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ISOLATION AND STRUCTURES OF EUONYMINE AND NEOEUONYMINE, ALKALOIDS FROM *EUONYMUS SIEBOLDIANA* BLUME

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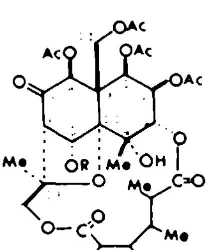
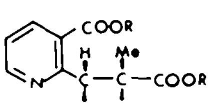
Abstract—Two new alkaloids, euonymine **5** and neoeuonymine **14** were isolated from *Euonymus Sieboldiana* Blume. The structures of euonymine and neoeuonymine were established to be **5** and **14**, respectively, by chemical and spectral means: the structural features of euonymine **5** are that it is a polyhydroxyl sesquiterpene of eudesman type (euonyminol) which is esterified with evoninic acid **3** and six moles of acetic acid. Neoeuonymine **14** was converted to euonymine **5**. Direct conversion of evonine **1** to euonymine **5** was also achieved.

Since the first publication appeared[1] on the presence of alkaloids in the plants of the *Celastraceae* family, isolation of alkaloidal components from those plants and their structural studies have been performed by a number of investigators.[2-6] Of those alkaloids, evonine **1** was one of the principal alkaloids obtained from *Euonymus europaea L.* (*Celastraceae* family) first by Doebel and Reichstein[5] and later by Pailer and Libiseller.[6a] The latter group established the structure of evoninic acid **3**,[6b] an alkaline hydrolysis product of evonine, and further revealed that evonine consisted of a C_{15}-polyhydroxy compound which was esterified by acetic acid (5 moles) and evoninic acid **3**.[6] During the course of our investigation on the alkaloidal components of *Euonymus Sieboldiana* Blume (Japanese name, Mayumi) evonine and neoevonine were isolated, and their whole structures were determined as **1** and **2**, respectively.[7,8]

Further investigation of the alkaloidal components of *Euonymus Sieboldiana* Blume resulted in the isolation of two new alkaloids, euonymine **5** and neoeuonymine **14**.[9] The full details of their structural studies are described in the present paper.



1: R Ac (evonine)
2: R = H (neoevonine)



3: R = H (evoninic acid)
4: R = Me

ISOLATION AND CHARACTERIZATION

The fruits of *Euonymus Sieboldiana* Blume were dried, powdered, and extracted with n-hexane and subsequently with ether. The extracts were concentrated to a small volume and shaken with dil. HCl solution. The aqueous phase was made basic (pH 9) with solid K_2CO_3 and extracted with AcOEt. The alkaloidal mixture obtained on concentration of the AcOEt solution was dissolved in EtOH. A crystalline mixture of evonine **1** and neoevonine **2** deposited in the EtOH solution was filtered, and the mother liquor, after concentration, was chromatographed on silicic acid to afford euonymine **5** and neoeuonymine **14**, in addition to evonine **1** and neoevonine **2**. Physical and spectral properties of the two new alkaloids are shown below.

Euonymine **5**: $C_{38}H_{47}NO_{18}$; amorphous powder (picrate m.p. 140–146°); $[\alpha]_D^{20}$ – 20° (c 0.32, $CHCl_3$); UV, λ_{max} (EtOH), nm 266, 230; IR ($CHCl_3$) 3550, 1750 (broad), 1585, 1565 cm^{-1}; NMR (Table); Mass, 805 (molecular ion peak).

Neoeuonymine **14**: $C_{36}H_{45}NO_{17}$; m.p. 259-262°; $[\alpha]_D^{20}$ – 11° (c 0.49, $CHCl_3$); UV, λ_{max} (EtOH), nm (ϵ) 266 (3500), 288 (4800); IR ($CHCl_3$) 3440, 1755 (broad), 1725 (shoulder), 1585, 1565 cm^{-1}; NMR (see Experimental); Mass, 763 (molecular ion peak).

Euonymine. Structural similarity between evonine **1** and euonymine **5** was indicated by comparison of the NMR spectra of both alkaloids (Table). Furthermore, the consideration of the molecular formula and the presence of six acetate groups (determined by NMR spectral analysis) in euonymine **5** suggested that a keto group of the C_{15}-part (evoninol) of evonine **1** would exist as a secondary acetoxyl group in euonymine **5**. A strong support for the above arguments was provided by the NMR spectral evidence: the signals in the NMR spectra of evonine **1** and euonymine **5** correspond well with each other except that there are observed the signals of an ABX type [H-6 (δ 2.33, dd, J = 3.8, 1.0 Hz), H-7 (δ 5.51, dd, J = 6.2, 3.8 Hz), H-8 (δ 5.34, d, J = 6.2 Hz)] in the NMR spectrum of eonymine **5**, while the signals due to H-6 and H-8 of evonine **1** appear as a doublet (δ 3.04, d, J = 1.0 Hz) and a singlet (δ 5.57), respectively. On the basis of the following chemical and spectral evidence the structure of euonymine was rigorously established as **5**.

Reduction of euonymine **5** with $LiAlH_4$ in THF–ether gave a diol **6**[6a,7d] and euonyminol **7**,[7d] the latter product being identified as its octaacetate **11**.[7d,10] This result indicates that euonymine **5** is an euonyminol derivative esterified with evoninic acid **3** and 6 moles of AcOH.

When euonymine **5** was subjected to the action of NaOMe in MeOH (30–35°, 6 h), two products were formed: hexadesacetyl euonymine **8**, $C_{26}H_{35}NO_{12}$ (m.p. 257–261°) and hexadesacetyl euonymine methyl ester **9**, $C_{27}H_{39}NO_{11}$ (amorphous powder). On acetylation of **8** with Ac_2O in pyridine, euonymine **5** was regenerated.

Table 1.* NMR spectral data (δ in ppm)

Compound	H-1	H-2	H-3	H-5	H-6	H-7	H-8	H-11 (CH_2)	H-15 (CH_2)
1**	5.71 (d 3.2)	5.29 (t 3.2)	4.78 (d 3.2)	6.72 (d 1.0)	3.04 (d 1.0)		5.57 (s)	4.58 4.82 (ABq 13.0)	3.76 (d 11.7) 6.04 (br.d 11.7)
5**	5.55 (d 4.0)	5.23 (dd 4.0, 2.5)	4.72 (d 2.5)	7.02 (d 1.0)	2.33 (dd 3.8, 1.0)	5.51 dd 6.2, 3.8	5.34 (d 6.2)	4.50 5.13 (ABq 13.5)	3.72 (d 12.0) 5.94 (br.d 12.0)
8			4.81 (d 3.0)	5.41 (d 1.0)					6.00‡ (br.d 12.0)
9			4.73 (d 3.0)	5.20 (d 1.0)					

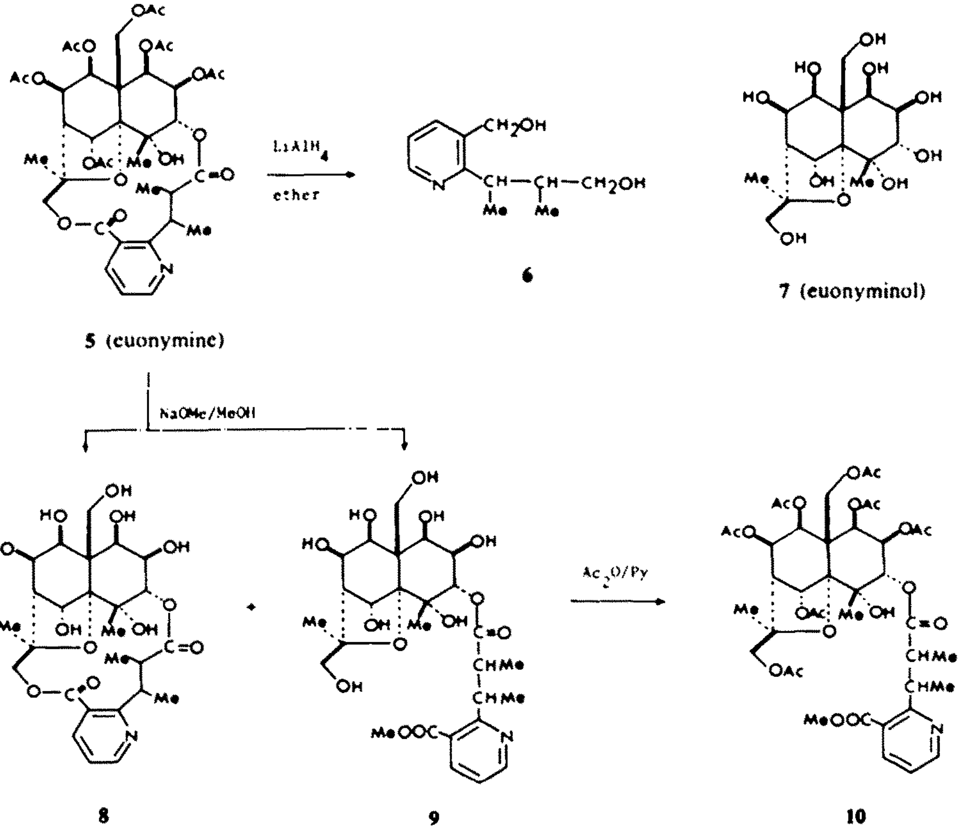
* Multiplicities and coupling constants (Hz) are given in parentheses. Spectra of 1 and 5 were taken in $CDCl_3$ at 100 MHz and those of 8 and 9 in acetone-D_6 at 60 MHz.

** All the couplings were confirmed by double resonance experiments.

‡ The location of the doublet in the region of δ 3 - 4 coupled to this signal could not be detected owing to the overlap with other signals.

For characterization, the product **9** was acetylated to give the crystalline heptaacetate **10**, $C_{41}H_{53}NO_{20}$ (m.p. 117–122°) (Scheme 1).

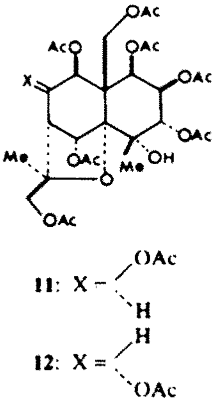
Comparison of the NMR spectra of euonymine **5** and the hexadesacetyl derivative **8** made it possible to determine the positions of six acetate groups in the sesquiterpene part (euonyminol) of euonymine **5**: whereas signals due to protons, H-1, H-2, H-5, H-7, H-8

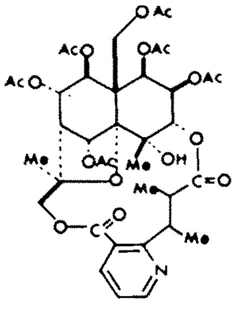


Scheme 1.

and H-11 shifted to a higher field region by conversion of **5** to **8**, essentially no change in the chemical shifts was observed as to protons, H-3 and H-15 in **5** and **8**. The above findings also reveal the location of evoninic acid residue in the sesquiterpene part: two hydroxyl groups on C-3 and C-15 are esterified with evoninic acid. Of the two ester linkages between evoninic acid and the euonyminol moiety, the ester group involved in methanolysis leading to **10** was shown to be the one formed between the aromatic carboxyl group of evoninic acid **3** and the primary hydroxyl group (C-15): while there was no significant difference in the chemical shifts of the signal due to H-3 in the NMR spectra of **5** and **9**, the signal of H-15 revealed an upfield shift in **9** in comparison with the corresponding one in **5**; and the singlet due to the methyl ester of **9** was observed at δ 3.99, indicating the presence of the aromatic methyl ester in **9**.[11] Based on these findings, the structure of euonymine is established to be **5**.

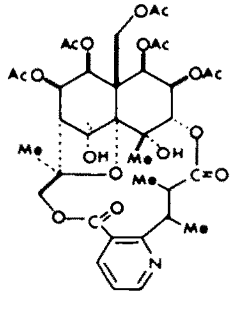
Transformation of evonine **1** into euonymine **5** was examined: reduction of evonine **1** by $NaBH_4$ in EtOH followed by acetylation with Ac_2O in pyridine afforded exclusively a diastereoisomer **13** of euonymine regarding C-7. However, when the $NaBH_4$ reduction was conducted in DMF as solvent and the product was acetylated, a mixture of euonymine **5** and its diastereoisomer **13** was obtained, which was separated by preparative TLC affording euonymine **5**. Thus, the direct correlation of evonine **1** to euonymine **5** was achieved. Inspection of the Dreiding models of evonine **1** suggests that the attack of a reagent towards the keto group at C-7 from the α-side would severely be hindered owing to the presence of the methyl group on C-13.





13

Neoeuonymine. Considering the molecular formula, neoeuonymine is a monodesacetyl derivative of euonymine **5**: actually neoeuonymine **14** was converted to euonymine **5** by acetylation with Ac_2O in pyridine. Since the signal corresponding to H-5 (δ 7.02) of euonymine **5** was not observed around δ 6–7 region in the NMR spectrum of neoeuonymine, the structure **14** was assigned to this alkaloid.



14 (neoeuonymine)

EXPERIMENTAL

Mp.s were uncorrected. UV spectra were measured in EtOH on a Perkin-Elmer Model 202 spectrophotometer. IR spectra were recorded with JASCO Model IRS and JASCO DS-402G instruments. NMR spectra were obtained using JNMC-60H, JNM 4H-100, and Varian HA-100 instruments; chemical shifts (δ) are reported in ppm downfield from internal TMS; signals arising from the sesquiterpene part are cited, unless otherwise stated. The mass spectra were determined on a Hitachi RMU-6C mass spectrometer equipped with a direct inlet system. Optical rotations were measured on an Oyo-denki Model MP-1 spectropolarimeter. For TLC silica gel GF_{254}, PF_{254} and alumina GF_{254}, PF_{254}-Type T (E. Merck, A.G., Germany) were used: thickness employed was 0.25 mm for analytical purpose, and 1.00 mm for preparative purpose. For column chromatography, silicic acid (100 Mesh, Mallinckrodt, U.S.A., and Silica Gel 60, No. 7734, E. Merck, A.G., Germany) and alumina (activity II–III, E. Merck, A.G., Germany) were used. The organic solutions were washed with saturated NaCl soln, dried over Na_2SO_4, and evaporated by vacuum rotary evaporator.

Isolation of euonymine **5** *and neoeuonymine* **14**. The seeds (*ca.* 225 kg) of *Euonymus Sieboldiana* Blume collected in November at Mt. Ibuki (Shiga Prefecture) were dried at room temp. for several days and then at 40° overnight, and ground. The powdered seeds were immersed in n-hexane (60 l) for a week at room temp. The mixture was filtered with suction. This procedure of n-hexane extraction was repeated twice. The combined filtrates were concentrated to give an orange soln (*ca.* 24 l), which was diluted with ether (*ca.* 24 l). The n-hexane–ether soln was extracted with 2.5% HCl five times (5 × 8 l). The combined aqueous layers were made alkaline (pH 10–11) with solid K_2CO_3. The aqueous mixture was extracted with AcOEt three times. The AcOEt extracts were washed with saturated NaCl soln, dried, and concentrated, yielding an oily alkaloidal mixture (*ca.* 42 g). After treatment with n-hexane, the powdered seeds were further extracted with ether twice (2 × 60 l). From the ethereal extracts an oily alkaloidal mixture (41 g) was obtained by the same procedure as described in the extraction using n-hexane. The amount of the total alkaloidal mixture was 83 g. The mixture was dissolved in EtOH, and the soln was allowed to stand, affording crude crystals (28 g) of **1** and **2**, which were filtered. The filtrate was concentrated to afford an amorphous residue, which was chromatographed on silicic acid (1500 g) with benzene–AcOEt (v/v, 1:1) as eluent. Early fractions gave crystalline evonine **1**, and subsequent fractions afforded colorless amorphous euonymine **5** (2.1 g). Further elution yielded neoevonine **2**. Then neoeuonymine **14** (0.5 g) was eluted. Euonymine **5** formed the picrate (prepared from benzene soln of picric acid), which was recrystallized from benzene. Neoeuonymine **14** was recrystallized from MeOH. Physical and spectral data of **5** and **14** are listed in the text. NMR (100 MHz, $CDCl_3$) of **14**: 5.57 (1H, d, J – 4.0, H-1), 5.28 (1H, dd, J = 4.5, 2.5, H-2), 4.73 (1H, d, J = 2.5, H-3), 2.42 (1H, dd, J = 4.0, 1.0, H-6), 5.48 (1H, dd, J = 6.0, 4.0, H-7), 5.34 (1H, d, J 6.0, H-8), 4.54 and 5.11 (2H, ABq, J = 13.5, H-11), 1.88 (3H, s, H-12), 1.66 (3H, s, H-14), 3.75 and 6.03 (2H, ABq, J – 12.0, H-15), 1.83, 1.95, 2.13, 2.15 and 2.19 (3H each, 5 × AcO). The picrate of **5** (Found: C, 50.79; H, 4.80; N, 5.25. $C_{44}H_{50}N_4O_{25}$ requires: C, 51.06; H, 4.87; N, 5.41%). **14** (Found: C, 56.77; H, 6.12; N, 1.62. $C_{36}H_{45}NO_{17}$ requires: C, 56.61; H, 5.94; N, 1.83%).

Reduction of **5** *with* $LiAlH_4$: *diol* **6**, *euonyminol* **7**, *and euonyminol octaacetate* **11**. A soln of **5** (720 mg) in dried THF (20 ml) was added with stirring to a soln of $LiAlH_4$ (940 mg) in THF (30 ml)–anhydrous ether (13 ml) under ice-bath cooling. The mixture was stirred at room temp. for 12 h. The excess reagent and the complex were decomposed by adding H_2O (4 ml) slowly. The mixture was filtered and the solid was washed with AcOEt. The combined filtrates were evaporated to give a residue, which was purified by preparative TLC (silica gel) with AcOEt, giving

6.[6a,7d] Identification of **6** with the authentic specimen [7d] was made by comparison of spectral (IR, NMR and MS) data and TLC behavior. The solid was dissolved in AcOH–H_2O (1:1) and the soln was passed through a column of ion-exchange resin Dowex 50W-X8 (H form). Evaporation of the eluate afforded a colourless amorphous residue (155 mg). A soln of the resinous product in Ac_2O (2.5 ml) and pyridine (3 ml) was stirred at 60° for 13 h. Concentration of the soln afforded a residue, which was purified by preparative TLC (silica gel) with benzene–AcOEt (1:1). Crude crystals were recrystallized from EtOH to give pure euonyminol octaacetate **11**[7d] (170 mg), m.p. 192.5–193°. Identification was carried out by m.m.p. and IR spectral comparison with the authentic specimen.[7d]

Hexadesacetyl euonymine **8** *and hexadesacetyl euonymine methyl ester* **9**. To a soln of **5** (126 mg) in anhydrous MeOH (10 ml) was added a soln (0.25 ml) of NaOMe in MeOH (prepared from 100 mg of Na and 20 ml of anhydrous MeOH) under N_2. The soln was stirred at 30° for 5 h. After cooling the soln was passed through a column of ion-exchange resin Amberlite IRC-50 (H form). The methanolic eluate was concentrated to give a mixture, which showed two spots on TLC (silica gel) (hexadesacetyl euonymine **8** was the less polar substance). The mixture was separated by preparative TLC (silica gel) with $CHCl_3$–MeOH (88:12), affording **8** and **9**. Recrystallization of **8** from EtOH gave pure **8** (35 mg). **9** was obtained as colourless amorphous powder (30 mg). **8**; m.p. 257–261°; UV λ_{max} nm (ϵ) 263 (2800), 228 (3900); IR (KBr) 3420 (broad), 1730, 1585, 1565 cm^{-1}; NMR (Table); Mass 553 (M^+). (Found: C, 56.74; H, 6.48; N, 2.53. $C_{26}H_{35}NO_{12}$ requires: C, 56.41; H, 6.37; N, 2.53%). **9**; IR (KBr) 3400 (broad), 1730, 1585, 1570 cm^{-1}; NMR (Table); Mass 567 (M^+-18).

Acetylation of hexadesacetyl euonymine methyl ester **9**. A soln of **9** (79 mg) in AC_2O (1.5 ml) and pyridine (1.5 ml) was stirred at 60° overnight, concentrated and diluted with H_2O (40 ml). The mixture was extracted with four 20 ml portions of AcOEt. The combined AcOEt layers were dried and evaporated to give an oily product (123 mg), which was chromatographed on neutral alumina (3 g) with AcOEt. Recrystallization from n-hexane–ether yielded **10** (87 mg), m.p. 117–122°; UV λ_{max} nm (ϵ) 266 (2900), 225 (5600); IR ($CHCl_3$) 3550, 1740 (broad), 1585, 1570 cm^{-1}; NMR (60 MHz, $CDCl_3$) 6.70 (1H, s, H-5), 5.69 (1H, d, J = 3.0, H-1), 5.22 (1H, t, J = 3.0, H-2), 4.72 (1H, d, J = 3.0, H-3), 3.90 (3H, s, COOMe), 2.25, 2.19, 2.13, 2.10, 2.08, 2.00 and 1.90 (3H each, s, 7 × AcO), 1.60 (3H, s, $\text{Me}-\overset{|}{\underset{|}{\text{C}}}-$), 1.47 (3H, s, $\text{Me}-\overset{|}{\underset{|}{\text{C}}}-$); Mass 879 ($M^+$). (Found: C, 55.73; H, 6.15; N, 1.57. $C_{41}H_{53}NO_{20}$ requires: C, 55.97; H, 6.07; N, 1.59%).

Conversion of evonine **1** *to euonymine* **5**. To a soln of **1** (30 mg) in DMF (3.6 ml) was added $NaBH_4$ (27 mg). The mixture was stirred at room temp. for 11 h, diluted with H_2O (6 ml), and extracted with four 15 ml portions of AcOEt. The combined AcOEt layers were dried and concentrated to give a resinous material (29 mg). A soln of the product in Ac_2O (1.5 ml) and pyridine (1.5 ml) was stirred at 60° for 14 h and concentrated, affording a residue, which showed two spots on TLC (alumina) (**5** was the more polar substance). The mixture was separated by preparative TLC (alumina) with benzene–AcOEt(3:1) to give **5** (amorphous powder, 5 mg) and **13** (amorphous powder, 14 mg) in pure state, respectively. Identification was performed by spectral (IR, NMR and MS) and TLC comparison.

Conversion of neoeuonymine **14** *to euonymine* **5**. A soln of **14** (55 mg) in Ac_2O (0.5 ml) and pyridine (0.7 ml) was stirred at 50° for 13 h, concentrated, and diluted with H_2O (20 ml). The mixture was extracted with four 15 ml portions of AcOEt. The AcOEt extracts were dried and concentrated to give a residue (63 mg), which was purified by preparative TLC (silica gel) with benzene–AcOEt (1:1). There was obtained an amorphous powder (53 mg), which was identified as **5** by IR spectral and TLC comparison. Further, the product was converted to the crystalline picrate, m.p. 138–145°, which was proved to be identical with the picrate of euonymine by m.m.p.

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[8] Pailer also reported the structure **1** for evonine: M. Pailer, W. Streicher and J. Leitch, *Monatsh. Chem.* **102**, 1873 (1971).

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[10] Stereochemistry of C-7 in euonyminol **7** was determined by the following evidence.[7c] Since the coupling constant of the doublet due to H-8 was 6.0 Hz in euonyminol octaacetate **11** and 10.0 Hz in isoeuonyminol octaacetate **12** in their NMR spectra, the relationship between H-7 and H-8 is *cis* (equatorial–axial) for the former compound and *trans* (axial–axial) for the latter (the detailed NMR spectra of both compounds are shown in Ref. 7*d*). Further, the nuclear Overhauser effect was observed between H-5 (δ 6.51) and H-7 (δ 5.57) in isoeuonyminol octaacetate **12**: on irradiation of H-7 the intensity of the H-5 signal was increased (13%).

[11] Two methyl ester singlets at δ 3.92 and 3.39 of dimethyl evoninate **4** were assigned to the aromatic one and the aliphatic one, respectively.[7d]