New Lanostane Triterpene Lactones from the Vietnamese Mushroom
Ganoderma colossum (Fr.) C. F. Baker

Riham Salah El Dine, a Ali Mahmoud El Halawany, a Norio Nakamura, b Chao-Mei Ma, a and Masao Hattory * a

 a Institute of Natural Medicine, University of Toyama; 2630 Sugitani, Toyama 930-019, Japan; and b Faculty of Pharmaceutical Sciences, Doshisha Women’s College of Liberal Arts; Kodo, Kyotanabe, Kyoto 610–0395, Japan.

Received November 21, 2007; accepted February 20, 2008; published online February 21, 2008

Four new lanostane tetraterpene lactones (colossolactone I, colossolactone II, colossolactone III and colossolactone IV) were isolated from the Vietnamese mushroom Ganoderma colossum (Fr.) C. F. Baker along with five known compounds. The structures of the new compounds were determined on the basis of MS, NMR and circular dichroism.

Key words Ganoderma colossum; Ganodermaeae; lanostane tetraterpene lactone; colossolactone

The fungal family Ganodermataceae is represented by more than 200 species, which mostly occur in subtropical and tropical regions. The fruiting bodies of Ganoderma species have been widely used in traditional Chinese, Japanese and Korean medicine to treat a variety of conditions from ancient time. 1–3 Interesting biological activities have been observed in these mushrooms; Ganoderma lucidum Karst. showed cytotoxic and antiviral activities, 4,5 Ganoderma colossum (Fr.) C. F. Baker showed anti-inflammatory, cytotoxic and antimicrobial activities. 6,7,8 Ganoderma applanatum (LeCyss. ex Fr.) Karst. 9 revealed antibacterial and aldose reductase inhibitory activities, Ganoderma concinnum RYV. Nov. sp. induced apoptosis in human promyelocytic leukemia HL-60 cells, 10 and Ganoderma pfeifferi Bres. showed antimicrobial activity. 11,12 These basidiomycetes are known to be prolific producers of lanostane type triterpenoids, and over 130 such compounds have been recognized from the genus Ganoderma. Colossolactones were isolated previously from the Vietnamese mushroom G. colossum, such triterpenoids being characterized by the presence of a six-membered α,β-unsaturated δ-lactone group in their side chain with or without a seven membered lactone ring as the ring A, and this type of triterpenoids has not yet been reported previously from fungal metabolites. However, representatives of this structural type such as schisanlactones, kadsulactone A and lancilactones were isolated previously from the stems and roots of plants such as Schisandra species, 13 Kadsura heteroclita ROXB. 14 and Kadsura lancilimba How 15 which are used as folk medicines for the treatment of rheumatism, stomachache and enterogastritis. 16–18 In a hope to isolate new secondary metabolites from the Vietnamese mushroom, which are effective for inhibiting viral protease from human immunodeficiency virus (HIV) and hepatitis C virus (HCV), we investigated phytochemically a triterpene fraction from the fruiting bodies of the cultivated G. colossum.

Results and Discussion

Repeated column chromatography (CC) of a methanol-soluble fraction of the chloroform extract of the fruiting bodies of G. colossum after defatting led to the isolation of four new lanostane triterpene lactones called colossolactone I (1), colossolactone II (2), colossolactone III (3) and colossolactone IV (4) in addition to five known compounds, ergosterol (5), 19 colossolactone B (6), colossolactone C (7), colossolactone G (8) 19 and schisanlactone A (9). 11 The known compounds were identified by comparison of the spectroscopic data with their reported ones and structures of new compounds were determined as follows.

Compound 1 was obtained as colorless needles (hexane–acetone), mp 266–268 °C with a positively optical rotation ([α] D 25.5 + 23.8°) (CHCl 3). A molecular formula of C 30H 46O 3 was estimated from the high resolution electron impact mass (HR–EI–MS) spectrum. The 1H–1H-correlation spectroscopy (COSY) spectrum showed signals for seven methyls with the most characteristic peaks including a doublet at δ H 1.02 (J = 6.3 Hz) and an allylic methyl at δ H 1.90, one oxymethine at δ H 3.20 (dd, J = 4.0, 11.5 Hz), one methine at δ H 4.50 (dd, J =4.0, 13.5 Hz) and an olefinic methine at δ H 6.59 (m). The 13C–NMR spectrum displayed 30 carbon signals, in which signals characteristic for seven methyls, six methines (including two oxymethines at δ C 78.8 and 80.2) and eight quaternary carbons (including one carbonyl at δ C 166.6) and four olefinic carbons at δ C 128.1, 134.1, 134.4 and 139.7 were assigned from the distortionless enhancement by polarization transfer (DEPT) spectra. These findings suggested that compound 1 was an oxygenated lanostane-type triterpene. The UV absorbance at 245 nm indicated the presence of an α,β-unsaturated lactone, 19 and the IR spectrum showed the presence of a hydroxyl group at 3530 cm −1 and one conjugated δ-lactone group at 1708 cm −1. Comparison of the 1H–1H-NMR spectrum of 1 with the closely related compound colossolactone B 19 resulted in the assignments for signals at δ H 4.50 as H-22, δ H 6.59 as H-24, δ H 1.52 as H-20 and δ H 1.90 as H-27 as well as 13C-NMR signals at δ C 166.6 as C-26 and δ C 80.2 as C-22. The presence of mass spectral fragment ions at m/z 111 19 and 314 [M–side chain–H] 19 indicated that this lanostane type triterpene contained an α-methyl, α,β-unsaturated δ-lactone group as a side chain. The main difference between 1 and colossolactone B was the presence of a methyl group as C-19 instead of an acetylated primary alcohol as in colossolactone B. In the 1H–1H-correlation spectroscopy (COSY) spectrum, the following correlations were found: H-23 (δ H 2.56, 1.98) with H-22 (δ H 4.50) and H-24 (δ H 6.59), H-27 (δ H 1.90) with H-24 (δ H 6.59), H-21 (δ H 1.02) with H-20 (δ H 1.52) and H-20 (δ H 6.59) with H-22 (δ H 4.50). In the heteronuclear
The UV absorption at 245 nm and IR spectra showed characteristic bands at 3434 cm⁻¹ (OH group) and 1708 cm⁻¹ (δ-lactone moiety). The 1H- and 13C-NMR spectra of 2 showed the presence of seven methyl groups including a secondary methyl (δH 0.94, d, J=6.3 Hz) and six tertiary methyl groups (δH 0.68, 0.81, 1.00, 1.13, 1.22, 1.87). The 1H-NMR spectra showed two protons on oxygenated carbons at δH 3.70 and 3.46. The 13C-NMR spectra showed four olefinic carbons (δC 134.1, 137.0, 140.5, 127.8) and one carbonyl (δC 166.2). The NMR spectral data resembled those of compound 1 except for the presence of an additional hydroxyl group (δH 3.70, δC 73.8). In the 1H-NMR and 1H-1H COSY spectra, the lower
field shift of H-2 [δH 2.35 and 2.39, δC 39.8] and the correlations of H-2 with H-1 (δH 3.70) and H-3 (δH 3.46) in comparison to those of 1 showed that a hydroxyl group was attached to C-1. Also we attempted the HMBC to confirm that the location of the hydroxyl group was at C-1 by detection of cross peaks between H-1 with C-2, C-3, C-5 and C-19. The β-orientation of the hydroxyl group at C-1 was deduced from the J-constants of H-1 (δH 3.70, dd, J=4.5, 11.1 Hz).25 Also the S configuration of C-22 was confirmed from the CD measurement (Δε250 -1.680, CHCl3). Accordingly 2 was determined to be (22S)-3β,13β-dihydroxylanosta-8,24-dien-26,22-olide and called colossolactone II.

Compound 3 was obtained as colorless needles, mp 245—250 °C with a positively optical rotation ([α]D 25° +54.9°) (CHCl3). A molecular formula C31H46O4 was estimated from the HR-EI-MS spectrum. The UV absorbance at 243 nm and a characteristic IR band at 1706 cm⁻¹ suggested the presence of a conjugated carbonyl group. The 13C-NMR spectrum displayed 31 carbon signals. Similarities in the spectra indicated that 3 was related in structure to compound 1. Comparison of the 1H- and 13C-NMR spectral data suggested that the most prominent differences were the absence of H3-19 and the lower field shift of H-19 (δH 4.60, s), appearance of a methoxy group at δC 3.40 and an acetal carbon at δC 104. The HMBC experiment showed that a signal of the acetal carbon at δC 104 was correlated with protons of OCH3 and H-5 and a signal due to the oxygenated tertiary carbon atom at δC 77.5 was correlated with those of H-19 (δH 4.60), H-28 (δH 0.97) and H-29 (δH 1.02). These findings suggested that the acetal carbon at C-19 was connected with C-3 through an oxygen atom.18 Thus, the connection of the A ring and the acetal ring (F ring) became clear as shown in structure 3 (Fig. 1). A nuclear Overhauser effect spectroscopy (NOESY) experiment was carried out to determine the relative configuration at C-19 (Fig. 3). The NOESY correlations observed between (H-19 and H-29) showed that an ether linkage between C-3 and C-19 was formed in the β side.18 CD measurements were used for the assignment of the absolute configuration at C-22. The CD spectrum showing the same pattern of the negative Cotton effect (Δε250 -1.284, CHCl3) at the same wavelength as in compound 1, revealed the S configuration at C-22. The remaining of the structure had the same pattern as in compound 1. Accordingly compound 3 was assigned as (22S)-3β,19-epoxy-lanosta-8,24-dien-26,22-olide and called colossolactone III.

Compound 4 was obtained as white amorphous powder, with a positively optical rotation ([α]D 25° +94.3°) and assigned the molecular formula C30H44O5 by HR-EI-MS. The UV spectrum showed λmax at 243 nm. The IR spectrum indicated the presence of a hydroxyl group (3449 cm⁻¹) and carbonyl groups (1763, 1717 cm⁻¹). The 13C-NMR spectrum displayed 30 carbon signals, in which nine low-field signals corresponded to two carbonyl (δC 166.5, 177.3), four olefinic (δC 121.7, 128.0, 139.2, 139.7) and three oxygenated carbons (δC 74.5, 80.1, 91.5) and high-field signals were assigned to six methyl, ten methylene, three methine and two quaternary carbons. The 1H-NMR data were similar to those of compound colossolactone D19 showing signals for one secondary (δH 1.01) and five tertiary (δH 1.92, 1.32, 1.28, 0.94, 0.74) methyls. These findings were consistent with the molecular formula determined by HR-EI-MS and suggested the structure of 4 in Fig. 1.

The seven membered lactone ring of 4 was assigned as the ring A based on the biogenetic and NMR spectral consideration but differ from colossolactone D in lacking the conjugated system shared between ring A and ring B and also the absence of a hydroxyl group at C-15. This assignment was supported by the downfield shifts at δH 1.28 and 1.32 for gem-dimethyl proton signals (Hδ3-28 and Hδ3-29) and, the downfield shifts of C-4 (δC 74.5) and C-3 (δC 177.3) carbon signals.19 A significant peak at m/z 111 in the mass spectrum as well as the 1H-NMR signals at δH 1.92 (s), 6.61 (m) and
4.52 (dd, J = 2.5, 13.0 Hz) indicated the presence of a six membered α,β-unsaturated lactone ring substituted at the δ position. The HMBC experiment showed that H-1 was correlated with a carbonyl carbon (δc 177.3) and H-5 was correlated with C-28, C-29 and the low field C-4 (δc 74.5). In the 1H- and 13C-NMR spectra, 4 had only six methyl groups and no cyclopropane ring, which confirmed the presence of a seven membered B ring. The presence of a hydroxyl group at C-10 was confirmed from the HMBC correlation between C-10 (δh 91.5) and down field shifted H-29 (δh 3.18) and the correlation between C-9 (δc 121.7) and the same proton. The stereochemistry of this compound was determined from the CD spectrum to confirm the S-configuration of C-22 (Δ2290 = 1.349, CHCl3) and also from the NOESY spectrum to indicate the configuration of a hydroxyl group at C-10; the proton of the OH group (δh 2.05) had a NOE correlation with H-29 (δh 1.32) indicating that the hydroxyl group was projected with β-orientation. Also the α-orientation of H-5 (δh 1.80) was determined from the NOESY spectrum (Fig. 3) through the correlation with H-28 (δh 1.29) leading to the trans-fusion between ring A and ring B, in which ring A is a seven-membered ring. Accordingly compound 4 was determined to be (22S)-A,B-dihomo-19-nor-4-oxalanosta-8,24-dien-26,22-olide, and called colossolactone IV. The absolute configuration of a hydroxyl group at C-5 in 8 was not yet determined by Kleinwachter et al., but the α-orientation of this hydroxyl group was established from a clear correlation of proton signals between the hydroxyl group (δh 1.82, br s) and H-28 in the NOESY experiments.

**Experimental**

**General Experimental Procedures** Melting points were measured on a Yanagimoto micro hot stage melting point apparatus. Optical rotations were measured with a DIP-360 automatic polarimeter (Jasco Co., Tokyo, Japan). UV spectra were measured with a UV2200/UV–VIS recording spectrophotometer (Shimadzu Co., Kyoto, Japan). 1H- and 13C-NMR spectra were measured with Varian UNITY 500 (1H, 500 MHz; 13C, 125 MHz) spectrometer and Jeol JNA-LAA 400WBF-FT (1H, 400 MHz; 13C, 100 MHz). HR-ESI-MS and EI-MS were measured with a JMX-AX 505 HAD mass spectrometer (Jeol Co., Tokyo) at an ionization voltage of 70 eV. IR spectra were measured with a Fourier transform (FT)IR-460 infrared spectrometer (Jasco Co., Tokyo). CD spectra were recorded in CHCl3 on a Jasco J-805 spectrometer. Column chromatography was carried out on silica gel (Kieselgel 60, 230 mesh, Merck). Medium pressure liquid chromatography (MPLC) was carried out on a LiChroprep Si 60 (Merck Co., Darmstadt). Thin layer chromatography (TLC) was carried out on pre-coated Silica gel 60 F254 plates (0.25 mm, Merck) and Rp-18 F254S (0.25 mm, Merck) and spots were detected under a UV light and by spraying with p-anisaldehyde/H2SO4 followed by heating.

**Fungal Material** The fruiting bodies of *Ganoderma colossum* (Fr.) F. Baker were obtained from Vietnam in September 2005, and a voucher specimen is deposited at the Museum of Ethnomedicines in the University of Toyama.

**Extraction and Isolation** The pulverized fruiting bodies of *G. colossum* (3.5 kg) were extracted with CHCl3 (8 l) at room temperature for 5 d. The combined extracts were filtered and concentrated to give a dark brown residue of 582 g. The chloroform extract was dissolved in MeOH (11) and defatted with hexane (21.3). The two were separately evaporated to give dark orange and dark brown extracts of 98 g and 474 g, respectively. An orange precipitate (10 g) formed at the interface between the MeOH and hexane layers was chromatographed over a silica gel column (5 × 55 cm). The elution was started with hexane (100%), then hexane–acetic mixtures (9.5: 0.5) with increasing the concentration of acetic till 20% to afford compounds 1 (170 mg) and 8 (2.9 g). Two hundred grams of the MeOH extract was chromatographed on silica gel (2 kg) with hexane–acetic acid mixtures (9: 1—9 —1) and 2 (200 mg) and 5 (3.5 g).


**22S)-A,B-Dihomo-19-nor-4-oxalanosta-8,24-dien-26,22-olide (4, Colossolactone IV)** White amorphous powder, [α]D25+94.3° (c = 0.18, CHCl3). CD: (Δ290 = 1.349, CHCl3) UV (CHCl3) nm λmax (log ε) 253 (5.81). IR cm−1 3449, 2963, 2345, 1763, 1717. 1H- and 13C-NMR (see Tables 1, 2, respectively). EI-MS m/z: 484 [M]+, 466 [M–H2O]+, 451
[M−H2O−Me]+, 408 [M−H2O−CH3CO]+, 393 [M−H2O−CH3CO−CH3]1+. 327, 311, 285, 252, 175, 139, 111 and 55. HR-EI-MS m/z 484.32313 (Calcd for C30H44O5, 484.31887).

Acknowledgment The authors are grateful to Reishi-Sogo Kenkyusho Co., Tokyo for providing the fruiting bodies of *Ganoderma colossum* (Fr.). C. F. Baker cultivated in Vietnam.

References