NOTE

(24 *E*)- and (24 *Z*)-24-Ethylidenecycloartanols from *Neolitsea sericea*

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Two new triterpene alcohols separated as a mixture from the unsaponifiable lipid of a stem extract of *Neolitsea sericea* were identified as (24 *E*)- and (24 *Z*)-24-ethylidenecycloartanols.

1 Introduction

We have recently demonstrated that the unsaponifiable lipid of the stem extract of *Neolitsea sericea* Koidz. (Japanese name : Shiroadamo) contain three Δ^8 -lanostene triterpene alcohols : (24 *E*)-24-ethylidenelanost-8-enol [**2a**, (24 *E*)-24-ethylidene-5 α -lanost-8-en-3 β -ol], its (24 *Z*)-isomer (**2b**) and 24-methylenelanost-8-enol (**2c**), in addition to the common plant triterpene alcohols such as 24-methylenecycloartanol (**1c**, 24-methylene-9 β , 19-cyclo-5 α -lanostan-3 β -ol), cycloartenol (24-dehydrocycloartanol), β -amyrin (5 α -olean-12-en-3 β -ol) and lupeol [5 α -lup-20(29)-en-3 β -ol]¹⁾. Occurrence of Δ^8 -lanostene triterpene alcohols in higher plants is very rare²⁾, and *N. sericea* constituted the first case among higher plants in which the occurrence of 24-ethylidenelanost-8-enols (**2a** and **2b**) has been demonstrated. This characteristic feature of *N. sericea* prompted us to undertake more thorough investigation, and this paper described that our further study on the triterpene alcohol fraction obtained from the *N. sericea* extract led to the detection and characterization of two 24-ethylidenecycloartanols, *i.e.*, (24 *E*)-24-ethylidenecycloartanol (**1a**) and its (24 *Z*)-isomer (**1b**), which were considered to be new natural products.

2 Experimental

2.1 General Methods and Materials

Argentation (silver nitrate-silica gel, 1 : 4)

preparative thin layer chromatography (TLC : 0.5 mm thick silica gel) was developed 3 times with $\text{CCl}_4\text{-CH}_2\text{Cl}_2$ (5 : 1). Preparative high performance liquid chromatography (HPLC) was carried out on a Partisil 5 ODS-2 column (25 cm $\times$ 10 mm i.d.) with MeOH as a mobile phase (flow rate, 4 mL/min) using an RI detector. Gas liquid chromatography (GLC) was performed on a SCOT OV-17 glass capillary column (30 m $\times$ 0.3 mm i.d.) at the column temp. of 255°C. R_f values on the argentation TLC and relative retention time (RRT) values on the HPLC and GLC were taken relative to cholesterol (cholest-5-en-3 β -ol) acetate. Low-resolution mass spectra (EI-MS, 70 eV) were taken by a combined gas chromatograph-mass spectrometer (GC-MS) under the following GLC conditions : column, DB-1 fused silica column (15 m $\times$ 0.26 mm i.d.), splitless mode, column temp. 175°C (2 min) then elevated to 270°C (32°C/min). High-resolution EI-MS (70 eV) were taken by means of a probe

injection. ^1H NMR spectra (400 MHz) were recorded in a CDCl_3 solution, with tetramethylsilane as an internal standard. Acetylation was performed in acetic anhydride-pyridine overnight at room temperature. The acetates of the four triterpene alcohols, **1c**, **2a**, **2b**, and **2c**, were used as the reference specimens¹⁾. The stems of *N. sericea* were collected at the bank of Akigawa river of Nishi-Tama area of Tokyo in 1986.

2.2 Isolation of Triterpene Alcohols

Dried and milled *N. sericea* stems (9.6 kg) were refluxed in CH_2Cl_2 for 8 h giving extracted lipid (40 g). Saponification of the lipid with 5 % KOH in MeOH under reflux followed by extraction with isopropyl ether gave unsaponifiable lipid (14 g). Column chromatography (CC) of the unsaponifiable lipid of silica gel (200 g) [hexane (1 L), hexane- Et_2O (9 : 1, 1 L, then 4 : 1, 1 L), hexane- EtOAc (6 : 1, 2 L)] gave the triterpene alcohol fraction (2.5 g) (The elution was monitored by TLC on precoated silica gel,) which was subjected again to CC [silica gel, 200 g; eluent, hexane- EtOAc (6 : 1)] yielding the purified triterpene alcohol fraction (1.5 g). This was acetylated, and the acetate fraction (1.4 g) was subjected to argentation TLC to give four major bands. The second least polar band ($R_f=0.51$) afforded a fraction (100 mg) which contained the acetates of **1a** and **1b** in addition to several other components. This was further fractionated by HPLC into two fractions (A and B) among the other fractions : fraction A ($RRT=0.94$ on HPLC, 12 mg, mixture of the acetates of **2a** and **2b**) and fraction B ($RRT=1.09$ on HPLC, 2 mg, mixture of the acetates of **1a** and **1b**).

2.3 The MS Data of the Acetates of **1a** and **1b**

Mixture of (24E)-(1a) and (24Z)-24-Ethylidenecycloartanol (1b) acetates (Fraction B). High-resolution MS : m/z 496.4277 ($[\text{M}]^+$, $\text{C}_{34}\text{H}_{56}\text{O}_2$, rel. int. 12 %, requires 496.4266), 481.4042 ($\text{C}_{33}\text{H}_{53}\text{O}_2$, 7 %), 436.4066 ($\text{C}_{32}\text{H}_{52}$, 39 %), 421.3882 ($\text{C}_{31}\text{H}_{49}$, 20 %), 398.3182 ($\text{C}_{27}\text{H}_{42}\text{O}_2$, 2 %), 396.3753 ($\text{C}_{29}\text{H}_{48}$, 5 %), 393.3518 ($\text{C}_{27}\text{H}_{45}$, 10 %), 367.3363 ($\text{C}_{27}\text{H}_{43}$, 7 %), 357.2790 ($\text{C}_{24}\text{H}_{37}\text{O}_2$, 3 %), 339.3049 ($\text{C}_{25}\text{H}_{39}$, 6 %), 338.2972 ($\text{C}_{25}\text{H}_{38}$, 6 %), 314.2971 ($\text{C}_{23}\text{H}_{38}$, 8 %), 297.2581 ($\text{C}_{22}\text{H}_{33}$, 11 %), 295.2424 ($\text{C}_{22}\text{H}_{31}$, 2 %), 255.2110 ($\text{C}_{19}\text{H}_{27}$, 5 %), 241.1955 ($\text{C}_{18}\text{H}_{25}$, 2 %), 55.0547 (C_4H_7 , 100 %).

(24E)-24-Ethylidenecycloartanol(1a)acetate.

Low-resolution MS (by GC-MS) : m/z 496 ($[\text{M}]^+$, rel. int. 12 %), 481 (9 %), 436 (62 %), 421 (30 %), 393 (13 %), 367 (10 %), 357 (4 %), 339 (12 %), 338 (7 %), 326 (11 %), 314 (15 %), 297 (13 %), 295 (7 %), 255 (3 %), 55 (100 %).

(24Z)-24-Ethylidenecycloartanol(1b)acetate.

Low-resolution MS (by GC-MS) : m/z 496 ($[\text{M}]^+$, rel. int. 12 %), 481 (6 %), 436 (47 %), 421 (28 %), 398 (4 %), 393 (15 %), 367 (8 %), 357 (3 %), 339 (11 %), 338 (15 %), 326 (4 %), 314 (9 %), 297 (13 %), 295 (7 %), 255 (7 %), 241 (3 %), 5 (100 %).

3 Results and Discussion

The mass spectrum of the HPLC fraction B ($RRT=1.09$ on HPLC) eventually separated from the acetylated triterpene alcohol fraction of *N. sericea* by virtue of the procedure described in the Experimental section showed $[\text{M}]^+$ at m/z 496, corresponding to $\text{C}_{34}\text{H}_{56}\text{O}_2$ as has been determined by high-resolution MS, accompanied with the fragmentation ions at m/z 481 $[\text{M}-\text{Me}]^+$, 436 $[\text{M}-\text{HOAc}]^+$ and 421 $[\text{M}-\text{Me}-\text{HOAc}]^+$ indicating that it was an acetate of C_{32} triterpene alcohol. Other ions at m/z 357 $[\text{M}-\text{C}_{10}\text{H}_{19}$ (side chain)] $^+$, 297 (m/z 357- HOAc), 295 (m/z 297-2 H), 255 [m/z 297- C_3H_6 (ring D)] and 241 (m/z 255- CH_2) suggested that the triterpene alcohol was tetracyclic with C_{10} side chain and two double bonds, one of which was located in the skeleton and the other was in the side chain. Further significant ions at m/z 398 $[\text{M}-\text{C}_7\text{H}_{14}]^+$ and 338 (m/z 398- HOAc) due to a McLafferty rearrangement involving cleavage of the C-22, C-23 bond with one H transfer from C-20, suggested that the side chain double bond was located at either the $\Delta^{24(25)}$ -or the $\Delta^{24(28)}$ -position^{3),4)}. Furthermore, the ion at m/z 314 $[\text{M}-\text{C}_{11}\text{H}_{18}\text{O}_2]^+$, corresponded to the loss of ring A and characteristic for the 9β , 19-cyclo tetracyclic triterpenes^{5),6)}, has suggested the presence of 9β , 19-cyclo group rather than the double bond in the skeleton. The skeletal proton signals observed in the ^1H NMR spectrum (Table -1) of fraction B at δ 0.340 and 0.577 (each 1 H and d , 19- H_2), 0.847 (3 H, s , 30- H_3), 0.889 and 0.899 (each 3 H and s , 31- H_3 and 32- H_3), 0.972 (3 H, s , 18- H_3), 2.052 (3 H, s , 3β - OAc) and 4.566 (1 H, dd , 3α -H), were almost consistent with the corresponding signals for 24-methylene-

Table-1 ^1H NMR data of the acetyl derivatives of some triterpene alcohols (400 MHz, CDCl_3 , TMS as int. standard)^{a)}

Protons	Acetate ; chemical shifts (δ , ppm)				
	Fraction B ^{b)}		Authentic triterpenes		
	1 a	1 b	1 c	2 a	2 b
18-H ₃ (s)		0.972	0.965	0.968	0.962
19-H ₂ (<i>d</i> each)/		0.340 (4.1)	0.341 (4.4)		
19-H ₃ (s)		0.577 (3.9)	0.578 (4.1)	1.007	1.005
30-H ₃ (s)		0.847	0.848	} 0.876 } 0.882 } 0.884	} 0.882
31-H ₃ (s)	}	0.889	0.889		
32-H ₃ (s)		0.899	0.903		
21-H ₃ (<i>d</i>)	0.971 (5.7)	0.936 (6.3)	0.896 (5.5)	0.964 (6.3)	0.921 (6.3)
26-H ₃ (<i>d</i>)	}	0.981 (7.1)	} 1.026 (6.9) } 1.031 (6.9)	} 0.981 (6.6) } 0.984 (6.9)	} 0.979 (6.9)
27-H ₃ (<i>d</i>)					
29-H ₃ (<i>d</i>)	1.576 (6.3)	1.592 (6.6)	—	1.576 (6.6)	1.591 (6.6)
3 β -OAc(<i>s</i>)		2.052	2.051	2.050	2.048
3 α -H(<i>dd</i>)	4.566 (10.2, 4.4)		4.567 (11.3, 5.8)	4.502 (11.5, 4.7)	4.502 (11.5, 4.7)
25-H(<i>septet</i>)	2.207 (7.0)	2.832 (7.2)	2.236 (6.7)	2.204 (6.9)	2.831 (6.9)
28-H(<i>q</i>)/28-H ₂	5.184 (6.6)	5.115 (6.6)	4.664(<i>d</i> , 1.4) 4.716(<i>s</i>)	5.184 (6.9)	5.113 (6.8)

a) Figures in parentheses denote J values (Hz).b) Mixture of the C-24 isomers (1 a : 1 b=40 : 60) separated from *Neolitsea sericea* in this study.

cycloartanol (1 c) acetate (Table-1)⁷⁾ and, hence, the triterpene of fraction B was regarded to possess a cycloartane (1) skeleton. The ^1H NMR suggested moreover that the triterpene (with skeleton 1) of fraction B possesses 24-ethylidene substituted side chain. Presence of both C-24 stereoisomers [1 a (24 *E*) and 1 b (24 *Z*)] was shown by exhibiting the following side chain ^1H signals (Table-1): 21-H₃ (d) (1 a : δ 0.971; 1 b : δ 0.936), 26-H₃ and 27-H₃ (d) (1 a and 1 b : δ 0.980), 29-H₃ (d) (1 a : δ 1.576; 1 b : 1.592), 25-H (septet) (1 a : δ 2.027; 1 b : δ 2.832) and 28-H (q) (1 a : δ 5.184; 1 b : 5.115). The signal assignment was made by comparison with the corresponding signals of (24 *E*)-(2 a) and (24 *Z*)-isomers (2 b) of 24-ethylidenelanost-8-enol acetate (Table-1)¹¹⁾. Thus, fraction B was a mixture of the acetates of C-24 isomeric (24 *E*)-24-ethylidenecycloartanol (1 a) and its (24 *Z*)-isomer (1 b).

Fraction B showed two peaks with RRT=2.65 (relative proportion, 40 %) and 2.77 (60 %) on GLC of which the former was regarded to be the (24 *E*)-isomer (1 a) and the latter was the (24 *Z*)-isomer (1 b)^{11,8)}. The GLC correlation supported the structure of 24-ethylidenecycloartanol. The cycloartane (1)/ Δ^8 -lanostene (2) separation

factor on GLC was calculated as 1.176 based on the retention data of the acetates of 1 c (RRT=2.07) and 2 c (RRT=1.76). Since the RRT of 24-ethylidenelanost-8-enols were 2.25 (2 a-acetate) and 2.35 (2 b-acetate), the RRT of 24-ethylidenecycloartanols were estimated to be 2.65 (1 a-acetate) and 2.76 (1 b-acetate) which were almost consistent with the observed retention data. The MS data of 1 a- and 1 b-acetates (see Experimental section) determined individually by GC-MS showed any discrepancy from that obtained as a mixture by a probe injection. The content of 1 a and 1 b in the total triterpene alcohol mixture of *N. sericea* was estimated as 0.2 % and 0.3 %, respectively, based on the GLC data of the acetylated triterpene alcohol fraction. The fraction A (RRT=0.94 on HPLC) separated eventually by HPLC from the acetylated triterpene alcohol fraction of *N. sericea* (see Experimental section) was found to be consisted of 2 a- and 2 b-acetates based on the GLC and GC-MS data.

This study has thus demonstrated the occurrence of both (24 *E*)-(1 a) and (24 *Z*)-isomers (1 b) of 24-ethylidenecycloartanol, which are considered to be new triterpene alcohols, in addition to their Δ^8 -isomers (2 a and 2 b) in

N. sericea. The presence of the 24-ethylidene triterpene alcohols in *N. sericea* is of interest from the viewpoint of sterol biogenesis since the second methylation at C-24 of 24-methylene compounds to give 24-ethylidene homologs occurs at first at the level of 4 α -monomethylsterol in most of higher plants⁹⁾. The occurrence of Δ^8 -lanostene compounds as well as the (24 *E*)-ethylidene compounds further constituted the characteristic feature of *N. sericea*.

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しろだも (*Neolitsea sericea*) からの (24 *E*)-
及び (24 *Z*)-24-エチリデン
シクロアルタノール

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しろだも (*Neolitsea sericea*, くすのき科) 幹部抽出油の不けん化物に 2 種の構造未知のトリテルペンアルコールを検出し、それらが (24 *E*)-及び (24 *Z*)-24-エチリデンシクロアルタノールの構造を持つ新化合物であることを明らかにした。

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