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A LANOSTANE-TYPE STEROID FROM THE FUNGUS *GANODERMA CARNOSUM*

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Key Word Index—*Ganoderma carnosum*, Ganodermataceae, steroids, lanostane, 26,27-dihydroxylanosta-7,9(11),24-trien-3,16-dione, carnosodione.

Abstract—A new lanostane-type triterpene, isolated from the fruiting body of the fungus *Ganoderma carnosum* together with ergosterol peroxide, ergosta-7,22-dien-3β-ol; oleic acid methyl ester and glycerol trioleate, was determined to be 26,27-dihydroxylanosta-7,9(11),24-trien-3,16-dione by spectroscopic methods. © 1997 Elsevier Science Ltd

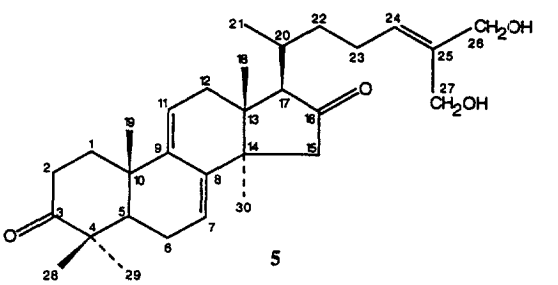
INTRODUCTION

Wood-rotting fungi are known to contain lanostane-type triperpenes, especially *Ganoderma lucidum* (Curt.:Fr.) Karst., which contains may polyoxygenated lanostane derivatives. This fungus, which grows in many different regions of the world, is widely used in Asian traditional medicine and regarded as a panacea for all types of diseases. Some of the lanostanes isolated from *G. lucidum* showed interesting properties, for example cytotoxic, antihepatotoxic or hypotensive activities [1]. *Ganoderma carnosum* Pat. (syn. *G. atkinsonii* Jahn, Kotl. and Pouz.), which is only found in Europe, is difficult to distinguish from *G. lucidum*. As *G. carnosum* has not yet been studied phytochemically, we describe here the isolation of a new constituent of this fungus, which has not been found in *G. lucidum*.

RESULTS AND DISCUSSION

Ground and lyophilized fruiting bodies of *G. carnosum* (300 g) were extracted successively with dichloromethane and methanol. the dichloromethane extract was fractionated on a silica gel column with a step-gradient of petrol–ethyl acetate, ethyl acetate and finally methanol to afford nine fractions (A–I). Further fractionation of B, F and I led to five compounds (**1**–**5**).

Compound **1** was identified as glycerol trioleate by comparison of its mass spectral, ^{1}H NMR and ^{13}C NMR data with literature data [2]. Oleic acid methyl ester (**2**) [3], ergosta-7,22-dien-3β-ol (**3**) [4] and ergosterol peroxide (**4**) [5] were identified by their spectral data and by direct comparison with authentic samples (co-TLC and co-HPLC).



5

The molecular formula $C_{30}H_{44}O_4$ of compound **5** was deduced from the ^{13}C NMR and DEPT spectra and confirmed by D/CI-mass spectrometry, in which the ammonium adduct ion $[M+NH_4]^+$ appeared at *m/z* 486. Furthermore, the EI-mass spectrum of compound **5** showed ions due to successive dehydrations at *m/z* 450 $[M-H_2O]^+$, 435 $[M-CH_3-H_2O]^+$ and 417 $[M-CH_3-2H_2O]^+$, indicating that **5** was a diol. The ^{1}H NMR spectrum exhibited five singlets attributable to tertiary methyl groups (δ 0.71, 1.02, 1.09, 1.13 and 1.22) and one doublet corresponding to one secondary methyl group (δ 0.97, *d*, *J* = 6.9 Hz). Two hydroxymethylene moieties (δ 4.20 and 4.32, *s*, 2H each) were also seen in the low field region. Finally, two triplets at δ 5.50 (1H) and 5.61 (2H) completed the spectrum. The ^{13}C NMR spectrum of **5** showed signals due to two carbonyl groups (δ 216.4 and 219.2), three olefinic quaternary carbons (δ 145.0, 139.9 and 137.3), three olefinic methines (δ 131.2,

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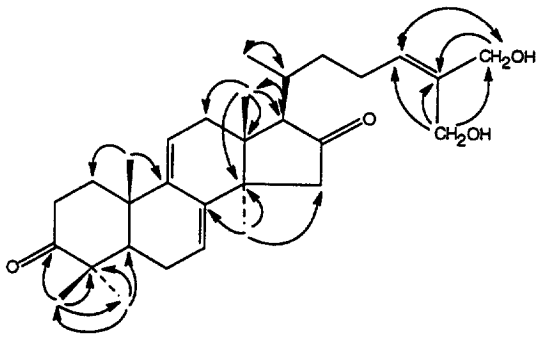

Fig 1. Slected correlations observed for compound **5** in ^{1}H-^{1}H DQF COSY ↔ abd ^{1}H-^{13}C FLOCK →

121.7 and 116.8), two hydroxymethylene groups (δ 67.7 and 60.5), one methine at δ 60.5 and one methylene at δ 47.0. The UV spectra showed characteristic absorption bands at 235, 242 and 252 nm, indicating the presence of a transoid heteroannular diene moiety. Comparison of the spectral data with those of known compounds [6] permitted the identification of a 3-oxo-lanosta-7,9(11),24-triene derivative having both hydroxyl groups at C-26 and C-27. The final structure was established by extensive 2D NMR experiments: ^{1}H-^{1}H COSY, ^{1}H-^{13}C HETCOR and ^{1}H-^{13}C FLOCK. The ^{1}H-^{1}H COSY spectral examination established the proton connections between C-20 and C-21, C-17 and C-18 and between C-23 and C-26. The assignments of the proton signals to their respective carbons were achieved by ^{1}H-^{13}C HETCOR analysis and the long range ^{1}H-^{13}C FLOCK spectrum clearly indicated correlations through two- or three-bond coupling (Fig. 1).

The ^{1}H-^{1}H COSY spectrum as well as the ^{1}H-^{13}C FLOCK long range correlations confirmed the positions of the two hydroxyl groups at C-26 and C-27. The position of the carbonyl group at δ 216.4. was also proved by long range correlation. The C-18 and the C-30 methyl groups showed long range correlation in the low field region with, respectively, one methine at δ 60.5 and one methylene at δ 47.0. These resonances are compatible with the presence of a vicinal carbonyl group.

Thus, the structure of **5** is 26,27-dihydroxylanosta-7,9(11),24-trien-3,16-dione, for which we propose the name carnosodione. Although C-16 oxygenated lanostanes are quite common within fungi [4], such compounds have not yet been described among the *Ganoderma* genus, despite the extensive investigations of *G. lucidum* [1]. For this reason, these derivatives might be of use as chemotaxonomic markers. Further studies to acertain this supposition has still to be done.

*,†: assignments may be reversed.

EXPERIMENTAL

General. TLC: Kieselgel 60F_{254} (Merck), detection with Godin reagent. Mp: Mettler-FP-80/82 hot stage apparatus, uncorr. UV: Varian DMS 100 spectrophotometer. ^{1}H and ^{13}C NMR: Varian VXR 200 at 200.06 and 50.03 MHz, respectively. EI-MS (70 eV) and D/CI-MS (NH_3, positive ion mode): Finnigan-MAT-TSQ-700 triple stage quadrupole instrument.

Fungal material. Mature carpophores of *Ganoderma carnosum* Pat. were collected in June 1993 near Neuchâtel, Switzerland, and identified by J. Keller, Institute de Botanique, Université de Neuchâtel, Switzerland. A dried voucher specimen is deposited at the Institut de Pharmacognosie et Phytochimie, Lausanne, Switzerland.

Extraction and isolation. Lyophilized fruiting bodies of *G. carnosum* (300 g) were extracted successively with CH_2Cl_2 and MeOH (3 × 3 l. each). The CH_2Cl_2 extract (9 g) was sepd on a Si 60 silica gel column with a step-gradient of petrol–EtOAc (1:0–95:5–9:1–7:1–5:1–3:1–1:1), EtOAc and finally MeOH to give nine frs (A–I). One portion (500 mg) of fr. B (2.4 g) was submitted to low pressure liquid chromatography (LPLC) on silica gel with petrol–EtOAc (49:1) to yield four frs (B1–B4). Further sepn of B3 by LPLC with *n*-hexane–$CHCl_3$ (6:1), followed by filtration on a Sephadex LH-20 column with $CHCl_3$–MeOH (1:1) afforded 84 mg of compound **1** and purification of B2 with *n*-hexane–$CHCl_3$ (6:1) by LPLC yielded 32 mg of compound **2**. One part (300 mg) of fr. F (900 mg) was sepd by LPLC with petrol–EtOAc (7:1) to give three frs (F1–F3). Fr. F3 was submitted to LPLC with petrol–EtOAc (3:1) to afford 18 mg of compound **3** and a final purification of fr. F2 gave 32 mg of compound **4**. Fr. I was sepd on an open column with a step-gradient of petrol–EtOAc (7:1–3:1–1:1–0:1) to give three frs (I1–I3). Fr. I2 was purified by LPLC with *n*-hexane–*iso*-PrOH (9:1) to yield 13 mg of compound **5**.

26,27-*dihydroxylanosta*-7,9(11),24-*trien*-3,16-*dione* (**5**). White amorphous powder, mp 136–139° UV (MeOH) λ_{max} nm (log ε): 235 (4.04), 242 (4.09), 252 (3.91). TLC: $CHCl_3$–*iso*-PrOH 9:1 $R_f = 0.29$. ^{1}H NMR ($CDCl_3$): δ 0.71 (3H, *s*, H-18), 0.97 (3H, *d*, $J = 6.9$ Hz, H-21), 1.02 (3H, *s*, H-30), 1.09 (3H, *s*, H-29), 1.13 (3H, *s*, H-28), 1.22 (3H, *s*, H-19), 1.78 (1H, *m*, H-20), 2.18 (1H, *m*, H-17), 4.20 (2H, *s*, H-26), 4.32 (2H, *s*, H-27), 5.50 (2H, *t*, H-7, H-11), 5.61 (1H, *t*, H-24). ^{13}C NMR ($CDCl_3$): 36.4 (C-1), 34.7 (C-2), 216.4 (C-3), 47.4 (C-4), 50.6 (C-5), 23.6 (C-6), 121.7 (C-7), 139.9 (C-8), 145.0 (C-9), 37.4 (C-10), 116.8 (C-11), 36.6 (C-12), 44.3 (C-13)*, 42.9 (C-14)*, 47.0 (C-15), 219.2 (C-16), 60.5 (C-17), 16.8 (C-18), 22.1 (C-19), 31.5 (C-20), 18.6 (C-21), 35.7 (C-22), 24.8 (C-23), 131.2 (C-24), 137.3 (C-25), 67.7 (C-26)†, 60.5 (C-27)†, 22.4 (C-28), 25.3 (C-29), 25.7 (C-30). EI – MS *m/z* (rel. int.): 450 (48), 435 (67), 417 (22), 311 (100), 269 (10), 157 (12), 106 (20). D/CI-MS *m/z*: 486 $[M+NH_4]^+$, 469 $[M+H]^+$, 451.

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REFERENCES

1. Jong, S. C. and Birmingham, J. M., *Advances in Applied Microbiology*, 1992, **37**, 101.
2. Della Greca, M., Mangoni, L., Molinaro, A., Monaco, P. and Previtera, L., *Gazetta Chimica Italia*, 1990, **120**, 391.
3. Gunstone, F. D., Pollard, M. R., Scrimgeour, C. M. and Vedanayagam, H. S., *Chemistry and Physics of Lipids*, 1977, **18**, 115.
4. Keller, A.-C., Maillard, M. P. and Hostettmann, K., *Phytochemistry*, 1996, **41**, 1041.
5. Magnus, V., Lacan, G., Aplin, R. T. and Thaller, V., *Phytochemistry*, 1989, **28**, 3047.
6. Nishitoba, T., Oda, K., Sato, H. and Sakamura, S., *Agriculture and Biological Chemistry*, 1988, **52**, 367.