Composite Constituent: Lactucenyl Acetate, a Novel Migrated Lupane Triterpenoid from *Lactuca indica* Revision of Structure of Tarolupenyl Acetate

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Lactucenyl acetate (1), a new member of migrated lupane triterpenoids was isolated from *Lactuca indica* and its structure was elucidated on the basis of spectral analyses. The structure of tarolupenyl acetate was revised as lup-19(21)-en-3β-yl acetate (2).

Key words triterpenoid; lactucenyl acetate; *Lactuca indica*; tarolupenyl acetate; Compositae

Composite plants of subfamily, Cichorioideae produce a significant amount of latex, in which several triterpenoids are reported. Our phytochemical investigations have revealed that these plants are rich sources of triterpenoids having extended structural diversity.[1—8] *Lactuca indica* L. (called as wild lettuce, "Akino-nogeshi" in Japanese) is widely distributed from Europe to Asia, and it is also found widely at the roadside by the fields and on the hills in Japan. Previously, Hui and Lee have reported that β-amyrin acetate (**3**), germanicyl acetate (**4**), taraxasteryl acetate (**5**) as well as some triterpenoid alcohols and sterols in this plant.[9] In this paper, we describe the detailed investigation of triterpenoid constituents of *L. indica*, especially in the triterpenoid acetates fraction, a novel migrated lupane triterpenoid, designated as lactucenyl acetate (**1**), together with six known triterpenoids were isolated. During the structure elucidation of **1**, we noticed that previously reported structure of tarolupenyl acetate, from *Taraxacum japonicum*,[1] was erroneously formulated as **1**, therefore structure of tarolupenyl acetate is revised as lup-19(21)-en-3β-yl acetate (**2**).

From the *n*-hexane extract of the fresh roots of *L. indica* by the method as described in experimental section, a novel triterpenoid, designated lactucenyl acetate (**1**) as well as six known triterpenoids such as β-amyrin acetate (**3**),[10] germanicyl acetate (**4**),[10] taraxasteryl acetate (**5**),[11] α-amyrin acetate (**6**),[12] bauerenyl acetate (**7**),[13] and lupenyl acetate (**8**)[14] were isolated. The structures of these compounds were determined by spectroscopic methods mainly 1D-NMR and MS and also comparing with in the literatures. The molecular formula of **1** was determined to be $C_{32}H_{52}O_2$ by high resolution (HR) MS. Its 600 MHz ^{1}H-NMR spectrum displayed signals for eight methyl groups, of which six are tertiary and two secondary. The equal coupling constant of 6.7 Hz of both the methyl doublets implied the presence of isopropyl moiety, thus **1** is triterpenoid with hopane or lupane skeleton. Besides, the spectrum showed the presence of an acetoxy methine proton and one vinylic methine proton. The splitting pattern of the vinylic proton (dd, J=2.8, 7.9 Hz) is diagnostic of pentacyclic triterpenoids with Δ^{14} double bond.[15] Moreover, the carbinyl proton signal at δ_H 4.46 appeared as a double doublet with J=5.3 and 10.8 Hz, demonstrating that the acetoxyl group is equatorially oriented. Its ^{13}C-NMR spectrum (Table 1) exhibited the presence of 30 skeletal carbons, of which eight were primary, nine secondary, seven tertiary and six quaternary carbons. The chemical shifts of C-1 to C-12 and C-23 to C-26 were found to be very close to those of taraxeryl acetate (**9**, Fig. 1).[1,8] But the chemical shifts of C-17 to C-22 and C-28 to C-30 in the ring E moiety differed from those of **9** and pteron-14-ene, migrated hopane triterpenoid.[16] Therefore **1** might be a migrated lupane triterpenoid with Δ^{14} double bond rather than a migrated hopane triterpenoid. The fragment ions in the low resolution (LR) MS at *m*/*z* 344, 257 and 204 (Fig. 2) are also diagnostic of pentacyclic triterpenoids with Δ^{14} double bond.[17]

The aforementioned observations indicated that **1** should be a migrated lupane or hopane derivative with Δ^{14} double bond. Detailed analysis of the heteronuclear multiple bond correlation (HMBC) spectrum of **1** led to the partial structure **1a** (Fig. 3) that clearly indicated a migrated lupane skeleton with Δ^{14} double bond. Although the connectivity between C-18 and C-19 could not be established from the HMBC spectrum, it could definitely be ascertained from the ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum. The remaining C–C bond connectivity was also determined from the ^{1}H–^{1}H COSY and ^{1}H–^{13}C correlation spectroscopy (^{1}H–^{13}C COSY) spectra (Fig. 3). The relative stereochemistry at most of the

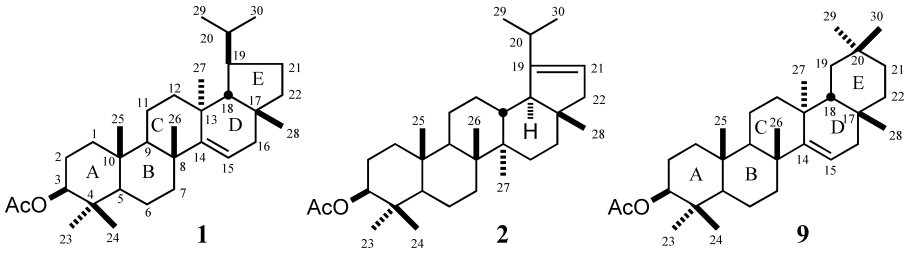

Fig. 1. Structures of Compounds **1**, **2** and **9**

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skeletal chiral centers, particularly the orientation of H_3-28, H-18 and H-19 in the E ring as β, β and α, respectively, were deduced from the nuclear Overhauser effect (NOE) interactions observed in its NOESY spectrum (Fig. 3). Thus, based on the above evidence, lactucenyl acetate was represented by structure **1**.

In an earlier communication,[1)] we reported the characterization of tarolupenyl acetate, isolated from *Taraxacum japonicum*, as structure **1** mainly on the basis of 100 MHz NMR and mass spectral analyses and its acid-induced rearrangement products. Recently, during the structure elucidation of **1** by HMBC spectral analysis, we noticed that the previously reported structure of tarolupenyl acetate must be erroneous. We, therefore, undertook a detailed analysis with the HMBC and other 2D-NMR spectra, and newly recorded MS of tarolupenyl acetate. All the ^{13}C- and ^{1}H-NMR chemical shifts were assigned on the basis of ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and HMBC spectra. The HMBC correlations clearly demonstrated the presence of the partial structure (**2a**) as shown in Fig. 3.

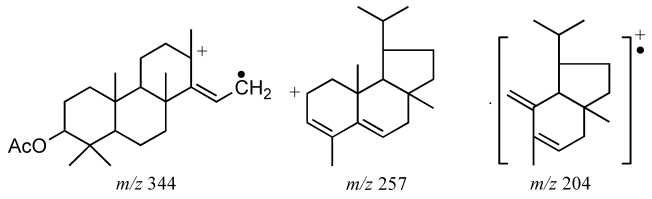

Fig. 2. Diagnostic Fragment Ions of Δ^{14} Triterpenoids

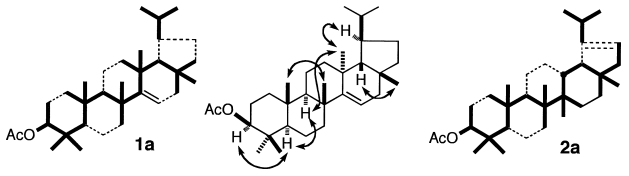

Fig. 3. 2D-NMR Correlations of Compounds **1** and **2**

It can be seen therein that both the protons of the secondary methyl groups (H_3-29 and H_3-30) have two- or three-bond correlations with the olefinic quaternary carbon (δ 155.01) unambiguously indicating that the tri-substituted double bond must be located at $\Delta^{19(21)}$ position in the E ring of a migrated lupane skeleton rather than at Δ^{14} position in the D ring as reported earlier. The vinylic methine carbon (δ 120.43) was also found to be correlated with H_2-22. The connectivities among the proton-bearing vicinal carbon atoms were confirmed by ^{1}H–^{1}H COSY (Fig. 3). On the basis of the above evidence, the structure of tarolupenyl acetate is revised to be lup-19(21)-en-3β-yl acetate (**2**).

It should be noted that the migrated lupane triterpenoids have limited distribution, which is in sharp contrast with the migrated oleanane triterpenoids. These have been found in Genera *Lactuca* and *Taraxacum*[1)] (Compositae), *Tylophora*[18)] and *Ceropegia*[19)] (Asclepidaceae), *Swertia*[20)] (Gentianaceae), and *Cymbopogon*[21)] (Gramineae). Biological activities of lu-

Table 1. ^{1}H- (600 MHz) and ^{13}C- (150 MHz) NMR Spectral Data for Compounds **1**, **2** and **9** in $CDCl_3$

Position	**1**		**2**		**9**	
	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)
1	37.32	1.03, 1.61	38.34	1.04, 1.69	37.32	1.03, 1.61
2	23.47	1.64, 1.64	23.71	1.64, 1.64	23.42	1.63, 1.63
3	81.04	4.46 (dd, 5.3, 10.8)	80.98	4.48 (dd, 5.3, 10.8)	80.99	4.46 (dd, 5.1, 10.0)
4	37.67	—	37.79	—	37.65	—
5	55.53	0.87	55.31	0.81	55.58	0.88
6	18.69	1.46, 1.58	18.26	1.40, 1.52	18.65	1.48, 1.60
7	41.57	1.35, 1.98	34.12	1.40, 1.40	41.15	1.36, 2.03
8	38.58	—	41.05	—	38.95	—
9	49.13	1.28	50.02	1.39	49.14	1.44
10	37.88	—	37.04	—	37.85	—
11	17.32	1.49, 1.65	20.96	1.34, 1.50	17.49	1.46, 1.63
12	33.45	1.55, 1.55	25.71	1.35, 1.95	33.63	1.54, 1.63
13	38.99	—	35.30	1.97	37.51	—
14	159.42	—	43.38	—	157.93	—
15	116.67	5.51 (dd, 2.8, 7.9)	27.48	1.01, 1.72	116.91	5.53 (dd, 3.1, 8.2)
16	38.22	1.94, 2.04	33.65	1.48, 1.61	37.65	1.64, 1.92
17	43.47	—	45.99	—	35.78	—
18	61.85	1.06	52.83	2.15	48.66	0.95
19	47.44	1.77	155.01	—	36.61	0.96, 1.31
20	30.81	1.69	29.09	2.38	28.79	—
21	25.61	1.47, 1.57	120.43	5.32 (ddd, 3.2, 2.8, 1.4)	33.05	1.25, 1.33
22	41.28	1.35, 1.47	45.27	1.82, 1.82	35.06	1.01, 1.37
23	28.00	0.85	27.95	0.85	27.95	0.88
24	16.62	0.87	16.48	0.84	16.58	0.86
25	15.29	0.94	16.05	0.86	15.49	0.95
26	26.04	1.04	15.94	1.04	25.92	1.09
27	18.94	0.94	14.41	0.97	21.26	0.90
28	32.38	0.85	19.15	0.82	29.80	0.82
29	17.59	0.83 (d, 6.7)	22.57	0.98 (d, 6.7)	33.33	0.95
30	22.97	0.90 (d, 6.7)	22.30	1.01 (d, 6.7)	29.91	0.91
$\underline{C}H_3CO$–	21.33	2.05	21.33	2.05	21.35	2.05
$CH_3\underline{C}O$–	171.02		171.03		171.04	

pane triterpenoids and their synthetic analogs have been studied intensively,[22] whereas those of migrated lupane triterpenoids have not been undertaken yet. Discovery of a novel migrated lupane skeleton from *L. indica* demonstrated that Compositae plants (especially, subfamily Cichorioideae) have ability to produce variety of migrated lupane triterpenoids, and further investigation of this class of plants will play crucial roles for establishment of triterpenoid library and for structure–activity relationships of lupane-series triterpenoids.

Experimental

General Experimental Procedures Melting point (mp) was measured on Yanagimoto Micro Melting Point Apparatus without correction. Specific rotation was determined using a JASCO DIP-140 digital polarimeter in $CHCl_3$ at 23 °C. IR spectra were recorded with KBr tablets on JASCO A-102 infrared spectrophotometer. HPLC was performed using Senshu Pak ODS-3251D (8 mm i.d.×250 mm) column with a JASCO 880-PU pump and JASCO 830 RI detector. NMR spectra were obtained with Bruker AV 600 spectrometer using tetramethylsilane as an internal standard. Mass spectra were obtained with a JEOL JMS-HX100 spectrometer under electronic impact at 30 eV.

Plant Material Fresh roots of *L. indica* were collected from Hino City, Tokyo in July, 1988, and taxonomically identified by Kohji Tanaka, Emeritus Professor of Showa Pharmaceutical University. Voucher specimens were deposited at the Herbarium of Showa Pharmaceutical University, Machida, Tokyo.

Extraction and Isolation Fresh roots of *L. indica* (2.0 kg) were extracted with *n*-hexane for 5 h three times each followed by filtration and evaporation to yield the extract (8.0 g) which have been kept in the freezer at −60 °C. The hexane extract was subjected to column chromatography (CC) on silica gel and eluted with a gradient solvent system of *n*-hexane, *n*-hexane–benzene (8 : 2), benzene, benzene–diethyl ether (9 : 1) and diethyl ether to yield 9 fractions, L1—L9. The fraction L3 (3.1 g) eluted with the *n*-hexane–benzene (8 : 2), was subjected to 20% $AgNO_3$-impregnated silica gel CC eluting with *n*-hexane–benzene (8 : 2) to give 4 fractions, L3A—L3D. The isolation of **3** (425 mg), **6** (418 mg), **7** (95 mg) and **4** (46 mg) were carried out by repeated re-crystallization of the fraction L3A (1.2 g) from acetone. Then, the filtrate L3A—F was subjected to HPLC [CH_3CN–$CHCl_3$ (9 : 1)] to yield **1** (19 mg). The fractions L3B and L3C repeatedly re-crystallized from acetone to afford **5** (135 mg). The fraction L3D was repeatedly re-crystallized from acetone and MeOH to yield **8** (141 mg).

Lactucenyl Acetate (**1**): Colorless plates. mp 213—214 °C. $[\alpha]_D^{23}$ +41 (c=0.4, $CHCl_3$). IR (KBr) cm^{-1}: 1733, 1249. ^{1}H- and ^{13}C-NMR date are shown in Table 1. HR-electron ionization (EI)-MS *m/z*: 468.3979 (Calcd for $C_{32}H_{52}O_2$: 468.3967, M^+). MS *m/z* (rel. int.): 468 (M^+, 9), 453 (7), 408 (3), 393 (7), 344 (74), 329 (36), 284 (19), 269 (31), 257 (10), 204 (100), 189 (26).

Tarolupenyl Acetate (**2**): MS *m/z* (rel. int.): 468 (M^+, 5), 453 (8), 425 (37), 365 (18), 249 (8), 204 (35), 203 (23), 191 (40), 189 (53), 135 (100). ^{1}H- and ^{13}C-NMR date are shown in Table 1. Other physical and spectral data have been reported previously.[1]

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