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THREE SESQUITERPENE ALKALOIDS FROM *EUONYMUS ALATUS* FORMA *STRIATUS*

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Key Word Index—*Euonymus alatus* forma *striatus*; Celastraceae; fruits; sesquiterpene alkaloids; alatusamine; neoalatamine; alatusinine; structure elucidation.

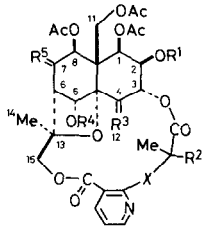
Abstract—Three new sesquiterpene alkaloids, alatusamine, neoalatamine and alatusinine, were isolated from the fruits of *Euonymus alatus* forma *striatus* and their structures elucidated by spectroscopic and chemical methods.

INTRODUCTION

Certain members of the Celastraceae contain various sesquiterpene alkaloids, which have been isolated and structurally determined [1, 2]. Our previous studies on the constituents of the fruits of *Euonymus alatus* forma *striatus* resulted in the isolation and structural elucidation of five sesquiterpene alkaloids, evonine, neoevonine, euonymine, alatamine (**4**) and wilfordine (**6**) [3, 4], and of two related sesquiterpene polyesters, euolalin and alatolin [5–7]. We now describe the isolation and structure elucidation of three new alkaloids, alatusamine (**1**), neoalatamine (**3**) and alatusinine (**5**) from the same plant material.

RESULTS AND DISCUSSION

Alatusamine (**1**) is an amorphous solid, $C_{38}H_{47}O_{17}N$ ($[M]^+$ at m/z 789). The molecular formula and absence of a hydroxyl group (IR spectrum) in **1** suggested that alatusamine (**1**) was a deoxy compound of euonine (**2**) [8], which was supported by the following ^1H NMR spectral evidence. In the ^1H NMR spectra (Table 1), the signals of alatusamine (**1**) corresponded well to those of euonine (**2**) except for (i) on going from **2** to **1** there was a change of the multiplicity for each of the signals due to H-12, H-2 and H-3; H-12 appeared at δ 1.55 (3H, *br s*) in **2** and at δ 1.26 (3H, *d*, J = 8 Hz) in **1**, whereas H-2 and H-3 were observed at δ 5.16 (1H, *dd*, J = 3, 4 Hz) and 4.94 (1H, *d*, J = 3 Hz), respectively in **2**, and at δ 5.30 (1H, *ddd*, J = 1, 2, 4 Hz) and 4.90 (1H, *dd*, J = 1, 2 Hz), respectively in **1**; and (ii) a new signal due to a methine proton (H-4) at δ 2.74 (1H, *ddq*, J = 1, 1, 8 Hz) was observed in **1**. These findings indicated that alatusamine (**1**) is 4-deoxyeuonine. The configuration of the 4-methyl group in **1** was assigned as β from the comparison of the ^1H NMR spectra of alatusamine (**1**) and evonoline (4-deoxyevonine, **8**) [9, 10]. The chemical shifts and the coupling constants of H-1, H-2, H-3 and H-4 in alatusamine (**1**) were quite similar to those of evonoline (**8**), and, in particular, the characteristic long-range coupling between H-2 and H-4 arising from a W-shaped diequatorial configuration was observed in both compounds (see Table 1). The structure (**1**) of alatusamine was unambiguously confirmed by the transformation of euonine (**2**) [8] into alatusamine (**1**).



	R¹	R²	R³	R⁴	R⁵	X
1	Ac	H	β-Me , α-H	Ac	β-OAc , α-H	$-(CH_2)_2-$
2	Ac	H	β-Me , α-OH	Ac	β-OAc , α-H	$-(CH_2)_2-$
3	COPh	OH	β-Me , α-OH	H	O	$-(CH_2)_2-$
4	COPh	OH	β-Me , α-OH	Ac	O	$-(CH_2)_2-$
5	Ac	OH	β-Me , α-OH	Ac	β-OAc , α-H	$-(CH_2)_2-$
6	COPh	OH	β-Me , α-OH	Ac	β-OAc , α-H	$-(CH_2)_2-$
7	Ac	H	CH_2	Ac	β-OAc , α-H	$-(CH_2)_2-$
8	Ac	H	β-Me , α-H	Ac	O	$-CHMe-$

Euonine (**2**) was dehydrated with thionyl chloride and 4-dimethylaminopyridine in pyridine at 50° to give an olefin (**7**), reduction of which with diimide in acetonitrile afforded a compound spectroscopically (IR, ^1H NMR and mass spectrum) and chromatographically identical with natural alatusamine (**1**).

Neoalatamine (**3**) is an amorphous solid, $C_{39}H_{43}O_{17}N$ ($[M]^+$ at m/z 797) and was deduced to be a deacetyl compound of alatamine (**4**) [3, 4], considering the molecular formula and the resemblence of the ^1H NMR spectra of both compounds (see Table 1). Since the signal corresponding to H-5 of alatamine (**4**) (δ 6.76) was observed at δ 5.40 in the spectrum of neoalatamine, structure **3** was assigned to this alkaloid. Actually neoalatamine (**3**) was converted to alatamine (**4**) by acetylation with acetic anhydride in pyridine.

Alatusinine (**5**) is an amorphous solid, $C_{38}H_{47}O_{19}N$ ($[M]^+$ at m/z 821). Structural similarity between alatusinine (**5**) and wilfordine (**6**) [3, 4] was indicated by comparison of the ^1H NMR spectra of both alkaloids (Table 1). Furthermore, consideration of the molecular formula, the presence of six acetate groups (determined by ^1H NMR spectral analysis) and the absence of a benzoate

Table 1. ^{1}H NMR spectral data of alkaloids from *Euonymus alatus* forma *striatus**

Compound	H-1	H-2	H-3	H-4	H-5	H-6	H-7	H-8	H-11	H-12	H-14	H-15
1	5.74 (*d* 4)	5.30 (*ddd* 1, 2, 4)	4.90 (*dd* 1, 2)	2.74 (*ddq* 1, 1, 8)	6.62 (*s*)	2.44 (*d* 4)	5.57 (*dd* 4, 6)	5.37 (*d* 6)	4.48, 5.22 (AB*q* 14)	1.26 (*d* 8)	1.62 (*s*)	3.61, 5.57 (AB*q* 12)
2	5.62 (*d* 4)	5.16 (*dd* 3, 4)	4.94 (*d* 3)	—	6.91 (*s*)	2.33 (*d* 4)	5.53 (*dd* 4, 6)	5.35 (*d* 6)	4.45, 5.24 (AB*q* 14)	1.55 (*br s*)	1.64 (*s*)	3.77, 5.74 (AB*q* 12)
3	5.86 (*d* 3)	5.40 (*dd* 3, 3)	5.23 (*d* 3)	—	5.40 (*br s*)	3.20 (*s*)	—	5.62 (*s*)	4.68, 4.95 (AB*q* 13)	1.92 (*s*)	1.57 (*s*)	3.74, 5.92 (AB*q* 12)
4	5.88 (*d* 4)	5.41 (*dd* 4, 4)	5.13 (*d* 4)	—	6.76 (*s*)	3.05 (*s*)	—	5.60 (*s*)	4.82 (*s*)	†	1.57 (*s*)	3.75, 5.90 (AB*q* 12)
5	5.63 (*d* 4)	5.16 (*dd* 3, 4)	4.96 (*d* 3)	—	6.89 (*s*)	2.37 (*d* 4)	5.53 (*dd* 4, 6)	5.36 (*d* 6)	4.47, 5.32 (AB*q* 14)	†	1.63 (*s*)	3.74, 5.82 (AB*q* 12)
6	5.75 (*d* 3)	5.49 (*dd* 3, 3)	5.08 (*d* 3)	—	6.84 (*s*)	2.39 (*d* 4)	5.58 (*dd* 4, 6)	5.39 (*d* 6)	4.40, 5.58 (AB*q* 14)	†	1.65 (*s*)	3.75, 5.81 (AB*q* 12)
7	5.85 (*d* 3)	5.26–5.30 (2H, *m*)		—	6.56 (*s*)	2.28 (*d* 4)	5.58 (*dd* 4, 5)	5.47 (*d* 5)	4.37, 4.79 (AB*q* 14)	5.43 (*s*), 5.75 (*s*)	1.65 (*s*)	3.85, 5.30 (AB*q* 11)
8	5.85 (*d* 3)	5.38 (*ddd* 1, 3, 3)	4.89 (*dd* 1, 3)	2.91 (*ddq* 1, 1, 8)	6.50 (*s*)	3.12 (*s*)	—	5.56 (*s*)	4.52, 4.86 (*ABq* 13)	1.30 (*d* 8)	1.48 (*s*)	3.94, 5.39 (AB*q* 11)

*Chemical shifts (in δ values) relative to internal TMS. Multiplicities and coupling constant (Hz) are given in parentheses. Spectra were taken in $CDCl_3$ at 100 MHz (**1**, **5**, **7** and **8**), at 90 MHz (**2**, **3** and **6**) and at 80 MHz (**4**).

†The signals due to H-12 and the methyl group of the hydroxywilfordic acid part appeared at 1.53 and 1.74 in **4**, 1.46 and 1.60 in **5**, and 1.50 and 1.73 in **6**, respectively, but their assignments could not be made.

group in alatusinine (**5**) revealed that the benzoate group at C-2 in wilfordine (**6**) was replaced by an acetate group in alatusinine (**5**).

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR, 100 MHz, 80 MHz and 90 MHz, $CDCl_3$, TMS as int. standard. MS, direct inlet system, 70 eV. CC, silica gel BW-80 (Fuji-Davison) and Al_2O_3 activity II–III (Merck). TLC, silica gel 60 PF_{254} and Al_2O_3 PF_{254}-Type T (Merck).

Extraction. The fresh fruits (*ca* 27 kg) of *E. alatus* forma *striatus* (Thunb.) Makino collected in November at Mount Ibuki, Shiga prefecture, Japan (voucher No. KY-21, deposited at the Herbarium of the Laboratory of Organic Chemistry, Department of Chemistry, Nagoya University) were ground mechanically in MeOH (40 l.). The mixture was filtered with suction and the conc. filtrate (2 l.) was extracted with Et_2O (5 × 2 l.). The conc. Et_2O soln (5 l.) was extracted with 2.5% HCl (5 × 1 l.) and the combined aq. extracts after being made basic (pH 9) with K_2CO_3 were extracted with EtOAc (6 × 1.5 l.) The residue obtained on evapn of the EtOAc extracts was again dissolved in Et_2O (4 l.) and the Et_2O soln extracted with 2.5% HCl (5 × 1 l.). The acidic aq. extracts were made basic (pH 9) with K_2CO_3 and extracted with EtOAc (6 × 1 l.). The EtOAc extracts were dried (Na_2SO_4) and concd to give an oily mixture (19.9 g).

Isolation. The alkaloidal mixture (19.9 g) was chromatographed on silica gel (600 g) developed successively with $CHCl_3$ and $CHCl_3$–MeOH (99:1, 49:1 and 9:1). Fractions eluted with $CHCl_3$–MeOH (49:1) gave an oil (12.7 g), which was rechromatographed on silica gel (400 g) with $CHCl_3$ and $CHCl_3$–MeOH (99.5:0.5, 99:1, 98.5:1.5, 49:1 and 19:1) successively. Fractions eluted with $CHCl_3$–MeOH (99:1 and 98.5:1.5) afforded 9.9 g of an oil (fraction A) and those eluted with $CHCl_3$–MeOH (49:1) gave 680 mg of an oil (fraction B). Fraction A was further chromatographed on Al_2O_3 (400 g) with EtOAc–C_6H_6 (1:1); early fractions and later fractions yielded 248 mg of an oil (fraction C) and 79 mg of an oil (fraction D), respectively. Separation of fraction C by prep. TLC on silica gel with EtOAc–Et_2O (1:1) and HPLC [250 × 4.6 mm Zorbax ODS, MeOH–H_2O (2:1), flow rate 1 ml/min] afforded alatusamine (**1**) (36 mg, 0.00013%). Separation of fraction D by HPLC [250 × 10 mm Megapack SIL C_8, MeOH–H_2O (2:1), flow rate 2 ml/min] gave neoalatamine (**3**) (23.9 mg, 0.00009%). The later fractions obtained by CC of fraction B on silica gel (25 g) with EtOAc–C_6H_6 (1:1) afforded an oily residue, which was further separated by prep. TLC on silica gel with MeOH–Et_2O (1:19) and subsequently by prep. TLC on Al_2O_3

with EtOAc–C_6H_6 (3:2) to give alatusinine (**5**) (31.9 mg, 0.00012%).

Alatusamine (**1**). Amorphous solid (picrate mp 138–144°); $[\alpha]_D^{22}$ −11° ($CHCl_3$; *c* 3.0); UV λ_{max}^{EtOH} nm (ε): 221 (9000), 268 (3500); IR ν^{CHCl_3} cm^{-1}: 1730 (br), 1575 (br); ^1H NMR (Table 1); MS *m/z* 789 $[M]^+$. HRMS: Found 789.2836 $[M]^+$; $C_{38}H_{47}O_{17}N$ requires 789.2841.

Neoalatamine (**3**). Amorphous solid; $[\alpha]_D^{26}$ +42° ($CHCl_3$; *c* 1.1); UV λ_{max}^{EtOH} nm (ε): 228 (17 000), 267 (4100); IR ν^{CHCl_3} cm^{-1}: 3380, 1755, 1745, 1725 (sh), 1585, 1575; ^1H NMR (Table 1); MS *m/z* 797 $[M]^+$. HRMS: Found 797.2553 $[M]^+$; $C_{39}H_{43}O_{17}N$ requires 797.2531.

Alatusinine (**5**). Amorphous solid; $[\alpha]_D^{26}$ −16° ($CHCl_3$; *c* 0.6); UV λ_{max}^{EtOH} nm (ε): 223 (7600), 268 (3200); IR ν^{CHCl_3} cm^{-1}: 3480, 1750 (br), 1590, 1575; ^1H NMR (Table 1); MS *m/z* 821 $[M]^+$. HRMS: Found 821.2711 $[M]^+$; $C_{38}H_{47}O_{19}N$ requires 821.2741.

Transformation of euonine (**2**) *into alatusamine* (**1**). A soln of 4-dimethylaminopyridine (3.6 mg) in pyridine (*ca* 0.1 ml) was added to a soln of **2** (13.1 mg) in $SOCl_2$ (0.05 ml)–pyridine (0.5 ml) at 0°. The mixture was stirred at 50° for 17 hr, diluted with ice-H_2O, made basic (pH 9) with solid K_2CO_3 and extracted with Et_2O (20 ml). On removal of solvent an oily material (14.6 mg) was obtained, which was separated by prep. TLC on silica gel with EtOAc to give **2** (1.9 mg) and an olefin **7** (10.7 mg, 98% based on reacted **2**), amorphous powder; IR ν^{CHCl_3} cm^{-1}: 1740, 1585, 1570; ^1H NMR (Table 1); MS *m/z* 787 $[M]^+$. HRMS: Found 787.2704 $[M]^+$; $C_{38}H_{45}O_{17}N$ requires 787.2687. A soln of olefin **7** (6.8 mg) in MeCN (0.4 ml) was added to a soln of potassium azodicarboxylate (35.8 mg) at −30°. To the mixture was added a soln of HOAc (0.1 ml) in MeCN (0.9 ml) gradually over 2 hr. The mixture was stirred for 2.5 hr at −30 to −25°; the yellow colour of the mixture disappeared at the end of the reaction. The mixture was concd under red. pres., diluted with ice-H_2O, made basic with saturated $NaHCO_3$ soln and extracted with Et_2O (20 ml). Evapn of solvent afforded **1** (5.9 mg, 87%), which was shown to be pure by HPLC analysis. Identification was made by spectral (IR, ^1H NMR and MS) and HPLC comparison.

Conversion of neoalatamine (**3**) *to alatamine* (**4**). A soln of **3** (11.2 mg) in pyridine (0.5 ml) and Ac_2O (0.05 ml) was kept at room temp. for 7 hr. Additional Ac_2O (0.5 ml) was added to the mixture, which was kept at room temp. for 16 hr. Evapn afforded **4** (9.2 mg, 78%), mp 184–191° (MeOH), mmp 184–191°. Identification was also made by spectral (IR, ^1H NMR and MS) comparison.

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REFERENCES

1. Smith, R. M. (1977) in *The Alkaloids* (Manske, R. H. F., ed.), Vol. 16, p. 215. Academic Press, New York.
2. Brüning, R. and Wagner, H. (1978) *Phytochemistry* **17**, 1821.
3. Shizuri, Y., Yamada, K. and Hirata, Y. (1973) *Tetrahedron Letters* 741.
4. Yamada, K., Shizuri, Y. and Hirata, Y. (1978) *Tetrahedron* **34**, 1915.
5. Sugiura, K., Shizuri, Y., Yamada, K. and Hirata, Y. (1975) *Chem. Letters* 471.
6. Sugiura, K., Shizuri, Y., Yamada, K. and Hirata, Y. (1975) *Tetrahedron Letters* 2304.
7. Sugiura, K., Shizuri, Y., Hirata, Y. and Yamada, K. (1982) *Tetrahedron* **38**, 3465.
8. Sugiura, K., Yamada, K. and Hirata, Y. (1973) *Tetrahedron Letters* 113.
9. Pailer, M., Streicher, W. and Leitch, J. (1971) *Monatsh. Chem.* **102**, 1873.
10. Budzikiewicz, H., Römer, A. and Taraz, K. (1972) *Z. Naturforsch. Teil B* **27**, 800.