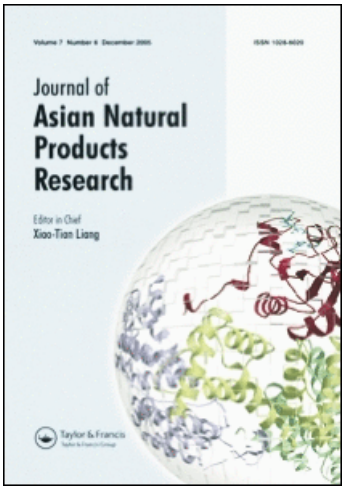

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Three lanostane triterpenoids, aeruginosols A, B and C, from the fruiting bodies of *Stropharia aeruginosa*

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Phytochemical analysis of the MeOH extract of the fruiting bodies of *Stropharia aeruginosa* resulted in the isolation of three lanostane triterpenoids, aeruginosols A, B and C. The structures of the new compounds were determined by spectroscopic analysis. The biological activities of aeruginosols A, B and C were examined by bioassay with lettuce seedling.

Keywords: *Stropharia aeruginosa*; Aeruginosols A, B and C; Lanostane triterpenoids; Plant-growth inhibitor

1. Introduction

During the course of our continuing phytochemical studies on the fruiting bodies of the family Strophariaceae, fasciculols A–F [1–3] and fascicularones A–K [4–7] were reported from *Hypholoma fasciculare*. Recently, we investigated the chemical constituents of the fruiting bodies of *Stropharia aeruginosa* (Strophariaceae) and isolated three new lanostane triterpenoid esters, designated as methyl aeruginosates A (**1**), B (**2**) and C (**3**) (figure 1) [8]. Further purification of the MeOH extract of this mushroom led to the isolation of three lanostane triterpenoids, named aeruginosols A (**4**), B (**5**) and C (**6**) (figure 1). This paper deals with the structural assignments of **4**, **5** and **6** on the basis of spectroscopic analysis, including two-dimensional NMR data, and chemical methods. The activities of the isolated compounds against lettuce are also reported.

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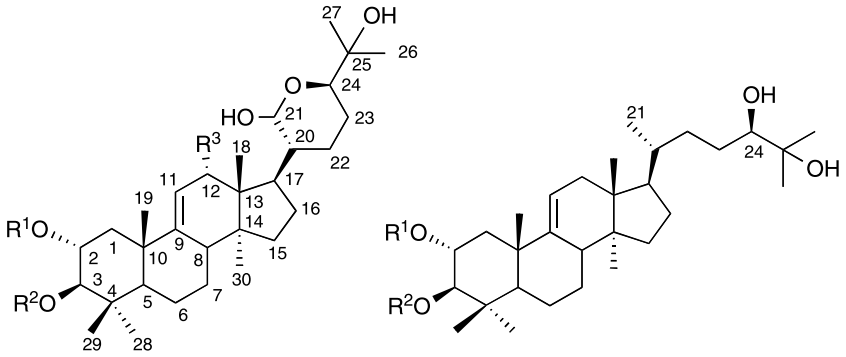

1 : R^1=Me-HMG, R^2=Ac, R^3=OH

2 : R^1=Me-HMG, R^2=Ac, R^3=H

4 : R^1=H, R^2=H, R^3=OH

5 : R^1=H, R^2=H, R^3=H

3 : R^1=Me-HMG, R^2=Ac

6 : R^1=H, R^2=H

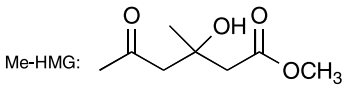

Figure 1. Structures of compounds **1**–**6**.

2. Results and discussion

The fruiting bodies of *S. aeruginosa* were dried and extracted with MeOH. Then, the MeOH extract was subjected to silica gel, Sephadex LH-20 column chromatography and preparative TLC to give compounds **4**, **5** and **6**.

The molecular formula of aeruginosol D (**4**) was found to be $C_{30}H_{50}O_6$ by HREI-MS. The IR spectrum of **4** showed an absorption band for hydroxyls group at 3340 cm^{-1}. ^{13}C NMR and DEPT spectra showed thirty carbon signals including characteristic signals due to seven methyls (δ 15.7, 16.9, 20.2, 23.5, 24.0, 26.0, 28.8), one double bond (δ 151.2, 116.4), seven methylene carbons (δ 21.1, 23.6, 25.8, 27.0, 28.4, 34.8, 44.2), and eight methines (δ 41.2, 42.9, 52.4, 69.3, 74.2, 74.8, 82.7, 93.1), five of which were linked to oxygen atoms. The ^1H NMR spectrum of **4** showed signals for seven tertiary methyl groups, one double bond and five oxygen-bearing methines. These data were characteristic for the lanostane-type triterpenoid. The ^1H NMR and ^{13}C NMR data of **4** were quite similar to those of **1** except for the Me-HMG [(3-hydroxy-3-methyl)-glutaryl-] and acetyl moieties. In the ^1H NMR spectrum of **4**, signals at δ_H 0.67, 1.00, 1.09, 1.15, 0.90, 0.84, and 1.17 were assigned to H_3—18, H_3—19, H_3—26, H_3—27, H_3—28, H_3—29 and H_3—30, respectively. Thus **4** was proposed to be the deacyl derivative of **1** and was confirmed by ^1H–^1H COSY and HMBC correlations (figure 2). Furthermore, the ^1H NMR and ^{13}C NMR spectra of **4** corresponded to those of deacyl derivative obtained by hydrolysis of **1** with 1 M KOH. Based on these results, the structure of **4** was assigned as deacyl derivative of methyl aeruginosate A (**1**).

The molecular formula of **5** was determined to be $C_{30}H_{50}O_5$ by HREI-MS, indicating that **5** has one oxygen less compared with **4**. The ^{13}C NMR spectral data (table 1) of **5** were very similar to those of **4**, except for a new methylene carbon signal at δ_C 36.6 in place of the signal assignable to the hydroxymethine (C-12) in **4**. Alkaline hydrolysis of methyl

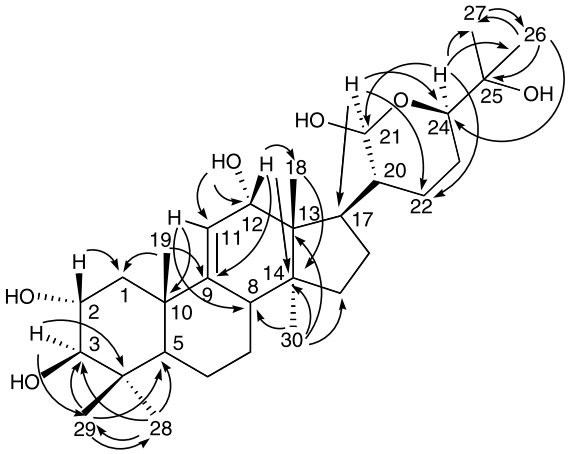

Figure 2. The key HMBC correlations of **4**.

aeruginosate B (**2**) with 1 M KOH afforded the deacyl derivative, whose spectral data was identical with that of **5**. Thus, **5** was determined to be a 12-deoxy-derivative of **4**.

The molecular formula of **6** was determined to be $C_{30}H_{52}O_4$ by HREI-MS. The ^{1}H NMR spectrum of **6** showed signals for seven tertiary methyl groups (δ 0.66, 0.75, 0.86, 1.03, 1.11, 1.17, 1.22), a methyl doublet (δ 0.89), an olefinic proton (δ 5.26) and three oxymethine protons (δ 3.00, 3.34, 3.77). The ^{1}H NMR and ^{13}C NMR spectral features of **6** were quite similar to those of **3**, except for the lack of the Me-HMG and acetyl signals in **3**, which was also supported by MS spectrum. Full assignment of the ^{1}H NMR and ^{13}C NMR spectroscopic data for **6** was accomplished in the same manner as with **4**. Furthermore, the ^{1}H NMR and ^{13}C NMR spectra of **6** corresponded to those of deacyl derivative obtained by alkaline hydrolysis of **3**.

The plant-growth inhibitory activities of aeruginosols A (**4**), B (**5**), and C (**6**) against lettuce seedling were tested. Aeruginosol C (**6**) had the inhibitory root growth activities of 75% of control at concentration of 100 ppm, respectively.

Table 1. ^{13}C NMR data of aeruginosols A (**4**), B (**5**) and C (**6**) (100 MHz, $CDCl_3$).

No.	***4***	***5***	***6***	*No.*	***4***	***5***	***6***
1	44.2 t	44.2 t	44.3 t	16	23.6 t	23.3 t	27.9 t
2	69.3 d	69.4 d	69.4 d	17	40.0 d	45.4 d	51.1 d
3	82.7 d	83.6 d	83.7 d	18	15.7 q	15.3 q	14.5 q
4	39.4 s	39.4 s	39.3 s	19	20.2 q	18.7 q	23.2 q
5	52.4 d	52.5 d	52.8 d	20	42.9 d	42.4 d	35.9 d
6	21.1 t	21.3 t	21.4 t	21	93.1 d	93.2 d	18.3 q
7	28.4 t	28.0 t	28.1 t	22	34.8 t	33.8 t	33.2 t
8	41.2 d	41.5 d	41.4 d	23	27.0 t	26.9 t	28.2 t
9	151.2 s	147.7 s	147.6 s	24	74.8 d	74.4 d	78.7 d
10	40.7 s	40.6 s	40.6 s	25	72.0 s	71.7 s	73.2 s
11	116.4 d	115.3 d	115.4 d	26	24.0† q	24.0† q	23.3† q
12	74.2 d	36.6 t	37.1 t	27	26.0† q	26.4† q	26.6† q
13	45.9 s	44.0 s	44.3 s	28	16.9 q	16.9 q	16.7 q
14	48.1 s	46.9 s	47.0 s	29	28.8 q	28.7 q	28.4 q
15	25.8 t	25.8 t	33.8 t	30	23.5 q	23.3 q	18.5 q

† Assignments with the same superscript may be reversed in each vertical column.

3. Experimental

3.1 General experimental procedures

Melting points (mp) are uncorrected. Optical rotations were measured with a Horiba model SEPA-300 polarimeter, whereas IR spectra were recorded with JASCO J-20A, Shimadzu UV mini-1240 spectrophotometer, respectively. 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. Chemical shifts are given on a δ (ppm) scale with TMS as an internal standard. Column chromatography was conducted on Sephadex LH-20 (Pharmacia), silica gel 60 (Kanto Chemical Co., Inc.). TLC was carried out using precoated silica gel plates (Merck), and spots were detected by spraying with 10% vanillin in H_2SO_4 followed by heating, or by UV irradiation.

3.2 Mushroom material

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

3.3 Extraction and isolation

Dried fruiting bodies (20 g) of *S. aeruginosa* were extracted with MeOH. The MeOH extract (2.0 g) was subjected to silica gel column chromatography using a mixture of hexane–EtOAc (1:2) to give six fractions (fr. 1.1–1.5). Fraction 1.4 (116 mg) was applied to silica gel column chromatography eluting with $CHCl_3$/MeOH (50:1) to give five fractions (fr. 2.1–2.5). Fraction 2.2 (17.5 mg) was subjected to column chromatography on silica gel (*n*-hexane/EtOAc, 50:1) to obtain aeruginosol A (**4**, 2.8 mg). Fraction 1.5 (945 mg) was chromatographed on silica gel with solvent system of $CHCl_3$/MeOH (10:1) to afford five fractions (fr. 3.1–3.5). Fraction 3.3 (26 mg) was subjected to Sephadex LH-20 with MeOH, and then separated by preparative TLC with $CHCl_3$/MeOH (20:1) to yield aeruginosol C (**6**, 1.8 mg). Fraction 5.5 (67 mg) was chromatographed on silica gel with $CHCl_3$/MeOH (10:1) and then subjected to Sephadex LH-20 with MeOH to yield aeruginosol B (**5**, 2.6 mg).

3.3.1 Aeruginosol A (4). White amorphous powder; mp.146–148°C. $[\alpha]_D^{20}$ + 3.2 (*c* 0.15, MeOH). HREI-MS: *m*/*z* 506.3601 $[M]^+$ (calcd for $C_{30}H_{50}O_6$, 506.3607). EI-MS: *m*/*z* 506 $[M]^+$. IR (KBr) ν_{max} cm^{-1}: 3340. For ^{1}H NMR and ^{13}C NMR: tables 1 and 2.

3.3.2 Aeruginosol B (5). White amorphous powder; mp.208–210°C. $[\alpha]_D^{20}$ + 5.2 (*c* 0.05, MeOH). HREI-MS: *m*/*z* 490.3666 $[M]^+$ (calcd for $C_{30}H_{50}O_5$, 490.3658). EI-MS: *m*/*z* 490 $[M]^+$. IR (KBr) ν_{max} cm^{-1}: 3420. For ^{1}H NMR and ^{13}C NMR: tables 1 and 2.

3.3.3 Aeruginosol C (6). White amorphous powder; mp.174–176°C. $[\alpha]_D^{20}$ + 2.4 (*c* 0.14, MeOH). HREI-MS: *m*/*z* 476.3878 $[M]^+$ (calcd for $C_{30}H_{52}O_4$, 476.3866). EI-MS: *m*/*z* 476 $[M]^+$. IR (KBr) ν_{max} cm^{-1}: 3330. For ^{1}H NMR and ^{13}C NMR: tables 1 and 2.

Table 2. ^{1}H NMR data of aeruginosols A (**4**), B (**5**) and C (**6**). (400 MHz, $CDCl_3$).†

No.	***4***	***5***	***6***
CH_3—18	0.67, 3H, *s*	0.70, 3H, *s*	0.66, 3H, *s*
CH_3—19	1.00, 3H, *s*	1.03, 3H, *s*	1.22, 3H, *s*
CH_3—21	–	–	0.89, 3H, *d* (6.4)
CH_3—26	1.09,‡ 3H, *s*	1.12,‡ 3H, *s*	1.11,‡ 3H, *s*
CH_3—27	1.15,‡ 3H, *s*	1.14,‡ 3H, *s*	1.17,‡ 3H, *s*
CH_3—28	0.90, 3H, *s*	0.86, 3H, *s*	1.03, 3H, *s*
CH_3—29	0.84, 3H, *s*	0.76, 3H, *s*	0.86, 3H, *s*
CH_3—30	1.17, 3H, *s*	1.18, 3H, *s*	0.75, 3H, *s*
H-2	3.75, 1H, *m*	3.78, 1H, *td* (12.2, 4.4)	3.77, 1H, *td* (11.7, 4.4)
H-3	3.00, 1H, *d* (9.3)	3.00, 1H, *d* (9.8)	3.00, 1H, *d* (9.3)
H-11	5.56, 1H, *d* (4.4)	5.27 1H, *br.s*	5.26, 1H, *br. s*
H-12	3.96, 1H, *m*	–	–
H-21	5.47, 1H, *s*	5.34, 1H, *br. S*	–
H-24	3.80, 1H, *m*	3.73, 1H, *br. d* (10.7)	3.34, 1H, *m*

† δ in ppm and J (parentheses) in Hz.
‡ Assignments with the same superscript may be reversed in each vertical column.

3.4 Alkaline hydrolysis of methyl aeruginosate A (1)

1 M KOH in MeOH (10 ml) was added to methyl aeruginoate A (**1**, 4 mg) and stirred at room temperature for 1 h. The mixture was neutralised with HCl, diluted with H_2O, and extracted with EtOAc. After evaporation of the EtOAc layer, the residue was subjected to silica gel column chromatography eluting with $CHCl_3$/MeOH (10:1) to yield white powders (2.6 mg). This product was identical with **4** in terms of EI-MS and NMR spectral comparisons.

3.5 Alkaline hydrolysis of methyl aeruginosate B (2) **and C (3)**

Hydrolysis of **2** (4 mg) and **3** (5 mg) were separately hydrolysed following the same procedure as that described for the hydrolysis of **1** to afford **5** (2 mg) and **6** (2.2 mg), respectively.

3.6 Lettuce seedling assay

Lettuce seeds (*Lactuca sativa* L.) were used for bioassay. Fifteen seeds were sown in filter paper containing a definite concentration of test compound in Petri dish (5 cm i.d.). Distilled water (1 ml, containing 100 ppm (w/v) Tween 80) was added to Petri dish and incubation was carried out at 25°C under continuous light for 7 days. The control experiments were conducted in the distilled water. The length of the roots were measured compared to those of the control.

References

[1] M. Ikeda, G. Niwa, K. Tohyama, T. Sassa, Y. Miura. *Agric. Biol. Chem.*, **41**, 1803 (1977).
[2] M. Ikeda, Y. Sato, M. Izawa, T. Sassa, Y. Miura. *Agric. Biol. Chem.*, **41**, 1539 (1977).
[3] M. Ikeda, H. Watanabe, A. Hayakawa, K. Sato, T. Sassa, Y. Miura. *Agric. Biol. Chem.*, **41**, 1543 (1977).
[4] Y. Shiono, R. Matsuzaka, H. Wakamatsu, K. Muneta, T. Murayama, M. Ikeda. *Phytochemistry*, **65**, 491 (2004).
[5] Y. Shiono, K. Wakamatsu, T. Murayama, M. Ikeda. *Z. Naturforsch*, **59b**, 119 (2004).
[6] Y. Shiono, H. Akasaka, F. Hiramatsu, K. Sato, T. Murayama, M. Ikeda. *Z. Naturforsch*, **60b**, 880 (2005).
[7] H. Akasaka, Y. Shiono, T. Murayama, M. Ikeda. *Helv. Chim. Acta*, **88**, 2944 (2005).
[8] Y. Shiono, H. Sugasawa, N. Kurihara, M. Nozarova, T. Murayama, K. Takahashi, M. Ikeda. *J. Asian. Nat. Prod. Res.*, **7**, 735 (2005).