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STEROID METABOLITES OF *GANODERMA APPLANATUM BASIDIOMYCETE*

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Abstract-Examination of the basidiomycete ***Ganoderma applanatum*(Fr.)** Pat ***(Fomes applanatus*** Gill) has disclosed the presence of a mixture of **(24S)-24-methyl-5α-cholest-7-ene-3β** 01 **(Ia)** and a new compound **(IIa).** Spectral and chemical evidence indicate that **IIa** is **(24S)-24-methyl-5α-cholest-7,16-dene-3β-ol.** The latter is the first natural sterol with a A 16-double bond.

RESULTS AND DISCUSSION

Ganoderma applanatum (Fr.) Pat. (***Fomes applanatus*** Gill) is a common basidiomycete parasitic on larchwood. According to **Bauchet,**[1] G. ***applanatum*** contains substances which have a stimulatory effect on living organisms, but unlike the similar ***Ganoderma lucidum*** (Fr.) Harst (***Polyporus lucidus*** **Fr.),**[2] G. ***applanatum*** does not contain ergosterol.

Following chromatography on Al_2O_3 and SiO_2–$AgNO_3$ and crystallization from methanol, the m.p. of the main component from the methanol extraction of the fungus, G_1, was 166–169·5°. Two absorption bands, specific for heteroannular (λ_{max}^{dioxan} 237, 245 and 251 nm) and homoannular (λ_{max}^{dioxan} 273, 284 and 296 nm) steroid diene systems, were detected in the UV spectrum of G,. The very low intensity of both bands indicated that this absorption is caused by an impurity of, probably, **7,9(II)-** and **5,7-dienes** (~ l-3 %). The 3620 cm^{-1} band in the IR spectrum of G_1 shows the presence of an **OH-**group, this being corroborated by the formation of an acetate (m.p. 168-170").

The MS shows G_1 to be a mixture of two components with mol. wt. **400 (Ia)** ~ 30 % and 398 **(IIa)** ~ 70 % respectively. The molecular ion fragmentation pattern is indicative that **Ia** and **IIa** are C_{28}**-steroids** with a methyl-isooctyl side chain (C_9H_{19}) which corresponds with the structural formulas **Ia** ($C_{28}H_{48}O$) and **IIa** ($C_{28}H_{46}O$). **Ia** and **IIa** contain one and two double bonds respectively. According to the UV spectrum, bonds in **IIa** do not form a diene system.

Hydrogenation of G_1 in ethyl acetate with the **Willstätter** catalyst led to the formation of **IIIa,** m.p. 132-134" **(MeOH).** IR **($CHCl_3$)**: 3620 cm^{-1}. NMR: **CH_3-19*s*** δ 0·68; **CH_3-18*s*** δ 0·85; **CH_3-21*d*** δ O-91; (CH,), CH-d δ O-83; and **CH_3-24*d*** S 0·75 ppm. The chemical shifts of the proton signals of **CH_3-18** and **CH_3-19** were due to the influence of the A **8(14)** double bond and **3β(OH),** and coincide with **theory.**[3] The corresponding acetate **IIIb** m.p. was 106-109" **(MeOH).** IR **(KBr)**: 1740 cm-' **(CH_3COO). IIIa** was identified by mixed melting point with (24 **S)-24-methyl-5α-cholest-8(14)-ene-3β-ol** (A **8(14)-ergostenol),**[4] obtained on hydrogenation of ergosterol under similar conditions. The MS for **IIIa** and A **8(14)-ergostenol** are identical.

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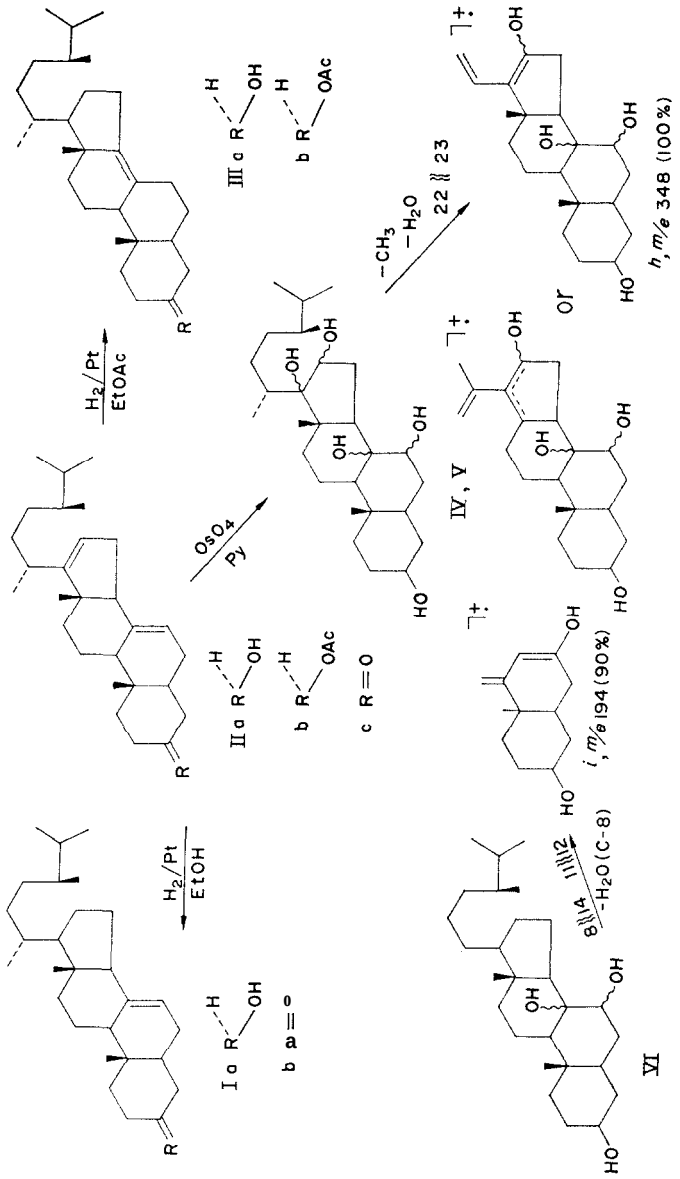
H2/Pt
EtOH
H2/Pt
EtOAc
OsO4
Py
-CH3
-H2O
22 ⇌ 23
-H2O(C-8)
I a R = H, OH
b a = O
II a R = H, OH
b R = H, OAc
c R = O
III a R = H, OH
b R = H, OAc
IV, V
or
h, m/e 348 (100%)
i, m/e 194 (90%)
VI

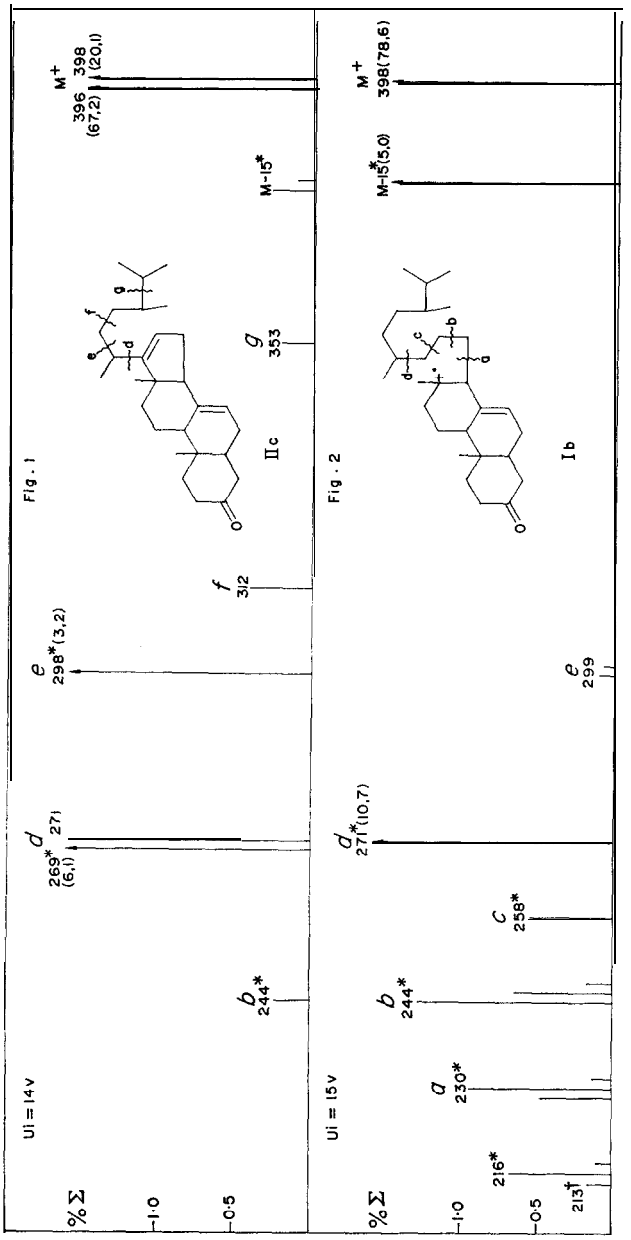

FIG. 1. MASS SPECTRUM IIc. FIG. 2. MASS SPECTRUM Ib.

* The appearance of this fragment from M^+ is corroborated by a corresponding metastable peak in the spectra at 20V.

† All the peaks except for *m/e* 213 in the spectra to the 2,2,4,4-d-analogs, shift by 4 m.e. upwards. The cited metastable peak values correspond to molecular ion fragmentation pattern.

The evidence for the double bond loci in Ia and IIa is based on the study of the MS of the starting compounds and their derivatives. Due to the low content of Ia in the mixture (~ 30 per cent) and lesser ionization efficiency, at 14 V the MS practically corresponds to IIc, the oxidation product of G_1 (Fig. 1). Its main fragments reflect the presence of the A 16-double bond, which is very seldom found in steroids of natural origin.' This is in agreement with the appearance in the m.s. recorded at 40 V of the peak with m/e 269 (100 per cent) corresponding to the polycyclic system ion *d*.[6] The A 16-double bond is additionally corroborated by the MS for IV (m.p. 181-182~5") and V (m.p. 172–174°), recorded at 30 V. The above two are the main hydroxylation products of G_1 with OsO_4. In the practically identical MS for IV and V, apart from the M+-466 molecular ion peaks, peaks with *m/e* 348 (100 per cent) are present. The latter correspond to ion *h*, the formation of which assumes the presence of the 16,17-α-glycol group in the initial compounds IV and V.

The positions of the unhydrogenated double bond in Ia and IIa, after correlation with A 8(14)-ergostenol, may be assigned to rings B/C. Of the three possible positions-A 8(9), A 8(14) and A 7(8)—the last is the most probable according to the NMR spectrum of G_1, in which the chemical shifts of the angular CH_3 group signals do not coincide with the signals of A 8(14)-ergostenol. G_1 NMR: CH_3-19*s* δ 0.76; CH_3-18*s* δ O-56; =CH *m* δ 5·08–5·2 ppm. Consequently, hydrogenation in ethyl acetate is accompanied by A 7-bond migration to the A 8(14) locus. Indeed, hydrogenation in ethanol leads to Ia (m.p. 145·5–147°).[7] NMR: CH_3-19*s* δ 0.8; CH_3-18*s* δ O-53; (CH,), CH*d* δ 0·86; CH_3-21*d* δ 0·88; CH_3-24*d* δ 0·76; =CH *m* δ 5·2 (IH) ppm. The proton signal shift in the angular CH_3 groups is due to the influence of the A 7-bond and 3β(OH), and is in agreement with the theoretical.[3] Oxydation of Ia with CrO_3 in pyridine gave Ib, m.p. 159–161° (Me-CO-Me). IR ($CHCl_3$): 1715 cm^{-1}. NMR: CH_3-19*s* S 1·00; CH_3-18*s* S 0·56; $(CH_3)_2$CH*d* S 0·83; CH_3-21*d* S 0.90; CH_3-24*d* δ *0.76;* ==CH *m* S 5·1 (IH) ppm. In the m.s. of Ib (Fig. 2), peaks of the a, ***b, c,*** and ***d*** fragments are present, characteristic for C-17 substituted steroids. A marked intensity of the ***d*** ion peak, *m/e* 271, corroborates the A 7(8) double bond.[8,9] Moreover, in the MS of the minor oxidation product of G_1 with OsO_4 (VI), corresponding to hydroxylation of Ia, there is another peak, second in intensity *(m/e* 194), apart from the M+-434 peak. This lower peak corresponds to ion *i*, the formation of which assumes the presence of a A 7,8-glycol group,

Thus, G_1 is a chromatographically inseparable mixture of two compounds, for which, based on the above evidence, the following structures are proposed: (24S)-24-methyl-5α-cholest-7-ene-3β-ol (Ia) and (24S)-24-methyl-5α-cholest-7,16-di-ene-3β-ol (IIa). The latter is the first sterol with a A 16-double bond, isolated from natural sources. Furthermore, a minor component termed G_2 (m.p. 168-170") was isolated from the methanol extract. The mass spectrum for G_2 showed, besides the M^+430 peak, the main peaks to be with *m/e* 412 and 398 respectively. Investigation of the structure of G_2 is now under way.

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EXPERIMENTAL

GLC on silica gel was used for analysing fractions. The following developing solvent systems were used: (a) hexane–EtOAc (1: 1), (b) hexane–EtOAc (1: 9). Saturated solution of $SbCl_3$ was used for spot detection. Silica gel 'KSK' (200–270 mesh) and aluminium oxide (activity II) were used for chromatography. The following solvent systems with gradient elution were used as eluants in column (c) hexane–EtOAc (1:0 → 2:1), (d) hexane–EtOAc (1:0 → 3:1), (e) hexane–EtOAc (1:0 → 0:1). MS for all substances were recorded on a 'MX-1303' spectrometer. Samples were admitted into the ionization chamber using the direct insertion technique.

Isolation of G_1 and G_2. *2* kg of grounded fruit body of G. ***applanatum*** was extracted with MeOH. The residue after the evaporation of MeOH extract (50 g) was chromatographed on alurninium oxide and then on silica gel impregnated with $AgNO_3$ [10] in system C. After chromatography and double crystallization 1·2 g of G_1 m.p. 166–169·5° (MeOH) and 0·05 g of G_2 m.p. 168-170" (MeOH) were obtained. The acetylation of the G_1 in the usual way led to acetate with m.p. 168-170" (MeOH) after chromatography on silica gel in system D.

Hydrogenation of G_1. (a) G_1 (0·1 g) was hydrogenated in EtOAc in the presence of Willstätter catalyst and the product chromatographed on silica gel; elution with system C. Separation afforded IIIa m.p. 132-l 34" (MeOH). The acetylation of IIIa in the usual way after chromatography on silica gel in system D led to acetate IIIb m.p. 106-108" (MeOH). (b) G_1 (0·1 g) was hydrogenated in EtOH as in (a). The reaction product after chromatography on silica gel; elution with system C, gave Ia m.p. 145.5-147" (MeOH).

Chromic acid oxidation of G_1. The solution of G_1 (0·45 g) in pyridine (20 ml) was combined with CrO_3 (0·45 g) in pyridine (25 ml) and the mixture was kept for 24 hr at room temp. Then 0·15 g of CrO_3 in 15 ml of pyridine was added and the mixture was left for another 24 hr. The reaction mixture was poured into H_2O and extracted with Et_2O. The crude products were submitted to chromatography on silica gel using system C as eluant. The separation gave the product with m.p. 173–175·5° (MeCOMe).

Chromic acid oxidation of Ia. Ia (0·1 g) obtained after hydrogenation of G_1 in EtOH, was oxydized with CrO_3 in pyridine as above. The reaction product was chromatographed on silica gel ; elution with system C. The separation gave Ib m.p. 159-161" (Me-CO-Me).

OsO_4 oxidation of G_1. G_1 (0·15 g) was dissolved in dry pyridine (10 ml), the soln. was added dropwise to a soln. of OsO_4 (0·4 g) in 10 ml of pyridine and the mixture left for 7 days at room temp. The pyridine was evaporated under reduced pressure, the residue treated with mannitol (1.25 g), KOH (2 g), EtOH (10 ml), benzene (5 ml), H_2O (5 ml) and the mixture was heated under reflux for 8 hr. The reaction mixture was dissolved in H_2O and extracted with $CHCl_3$. The crude product was chromatographed on silica gel; elution with system E. The separation gave IV (0·03 g) m.p. 181–182·5° (EtOAc), V (0.02 g) m.p. 172-174" (EtOAc) and VI (trace amount).

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