TRITERPENES AND STEROIDS FROM *Ganoderma applanatum**

Jiří Protiva[a], Hana Skorkovská[a], Jiří Urban[b] and Alois Vystrčil[a]

[a] *Department of Organic Chemistry, Charles University, 128 40 Prague 2 and*
[b] *Institute of Microbiology, Czechoslovak Academy of Sciences, 142 20 Prague 4*

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Five already described triterpenes and steroids were isolated from the fungus *Ganoderma applanatum*. Its main components are friedelin, ergosta-7,22-dien-3-one and ergosta-7,22-dien-3β-ol, which are accompanied by small amounts of friedoolean-5-en-3-one and ergosterol. Palmitic acid is a further component.

The components of the light petroleum extract of the fungus *Ganoderma applanatum* were already investigated by several groups of research workers. Thus, Petit and Knight[1] proved the presence of ergosta-7,22-dien-3β-ol (*I*) and ergosta-7,22-dien-3-one (*II*), while Strigina and coworkers[2] detected isomeric ergosta-7,16-dien-3β-ol (*III*) in addition to the dienol *I*. However, the existence of dienol *III* was contradicted by Ripperger and Budzikiewicz[3] who identified the substances isolated by mass spectrometry and chemical conversion as being *I* and *II*, in agreement with the ref.[1]. The papers cited do not specify the material employed (vegetation phase, locality, *etc.*), but state concordantly that the isolation of possible additional substances from the light petroleum extract was unsuccessful. Therefore we considered it purposeful to investigate the contradiction in the facts mentioned.

According to thin layer chromatography the ethanolic extract of the fungus contains 8 substances. Column chromatography of the extract on silica gel gave by gradual elution the following components: The substance isolated in 1·13% yield was identified as alnusenone (friedoolean-5-en-3-one; *IV* (ref.[4,5]) on the basis of its melting point, optical rotation, IR and mass spectrum. The next component, isolated in 6% yield, was found identical with friedelin (*V*) on the basis of identical IR and mass spectra, m.p. and optical rotation with an authentic sample. For further characterization this substance was also reduced with lithium aluminum hydride to the known alcohols *VI* and *VII*, ref.[6–8]). Further elution gave steroids *II* (5·9%) and *I* (24·5%), identical with the substances[1,3] identified already earlier in *G. applanatum*. In the ^{13}C-NMR spectrum of ketone *II* the assignment of the carbon atoms was done on the basis of known analogies[9]. Derivative *I* was hydrogenated selectively

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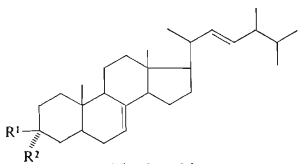
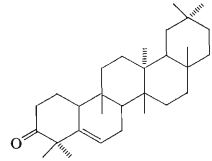
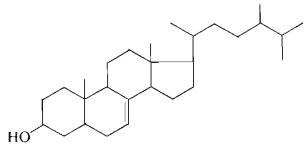


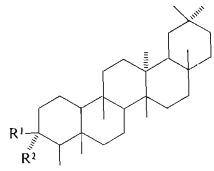
I, $R^1 = OH$, $R^2 = H$
II, $R^1 + R^2 = O$



IV



IX



V, $R^1 + R^2 = O$
VI, $R^1 = OH$, $R^2 = H$
VII, $R^1 = H$, $R^2 = OH$

on tris(triphenylphosphine)rhodium chloride to fungisterol *IX*, with the saturated side chain. From further eluate palmitic acid (6·6%) was isolated and identified by direct comparison with an authentic specimen, *i.e.* on the basis of IR and mass spectra[10]. The methyl ester prepared from it was also identical with an authentic specimen. The structure of ergosterol (*VIII*; 4·1%) was assigned to the most polar substance on the basis of comparison with an authentic sample.

Totally, 6 substances were identified (*i.e.* 50% of the light petroleum extract), but the substance with the properties of compound *III* could not be isolated.

EXPERIMENTAL

The melting points were measured on a Kofler block and they were not corrected. Optical rotation was measured in chloroform on a polarimeter ETL-NPL (Bendix–Ericsson) with a ±2% accuracy. The infrared spectra were measured in chloroform on a UR 20 (Zeiss, Jena) instrument. ^1H and ^{13}C-NMR spectra were measured on a Jeol FX-60 instrument in deuteriochloroform, chemical shifts are in δ-scale. The mass spectra were measured on a Varian MAT 311 spectrometer, energy of ionizing electrons 70 eV, ionizing current 1 mA, ion source temperature 200°C, temperature of direct inlet system 80—160°C.

Extraction of *Ganoderma applanatum*

The material employed was collected in south-western Bohemia peak Čkyně, in March and April.

a) The dry fungus (710 g), crushed to small pieces, was extracted with ethanol (4 l) at room temperature for 14 days. The extract was evaporated (6·2 g) and the residue extracted with boiling light petroleum (200 ml, b.p. 30—70°C) for 16 h; the extract was filtered and evaporated to give 4·9 g of an oil.

b) The dry fungus (620 g), crushed to small pieces, was extracted with boiling light petroleum (4 l) for 40 h. The extract obtained was evaporated (4·84 g of brown oil). Both extracts had identical composition according to TLC.

Separation of the Components

The oil obtained (4·84 g) was chromatographed on a column of silica gel (250 g) with light petroleum. After elution of 2 l with light petroleum, it was continued with light petroleum–benzene mixture (9 : 1). As first two unpolar substances were eluted, which could not be obtained in pure state owing to the scarcity of the material.

Further elution gave 55 mg of alnusenone (*IV*), m.p. 230—233°C (light petroleum), $[\alpha]_D$ +24° (*c* 0·05); lit.[4] gives m.p. 247°C, $[\alpha]_D$ +31°. IR spectrum: 1712 cm^{-1} (C=O); mass spectrum: *m/e* (%): 424 (M^+, 12, $C_{30}H_{48}O$), 409 (6, $C_{29}H_{45}O$), 274 (55, $C_{20}H_{34}$), 259 (37, $C_{19}H_{31}$), 205 (36), 69 (100).

The next fraction contained friedelin (*V*, 292 mg), m.p. 255—257°C (light petroleum), $[\alpha]_D$ —25° (*c* 1·2); lit.[6] m.p. 262—263°C, $[\alpha]_D$ —21°. IR spectrum: 1712 cm^{-1} (C=O). Mass spectrum: *m/e* (%): 426 (M^+, 4, $C_{30}H_{50}O$), 411 (3, $C_{29}H_{47}O$), 341 (2, $C_{25}H_{41}$), 302 (5, $C_{21}H_{34}O$), 273 (12, $C_{19}H_{29}O$), 205 (13), 69 (100).

From further fractions ergosta-7,22-dien-3-one (*II*) (290 mg) was obtained, with m.p. 172 to 174°C (light petroleum), $[\alpha]_D$ +5° (*c* 0·6); lit.[3] m.p. 178°C, $[\alpha]_D$ +4·1°. The mass spectrum is in accordance with the literature[3]. ^{1}H-NMR spectrum: 0·58; 0·78; 0·87; 0·97; 1·03; 1·08 (6 × CH_3), 5·14—5·25 m (3-H). ^{13}C-NMR spectrum: $C_{(1)}$ 38·7; $C_{(2)}$ 38·1; $C_{(3)}$ 211·9; $C_{(4)}$ 44·2; $C_{(5)}$ 42·8; $C_{(6)}$ 29·6; $C_{(7)}$ 117·0; $C_{(8)}$ 139·6; $C_{(9)}$ 48·9; $C_{(10)}$ 34·4; $C_{(11)}$ 21·7; $C_{(12)}$ 39·2; $C_{(13)}$ 43·3; $C_{(14)}$ 55·0; $C_{(15)}$ 22·9; $C_{(16)}$ 28·1; $C_{(17)}$ 55·9; $C_{(18)}$ 12·1; $C_{(19)}$ 12·3; $C_{(20)}$ 40·4; $C_{(21)}$ 21·0; $C_{(22)}$ 135·5; $C_{(23)}$ 132·0; $C_{(24)}$ 42·8; $C_{(25)}$ 33·0; $C_{(26)}$ 19·6; $C_{(27)}$ 19·9; $C_{(28)}$ 17·5 ppm.

Further elution gave palmitic acid (320 mg). Mass spectrum: M^+ 256, in accordance with the literature[10].

Elution with a mixture of benzene and ether (9 : 1) gave ergosta-7,22-dien-3β-ol (*I*), which is the main component of the separated mixture (1·2 g), m.p. 165—167°C (benzene–ethanol), $[\alpha]_D$ —20° (*c* 0·56); ref.[3] m.p. 168—172°C, $[\alpha]_D$ —20°C. The mass and the ^{1}H-NMR spectrum were in accordance with the literature[3]. The ^{13}C-NMR spectrum was in accordance with lit.[9].

Elution with ether gave eventually ergosterol (*VIII*, 200 mg) m.p. 142—145°C, $[\alpha]_D$ —120° (*c* 0·62). Mass spectrum *m/e* (%): 396 (M^+, 59, $C_{28}H_{44}O$), 381 (5), 378 (8, $C_{28}H_{42}$), 363 (32, $C_{27}H_{39}$), 337 (18, $C_{25}H_{37}$), 271 (13, $C_{19}H_{27}O$), 253 (22, $C_{19}H_{25}$), 69 (100).

Reduction of Friedelin

Friedelin (50 mg) was dissolved in ether (10 ml) and refluxed with lithium aluminum hydride (50 mg) for 2 h. After working up in the conventional manner, the mixture obtained was separated by thin layer chromatography on silica gel. The β-hydroxy derivative obtained (10 mg) had m.p. 271—275°C, $[\alpha]_D$ +19° (*c* 0·1). Mass spectrum: M^+ 428; lit.[6] m.p. 275—276°C, $[\alpha]$ +12. 3α-Hydroxy derivative had m.p. 301—304°C, $[\alpha]_D$ +21° (*c* 0·15); lit.[7] gives m.p. 304°C, $[\alpha]_D$ +18°.

Hydrogenation of Ergosta-7,22-dien-3β-ol (I) on Tris(triphenylphosphine)rhodium Chloride

Dienol *I* (50 mg) was dissolved in 10 ml of benzene with 10 mg of $((C_6H_5)_3P)_3RhCl$ and shaken with hydrogen for 16 h. The solution was filtered through a silica gel column which was then washed with ether. Crystallization of the residue from chloroform–methanol gave 24 mg of fungisterol *IX*, m.p. 146°C, $[\alpha]_D$ —5° (*c* 0·43); lit.[11] gives m.p. 149°C, $[\alpha]_D$ —1°.

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