (log ε): 236 (4.17), 244 (4.23), 251 (4.07). IR ν_{max} cm^{-1}: 3290 (OH), 810 (>C=CH–). NMR: 9.44—9.01 (24H), 6.76 (1H, m), 4.70 (1H, m), 4.55 (1H, m).

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