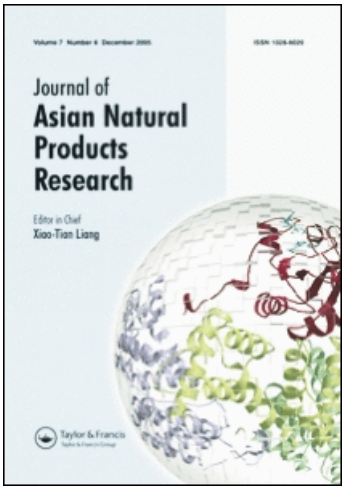

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Shiono, Yoshihito , Sugasawa, Hiromi , Kurihara, Naomi , Nazarova, Margarita , Murayama, Tetsuya , Takahashi, Koetsu and Ikeda, Michimasa(2005) 'Three lanostane triterpenoids from the fruiting bodies of *Stropharia aeruginosa*', Journal of Asian Natural Products Research, 7: 5, 735 — 740
To link to this Article: DOI: 10.1080/1028602042000325546
URL: http://dx.doi.org/10.1080/1028602042000325546

Journal of Asian Natural Products Research, Vol. 7, No. 5, October 2005, 735–740

Three lanostane triterpenoids from the fruiting bodies of *Stropharia aeruginosa*

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(Received 11 May 2004; revised form 20 July 2004; in final form 30 July 2004)

Three new lanostane triterpenoids, aeruginosic acid derivatives, were isolated from the fruiting bodies of *Stropharia aeruginosa* and their structures were determined on the basis of spectral data.

Keywords: *Stropharia aeruginosa*; Methyl aeruginosates A; B; and C; Lanostane triterpenoids; Column chromatography

1. Introduction

Mushrooms of the family Strophariaceae are widely distributed in the world. Phytochemical investigation of some species belonging to the family Strophariaceae revealed some biologically active substances which included ergosterol, lonpleic acid and some free amino acids from *Stropharia aeruginosa, Stropharia coronilla* and *Stropharia semiglobata* [1]. We previously have reported the isolation and structure elucidation of some new sesquiterpenes, fascicularones A–D from the liquid culture filtrates of *Naematoloma fasciculare*, belonging to Strophariaceae [2,3]. In the continuing search for biologically active compounds from the basidiomycetes fungus (Strophariaceae), we have isolated three new triterpenoids as the methyl esters, named methyl aeruginosates A (**1**), B (**2**) and C (**3**) from the Russian mushroom *S. aeruginosa*. This paper describes the isolation and structure elucidation of three new triterpenoids.

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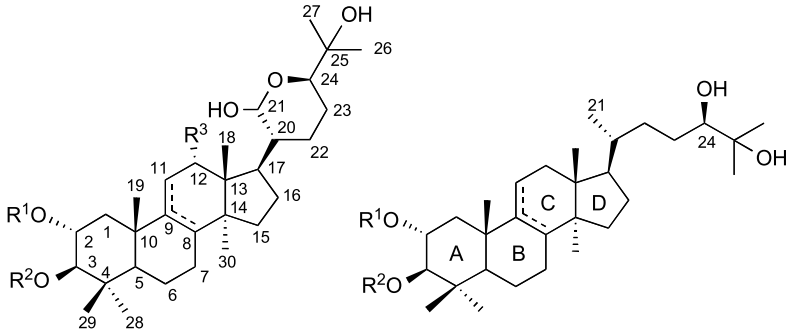
Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2005 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/1028602042000325546

2. Results and discussion

Purification of these metabolites was guided by characteristic TLC behaviour. The MeOH extract of dried fruiting bodies of *Stropharia aeruginosa* was methylated with diazomethane. The crude product was separated repeatedly by a combination of silica gel and Sephadex LH-20 column chromatography to give the methyl esters of three new $\Delta^{9(11)}$-lanostane-type triterpenoid acids, methyl aeruginosates A (**1**), B (**2**) and C (**3**) (figure 1).

Methyl aeruginosate A (**1**) was indicated to possess the molecular formula of $C_{39}H_{62}O_{11}$ by HRFAB-MS. The IR spectrum of **1** showed hydroxyls absorption at 3455 cm^{-1} and an ester carbonyl absorption at 1743 cm^{-1}. The ^1H NMR and ^{13}C NMR spectra of **1**, shown in tables 1 and 2, respectively, were quite similar to those of 3β-*O*-acetyl-2α-*O*-(3-hydroxy-3-methyl) glutarylcrustulinol (**4**), suggesting that **1** possessed the same lanostane-type triterpene ester [4]. In the ^{13}C NMR spectrum of **1**, almost all the signals observed in **4** were found in **1**, with the exception that signals assigned to C-8, C-9 and C-11 in **4** were replaced by the signals at δ_C 41.3, 118.1 and 149.2, indicating that the olefine moiety of **1** was constructed with C-9 and C-11. The full assignments of ^1H and ^{13}C NMR signals of **1** were made by performing a combination of $^1H-^1H$ COSY, HSQC and HMBC experiments. The long-range correlation cross-peaks (figure 2) between H-3 and an acetyl carbon, and between H-2 and C-1 resulted in the acetyl group at C-3 and the Me-HMG group at C-2. The hydroxyl was determined to be at C-12 because of a correlation peak of H-12 with a methyl at C-13, and correlation peaks of H-12 with C-9 and C-14. The signal of H-21 was correlated with C-17, C-22 and C-24, the signal of H-24 with C-21 and C-22. These data suggested the presence of a tetrahydropyran ring (C-20, C-21, C-22, C-23 and C-24). The stereostructure of **1** was characterised by careful comparison of the ^1H NMR and ^{13}C NMR data with those of **4**. In the ^1H NMR spectrum of **1**, H-21 proton appeared as a singlet at δ_H 5.93. Based on the generaliSed Karplus relationship, the observed singlet showed the dihedral angle for H-20



1 : R^1=Me-HMG, R^2=Ac, R^3=OH, $\Delta^{9(11)}$

2 : R^1=Me-HMG, R^2=Ac, R^3=H, $\Delta^{9(11)}$

3 : R^1=Me-HMG, R^2=Ac, $\Delta^{9(11)}$

5 : R^1=H, R^2=H, Δ^8

4 : R^1=HMG, R^2=Ac, R^3=OH, Δ^8

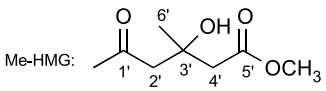


Figure 1. Structures of compounds **1**–**5**.

Table 1. ^{13}C NMR data of methyl aeruginosates A (**1**), B (**2**), and C (**3**). (100 MHz, pyridine-d_5).

No.	***1***	***2***	***3***	*No.*	***1***	***2***	***3***
1	41.7 *t*	41.9 *t*	42.0 *t*	21	93.3 *d*	93.0 *d*	18.6 *q*
2	70.4 *d*	70.5 *d*	70.7 *d*	22	24.6 *t*	24.5 *t*	34.0 *t*
3	79.8 *d*	80.0 *d*	79.8 *d*	23	26.0 *t*	26.2 *t*	28.4 *t*
4	39.7 *s*	39.7 *s*	39.8 *s*	24	75.0 *d*	74.6 *d*	79.1 *d*
5	51.8 *d*	52.2 *d*	52.2 *d*	25	71.1 *s*	71.2 *s*	72.7 *s*
6	21.1 *t*	21.3 *t*	21.3 *t*	26	26.1[a] *q*	26.3[a] *q*	26.0[a] *q*
7	28.6 *t*	28.5 *t*	28.9 *t*	27	26.7[a] *q*	26.8[a] *q*	26.1[a] *q*
8	41.3 *d*	43.7 *d*	41.5 *d*	28	17.9 *q*	17.8 *q*	17.9 *q*
9	149.2 *s*	147.3 s	147.5 *s*	29	28.5 *q*	28.0 *q*	28.5 *q*
10	40.8 *s*	40.5 *s*	40.6 *s*	30	23.0 *q*	22.9 *q*	18.6 *q*
11	118.1 *d*	116.0 *d*	116.0 *d*	1	170.6[b] *s*	170.7[b] *s*	170.7[b] *s*
12	74.1 *d*	33.9 *t*	37.1 *t*	2	46.0[c] *t*	46.0[c] *t*	46.1[c] *t*
13	48.3 *s*	44.4 *s*	44.5 *s*	3	69.8 *s*	69.8 *s*	69.8 *s*
14	46.2 *s*	47.1 *s*	47.2 *s*	4	46.5[c] *t*	46.5[c] t	46.6[c] *t*
15	35.0 *t*	36.8 *t*	34.1 *t*	5	171.1[b] *s*	171.1[b] *s*	171.2[b] *s*
16	27.4 *t*	27.2 *t*	28.0 *t*	6	28.3 *q*	28.3 *q*	28.4 *q*
17	40.6 *d*	41.5 *d*	51.4 *d*	$COCH_3$	21.0 *q*	20.9 *q*	21.0 *q*
18	15.8 *q*	15.5 *q*	14.7 *q*	$COCH_3$	171.9[b] *s*	171.9[b] *s*	171.9[b] *s*
19	20.3 *q*	18.8 *q*	22.9 *q*	OCH_3	51.3 *q*	51.2 *q*	51.3 *q*

[a–c] Assignments with the same superscript may be reversed in each vertical column.

/H-21 was approximately 90°. The appearance of the H-24 signals as a broad doublet δ_H 4.21 ($J = 10.3$ Hz) implied the proton is coupling only to one proton of the 23-methylene. The configurations of the hemiacetal pyran ring was considered to be the same as those in **4**, because protons at C-21 and C-24 in **1** had chemical shifts and coupling constants quite similar to those of **4**. Additional detailed assignments of acyl and methyl groups were obtained with NOE experiments, and the results are shown in figure 3.

Methyl aeruginosate B (**2**) showed spectral characteristics quite similar to those of **1**. The molecular formula of **2**, $C_{39}H_{62}O_{10}$, was determined by HRFAB-MS, corresponding

Table 2. ^{1}H NMR data of methyl aeruginosates A (**1**), B (**2**) and C (**3**) (400 MHz, pyridine-d_5).[a]

No.	***1***	***2***	***3***
CH_3-18	0.72, 3H, *s*	0.82, 3H, *s*	0.66, 3H, *s*
CH_3-19	1.11, 3H, *s*	1.11, 3H, *s*	1.14, 3H, *s*
CH_3-21	–	–	1.02, 3H, *d*, (6.3)
CH_3-26	1.40,[b] 3H, *s*	1.42,[b] 3H, *s*	1.52,[b] 3H, *s*
CH_3-27	1.40,[b] 3H, *s*	1.42,[b] 3H, *s*	1.55,[b] 3H, *s*
CH_3-28	0.94, 3H, *s*	0.94, 3H, *s*	0.95, 3H, *s*
CH_3-29	0.93, 3H, *s*	0.91, 3H, *s*	0.93, 3H, *s*
CH_3-30	1.17, 3H, *s*	0.77, 3H, *s*	0.74, 3H, *s*
CH_3-6′	1.69, 3H, *s*	1.69, 3H, *s*	1.72, 3H, *s*
H-2	5.50, 1H, *td* (10.7, 4.4)	5.51, 1H, *td* (10.7, 4.0)	5.54, 1H, *td* (10.7, 4.0)
H-3	5.06, 1H, *d* (10.7)	5.08, 1H, *d* (10.7)	5.11, 1H, *d* (10.7)
H-12	4.10, 1H, *m*	–	–
H-11	5.53, 1H, *d* (4.4)	5.13, 1H, *br. S*	5.10, 1H, *br. s*
H-21	5.93, 1H, *s*	5.79, 1H, *br. S*	-
H-24	4.21, 1H, *br. d*, (10.3)	4.30, 1H, *br. d*, (10.3)	3.78, 1H, *m*
H_2-2, 4	3.06, 4H, *m*	3.06, 4H, *m*	3.09, 4H, *m*
AcO-3β	2.15, 3H, *s*	2.16, 3H, *s*	2.17, 3H, *s*
OCH_3	3.61, 3H, *s*	3.60, 3H, *s*	3.61, 3H, *s*
OH-12	5.35, 1H, *d*, (2.4)	–	–

[a] δ in ppm and *J* (parentheses) in Hz.
[b] Assignments with the same superscript may be reversed in each vertical column.

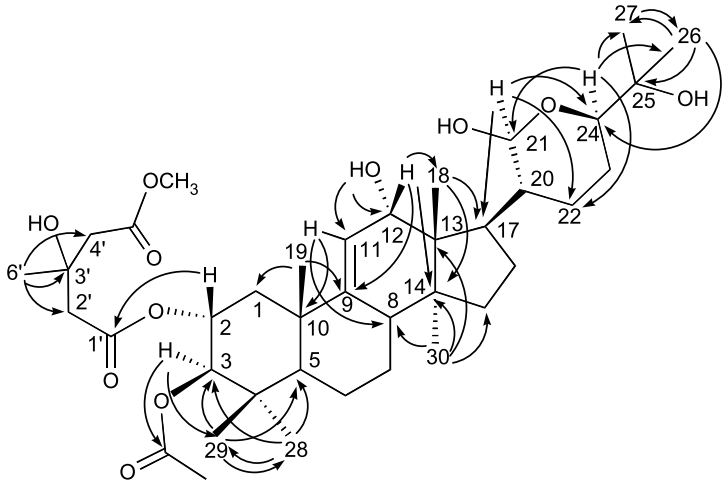


Figure 2. $^{13}C-^{1}H$ long-range correlations of **1**.

to one oxygen atom less than **1**. On comparison of the ^{1}H NMR, ^{13}C NMR and DEPT spectra of **2** with those of **1** (Tables 1 and 2), the signals due to the pyran ring, the monomethyl 3-hydroxy-3-methylglutarate group (Me-HMG) and acetyl moieties remained unaffected, while a methylene group was newly observed at δ_C 33.9, instead of a hydroxyl methine in **1**. In the HMBC spectrum of **2**, a long-range correlation between H_3-18 (δ_H 0.82) and C-12 supported the presence of a methylene at C-12. Thus the structure of **2** was determined to be 12-deoxyderivative of **1**.

Methyl aeruginosate C (**3**) had the molecular formula of $C_{39}H_{64}O_9$, which was determined by HRFAB-MS. A comparison of ^{13}C NMR signals of **3** with those of **2** revealed that some signals were almost superimposable over the signals attributed to carbons on the A–B–C–D ring, Me-HMG and acetyl group. But signals due to hemiacetal carbon present in the tetrahydropyran moiety of **2** were not observed, while new signals of one methyl doublet at δ_H 1.02 (*d*, $J = 6.3$ Hz) and δ_C 18.6 (C-21) were seen in the ^{1}H NMR and ^{13}C NMR spectra of **3**, implying cleavage of the pyran ring. Full assignment of the ^{1}H NMR and ^{13}C NMR spectroscopic data for **3** was accomplished by HMBC correlations, while the NOE experiments revealed the presence of the same stereochemistry on ring A–D as **2**. Comparing the ^{1}H NMR and ^{13}C- NMR data of fasciculol A (**5**) (24 *R*) [δ_H 3.77 (1H, *br. d*, $J = 6.8$ Hz, H-24), δ_C 79.0 (d, C-24)] with those of **3** revealed that C-24 chirality could

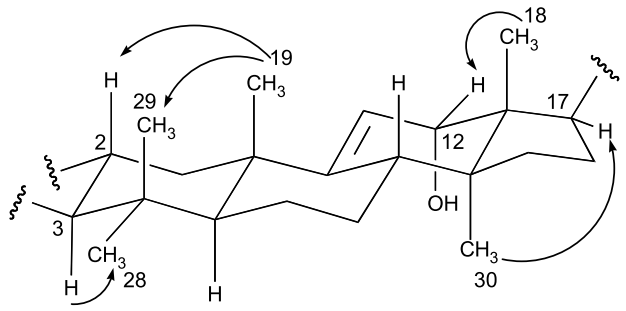


Figure 3. Significant correlations observed in the NOE difference spectra of **1**.

be assigned as the *R* configuration due to the fact that the *R* and *S* configuration at C-24 can be distinguished by the chemical shift of the hydroxyl methine at C-24 in the NMR spectrum [5]. Therefore, the structure of **3** was elucidated to be that as shown in figure 1.

3. Experimental

3.1 General experimental procedures

Melting point (mp) data were determined on Yanagimoto micro hot-stage melting point apparatus and are not corrected. Optical rotations were measured with a Horiba model SEPA-300 polarimeter, whereas IR spectra were recorded with a JASCO J-20A spectrometer. Mass spectra were obtained using a JEOL JMS-SX102A instrument, and ^{1}H NMR and ^{13}C NMR spectra were acquired with a JEOL EX-400 spectrometer. Column chromatography was conducted on Sephadex LH-20 (Pharmacia), silica gel 60 (Kanto Chemical Co., Inc.) and Amberlite XAD-2 (Organo Corp.).

3.2 Mushroom material

The fruiting bodies of *Stropharia aeruginosa* were collected from Vladivostok, Russia in 1993 and identified by one of authors (M.N.). The voucher specimen has been deposited at our laboratory.

3.3 Extraction and isolation

Dried fruiting bodies (22 g) of *S. aeruginosa* were extracted with MeOH. The methanolic extract was evaporated *in vacuo* to give a residue (2.3 g) which was methylated with excess ethereal CH_2N_2 in the usual way. The methylated acid fraction (2.1 g) was subjected to silica gel column chromatography eluting with a mixture of hexane/EtOAc (1:2) to give six fractions (fr. 1.1–1.6). Fraction 1.2 (109 mg) was subjected to silica gel column chromatography eluting with $CHCl_3$-MeOH (50:1) to yield methyl aeruginosate C (**3**, 3.5 mg). Fraction 1.3 (42 mg) was subjected to Sephadex LH-20 with MeOH, then chromatographed on silica gel with hexane- EtOAc (2:3) to yield methyl aeruginosate B (**2**, 11 mg). Fraction 1.4 (116 mg) was chromatographed on silica gel eluting with $CHCl_3$-MeOH (50:1) to afford methyl aeruginosate A (**1**, 27 mg).

3.3.1 Methyl aeruginosate A (1). White amorphous powder; mp.117–118°C. $[\alpha]_D^{20} + 5.2$ (*c* 1.5, MeOH). HRFAB-MS (neg.): *m/z* 705.4242 $[M-H]^-$ (calcd for $C_{39}H_{61}O_{11}$, 705.4194). FAB-MS (neg.): *m/z* 705 $[M-H]^-$. IR (KBr) ν_{max} cm^{-1}: 3455, 2975, 2950, 1743, 1375, 1238, 1031. ^{1}H NMR and ^{13}C NMR: See tables 1 and 2.

3.3.2 Methyl aeruginosate B (2). White amorphous powder; mp112–114°C. $[\alpha]_D^{20} + 1.0$ (*c* 0.65, MeOH). HRFAB-MS (neg.): *m/z* 689.4260 $[M-H]^-$ (calcd for $C_{39}H_{61}O_{10}$, 689.4248). FAB-MS (neg.): *m/z* 689 $[M-H]^-$. IR (KBr) ν_{max} cm^{-1}: 3450, 2975, 2950, 1743, 1375, 1238, 1033. ^{1}H NMR and ^{13}C NMR: See tables 1 and 2.

3.3.3 Methyl aeruginosate C (3). White amorphous powder; mp 157–158°C. $[\alpha]_D^{20} + 22$ (*c* 0.14, MeOH). HRFAB-MS (pos.): *m/z* 677.4597 $[M + H]^+$ (calcd for $C_{39}H_{65}O_9$, 677.4611). FAB-MS (pos.): *m/z* 677 $[M + H]^+$. IR (KBr) ν_{max} cm^{-1}: 3434, 2977, 2944, 1745, 1375, 1238, 1043. For ^1H NMR and ^{13}C NMR: See table 1 and 2.

References

[1] F. Senatore. *Biochem. Syst. Ecol.*, **18**, 103 (1990).
[2] Y. Shiono, R. Matsuzaka, H. Wakamatsu, K. Muneta, T. Murayama, M. Ikeda. *Phytochemistry*, **65**, 491 (2004).
[3] Y. Shiono, K. Wakamatsu, T. Murayama, M. Ikeda. *Z. Naturforsch.*, **59b**, 119 (2004).
[4] M.D. Bernardi, G. Fronza, M.P. Gianotti, G. Mellerio, G. Vidari, P. Vita-Finzi. *Tetrahedron lett.*, **24**, 1635 (1983).
[5] A.H. Banskota, Y. Tezuka, K.Q. Tran, K. Tanaka, I. Saiki, S. Kadota. *J. Nat Prod.*, **63**, 57 (2000).