# Isolation of Magnolialide and Artesin from *Cichorium intybus*: Revised Structures of Sesquiterpene Lactones

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**Abstract** – Two known eudesmanolides, magnolialide and artesin, were isolated from the roots of *Cichorium intybus*. Their structures were confirmed by HMBC and NOESY NMR spectral interpretation. Therefore, guaianolides and eudesmanolides that have been previously reported should be revised.
**Key words** – *Cichorium intybus*, Compositae, eudesmanolide, magnolialide, artesin, NMR studies

## Introduction

The processed roots of *Cichorium intybus* (Chicory) have been used as a substitute for coffee in Europe. While this herbaceous plant is not native to Korea, it is now cultivated in the alpine area of Korea. Sultana *et al.* (1995) reported that crude extracts of this plant inhibit free radical-mediated DNA damage. Seto *et al.* (1988) reported that various types of sesquiterpene lactones were responsible for its cytotoxic activity, and discussed the structure-cytotoxicity relationships. Various triterpenoids, such as triterpene alcohol, triterpene acetate and triterpene ester, and phytosterols have been isolated from this plant (Josephy & Radt, 1940). In addition, several sesquiterpene lactones with guaiane, eudesmane and germacrane skeletons have been reported. (El-Masry *et al.*, 1984; Monde *et al.* 1990; Pyrek, 1985; Seto *et al.*, 1988; Peters *et al.*, 1996)

To examine the cytotoxic mechanisms and the contents of several varieties of *Cichorium* spp. in Korea, we isolated two known eudesmanolides. The structures of these compounds were identified as magnolialide (1β-hydroxyeudesma-4,13-dien-6,12-olide, **1**) and its 11β,13-dihydro derivative (artesin, **2**) by interpretation of their 2D-NMR spectra and mass fragmentation patterns. Although ^{1}H-NMR

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spectra and molecular ions in the mass spectra of these two compounds were identical to those of eudesmanolide (called cichoriolide A) isolated by Seto *et al.* (1988) and those of guaianolides isolated by El-Masry *et al.* (1984) from *Cichorium intybus*, we propose here different sesquiterpene lactones structurally different from them. Hence, we are convinced that previously reported structures should be revised to be consistent with the structures reported here.

## Materials and Methods

**General experimental procedures** – Melting points were uncorrected. Optical rotation was measured on a JASCO DIP-360 digital polarimeter at 25°C. IR spectra were recorded on a Hitachi 260-01 spectrometer in KBr disks. EI-MS (ionization voltage, 70 eV) were measured with a Finnigan Mat TSQ-700. ^{1}H-, ^{13}C-NMR, HMBC and NOESY spectra were taken on a Bruker DRX-300 spectrometer with TMS as the internal standard.

**Plant material** – The roots of *Cichorium intybus* were collected in September at the National Alpine Agricultural Experiment Station, RDA, Pyongchang, Korea, and the plant was identified by Dr. K.O. Yoo (National Alpine Agricultural Experiment Station of Korea). A voucher specimen was also deposited in the same location.

**Extraction and Isolation** – Dried roots (1.5 kg) of *Cichorium intybus* were pulverized and extracted three times with MeOH under reflux. The MeOH extract was filtered and evaporated under reduced pressure to give a viscous residue (390 g). This material was suspended in $H_2O$ and then partitioned with a $CHCl_3$-soluble fraction, an EtOAc-soluble fraction and a *n*-BuOH-soluble fraction, respectively, to give a $CHCl_3$-soluble fraction (245 g) an EtOAc-soluble fraction (46 g) and an *n*-BuOH-soluble fraction (30 g). Part of the $CHCl_3$-soluble fraction (20 g) was subjected to column chromatography on silica gel (Merck, Art No. 7734). The column was eluted with a gradient of *n*-hexane-EtOAc (10:1→10:3), and eight subfractions were collected. Subfraction 4 (83 mg) was found to be a mixture of germanicol and taraxasterol by GC-MS analysis with authentic samples. Subfraction 2 (95 mg) was revealed to be a mixture of the acetate of germanicol and taraxasterol by GC-MS analysis in comparison with authentic samples. Subfraction 1 (37 mg) was found to be a mixture of palmitate of germanicol and taraxasterol by GC-MS analysis of its alkaline hydrolysate. Subfraction 5 was found to be composed of β-sitosterol, campesterol and stigmasterol by NMR and GC analyses. Subfraction 8 was also found to be a mixture of oleanolic acid and ursolic acid by co-TLC, HPLC and NMR analysis. (Ahmad *et al.*,

1994) Subfraction 7 was found to be a mixture of two compounds by GLC, with retention times of 5.18 min and 5.41 min. {column: OV-1 (15 m×0.53 mm×2.65 μm capillary; oven temp.: 40°C (4 min, 10°C/min) −220°C (final temp.); Injection temp.: 300°C; Detection Temp.: 300°C; carrier: 20 ml/min (helium)) From subfraction 7, compounds **1** (15 mg, 25% yield) and **2** (26 mg, 43% yield) were isolated by RP-18 reversed-phase column chromatography (YMC GEL ODS-A, 5×50 cm, elution: MeOH/H$_2$O (2:5, v/v) by HPLC (CAPCELL PAK C-18, 4×250 mm, Shiseido, elution: MeOH/H$_2$O, 2:3, v/v).

Part of the ethyl acetate fraction (20 g) was subjected to column chromatography on silica gel to give subfraction 9. This subfraction was recrystallized from MeOH to give colorless needles. This compound was identified as 11β,13-dihydrolactucin by comparison of the NMR data with literature values. (Kisiel *et al.*, 1993; Sarg *et al.*, 1982) $^{13}$C-NMR spectral data of 11β,13-dihydrolactucin were fully assigned by 2D-NMR spectral data for the first time.

**Compound 1** – Colorless solid; 152-153°, $[\alpha]_D$ +74.6° (c, 0.16, CHCl$_3$); IR (KBr) cm$^{-1}$: 3400 (OH), 1780 (lactone); MS *m/z* (%): 248.2 [M]$^+$ (36), 230.2 [M-H$_2$O]$^+$ (96), 204.2 [C$_{13}$H$_{16}$O$_2$]$^+$ (100), 191.2 (37), 163.2 [C$_{10}$H$_{11}$O$_2$]$^+$ (57); $^1$H-NMR and $^{13}$C-NMR δ: Table 1.

**Compound 2** – Colorless solid; IR (KBr) cm$^{-1}$:

**Table 1.** NMR spectral data of compounds **1** and **2** (300 MHz, CDCl$_3$)

| Position | 1 | | 2 | |
|---|---|---|---|---|
| | $^1$H | $^{13}$C (DEPT) | $^1$H | $^{13}$C (DEPT) |
| 1 | 3.55 (dd, J=11.4, 3.0 Hz) | 78.1 (CH) | 3.53 (dd, J=11.4, 3.0 Hz) | 78.1 (CH) |
| 2 | 1.72 (m) | 27.4 (CH$_2$) | 1.72 (m) | 27.4 (CH$_2$) |
| 3α | 1.97 (m) | 33.6 (CH$_2$) | 1.97 (m) | 33.6 (CH$_2$) |
| 3β | 2.17 (m) | | 2.17 (m) | |
| 4 | – | 126.7 (C) | 1.94 (m) | 126.3 (C) |
| 5 | – | 129.3 (C) | – | 129.3 (C) |
| 6 | 4.57 (m) | 83.5 (CH) | 4.59 (m) | 83.5 (CH) |
| 7 | 2.58 (dt, J=3.1, 9.3) | 50.0 (CH) | 1.71 (m) | 53.2 (CH) |
| 8β | 1.62 (m) | 23.4 (CH$_2$) | 1.50 (m) | 24.7 (CH$_2$) |
| 8α | 2.14 (m) | | 1.94 (m) | |
| 9 | 1.31 (m) | 38.8 (CH$_2$) | 1.31 (m) | 38.7 (CH$_2$) |
| | 2.10 (m) | | 2.10 (m) | |
| 10 | – | 42.4 (C) | – | 42.3 (C) |
| 11 | – | 139.4 (C) | 2.25 (dq, J=11.5, 7.0 Hz) | 41.5 (CH) |
| 12 | – | 171.0 (C) | – | 179.4 (C) |
| 13α | 6.15 (d, J=3.1 Hz) | 119.0 (CH$_2$) | 1.23 (d, J=7.0 Hz) | 13.1 (CH$_3$) |
| 13β | 5.48 (d, J=3.1 Hz) | | – | |
| 14 | 1.10 (d, J=2.4) | 18.8 (CH$_3$) | 1.11 (d, J=2.4 Hz) | 18.8 (CH$_3$) |
| 15 | 1.85 (br s) | 20.0 (CH$_3$) | 1.87 (br s) | 20.0 (CH$_3$) |

–: protons absent

3400 (OH), 1770 (lactone); MS $m/z$ (%): 250.2 $[M]^+$ (33), 232.2 $[M-H_2O]^+$ (18), 206.2 $[C_{13}H_{18}O_2]^+$ (100), 193.2 (62), 165.2 $[C_{10}H_{13}O_2]^+$ (34); $^1$H-NMR and $^{13}$C-NMR δ: Table 1.

**Compound 3 – 11β,13-dihydrolactucin (3)**: Colorless needles; 3450 (OH), 1770 (lactone); $^1$H-NMR (CDCl₃) δ: 1.17 (3H, d, $J = 6.9$ Hz), 1.87 (1H, m, H-7), 2.14 (1H, dd, $J = 10.7$ & 3.0, H$_b$-9), 2.15 (3H, s, H-14), 2.31 (1H, q, H-11), 2.50 (1H, dd, $J = 10.7$ & 13.7, Ha-9), 3.27 (1H, d, $J = 10.1$ Hz, H-5), 3.37 (1H, m, H-6), 3.39 (1H, m, H-8), 4.10 (1H, d, $J = 18.4$ Hz, Ha-15), 4.61 (1H, d, $J = 18.4$ Hz, H$_b$-15), 6.19 (1H, s); $^{13}$C-NMR (CDCl₃) δ: 132.2 (C-1), 195.3 (C-2), 132.9(C-3), 173.8 (C-4), 49.0(C-5), 81.0 (C-6), 61.3 (C-7), 68.8 (C-8), 49.0 (C-9), 147.7 (C-10), 41.6 (C-11), 178.0 (C-12), 15.6 (C-13), 21.8 (C-14), 62.3 (C-15): MS m/z: 278 $[M]^+$

## Results and Discussion

The CHCl₃ fraction of the MeOH extract of *Cichorium intybus* roots was chromatographed over Si gel to give subfraction 1-8. Several kinds of triterpenoids were identified, such as germanicol and taraxasterol (subfraction 1), their acetates (subfraction 2) and their palmitates (subfraction 1) by GC-MS data in comparison with authentic specimens. Subfractions 7 and 8 showed cytotoxic activity against various cell lines among all of the tested subfractions. (data not shown) Subfraction 8 was found to be a mixture of oleanolic acid and ursolic acid by comparison of NMR spectral data with the literature. (Ahmad *et al.*, 1994) Subfraction 7 was separated by preparative HPLC to yield compounds 1 and 2.

When the $^1$H-NMR spectral data of 1 were compared with those of 10β-hydroxyguaia-4,13-dien-6,12-olide reported by El-Masry *et al.* (1984) and 3β-hydroxyeudesma-4,13-dien-6,12-olide (cichoriolide A) reported by Seto *et al.*, (1988), our data agreed with the previous reports. The structures suggested by El-Masry are 10β-hydroxyguaianolides with an α-methylene-γ-lactone and a 13α-methyl-γ-lactone, respectively. In that paper, abnormal downfield shift of the resonance at δ 3.55 due to H-1 in the $^1$H-NMR spectrum was attributed to the presence of the 10β-hydroxy group. However, we clarified that the signal at δ 3.55 should be assigned to H-1 in a eudesmane skeleton by a thorough interpretation of the 2D-NMR spectra (Table 1). A 1β-hydroxy linkage in a eudesmanolide skeleton can be explained by the
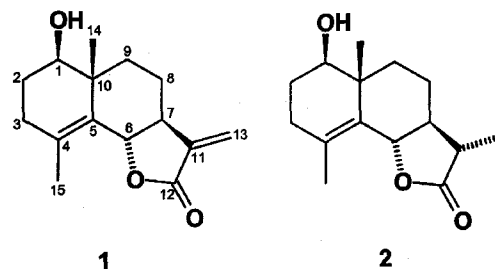


**Fig. 1.** Chemical structures of 1 and 2.

HMBC correlation shown by Fig.1. Therefore, the chemical shift at δ 3.55 is in a comparatively lower-field region due to the inductive effect of 1-hydroxy group in a eudesmanolide, rather than to an angular proton neighboring $C_{10}$-OH in a guaianolide. In the HMQC NMR spectrum of 1, the signal at δ$_H$ 3.55 was correlated to the resonance at δ$_C$ 78.1, indicating a secondary alcohol. A peak at δ 42.4 in the $^{13}$C-NMR spectrum was assigned to C-10 because it was found to be a quaternary carbon in the HMQC and DEPT NMR spectra. The signals at δ 4.57 and δ 2.58 were assigned to 6β-H and 7α-H, respectively, due to the *trans* junction of the α-methylene-γ-lactone with the B-ring of a eudesmane skeleton. The protons of 13-exomethylene were observed at δ 6.15 (d, $J = 3.1$ Hz) and δ 5.48 (d, $J = 3.1$ Hz). Thus, the third ring was shown to be an α-methylene-γ-lactone.

Meanwhile, the signals at δ$_C$ 126.7 and δ$_C$ 129.3 were found to be quaternary carbons in the DEPT NMR spectrum. Therefore, these two olefinic carbons must be located at C-4 and C-5, and this was confirmed by the HMBC correlation of H-6 (δ$_H$ 2.58) to these olefinic carbons (Fig. 1). In the mass spectrum of 1, a molecular ion peak and a $[M-H_2O]^+$ ion were found at $m/z$ 248 and $m/z$ 230, respectively. As shown in Fig. 3, the presence of the double bond between C-4 and C-5, and the absence of a 10-hydroxy group were revealed by a *retro* Diels-Alder
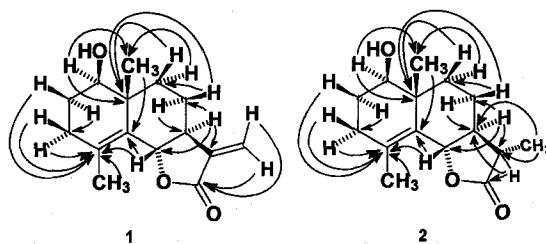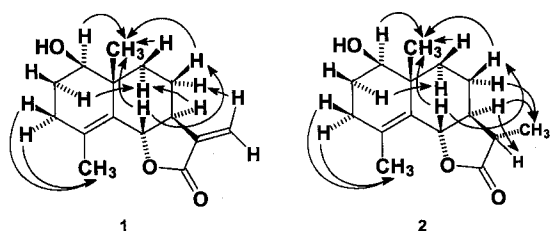


**Fig. 2.** HMBC correlations of 1 and 2.

**Fig. 3.** NOESY correlations of **1** and **2**.

fragmentation to yield an *m/z* 204. $^{13}$C-NMR spectral data were assigned, as shown in Table 1, on the basis of $^1$H-$^1$H-COSY, DEPT, HMQC, HMBC and NOESY spectral data. All chemical shifts were in accordance with those of cichoriolide A (3-hydroxy-eudesma-4,13-dien-6,12-olide), while the assignments were quite different from those in the literature. (Seto *et al.*, 1988) Meanwhile, H$_\alpha$-1 and H$_\beta$-9 are both equatorial in the steric structure since these two protons were correlated each other in NOESY spectrum, as shown in Fig. 2. Further, H$_\alpha$-1 was not correlated with H-14 or H$_\alpha$-9 in this spectrum. Thus, the configuration of the hydroxy attachment at C-1 was found to be β. Finally, the structure of **1** was determined to be 1β-hydroxyeudesma-4,13-dien-6,12-olide. Although this compound called magnolialide (El-Ferlay *et al.*, 1979) was isolated from *Magnolia grandiflora* as a novel eudesmanolide, it has never been reported from Cichorium species. Therefore, a eudesmanolide and a guaianolide suggested by Seto (1988) and El-Masry (1984), respectively, should be revised to magnolialide. All assignments for the $^{13}$C-NMR spectral data of magnolialide are given for the first time in the present study.

The other sesquiterpene lactone isolated from subfraction 7 by preparative HPLC was revealed to be an 11β,13-dihydro derivative of **1** by the interpretation of $^1$H-$^1$H COSY, HMQC, HMBC and NOESY NMR spectra as well as the mass spectrum. The correlations in the HMBC and NOESY spectra are shown in Fig. 1 and Fig. 2, respectively. In the NOESY correlation of **2** (Fig. 2), the configuration of 13-methyl was found to be α because 6β-H was correlated with 11β-H. The prominent ions in the mass spectrum, such as *m/z* 250 [M$^+$], *m/z* 232 [M-H$_2$O]$^+$ and *m/z* 206 [C$_{13}$H$_{18}$O$_2$]$^+$ were found by *retro* Diels-Alder fragmentation, and the ion at *m/z* 165 [C$_{13}$H$_{18}$O$_2$-isopropenyl]$^+$, indicates 1-hydroxyeudesmanolide. Thus, compound **2** was elucidated to be 1β-hydroxyeudesma-4-en-6,12-olide. This structure has been reported
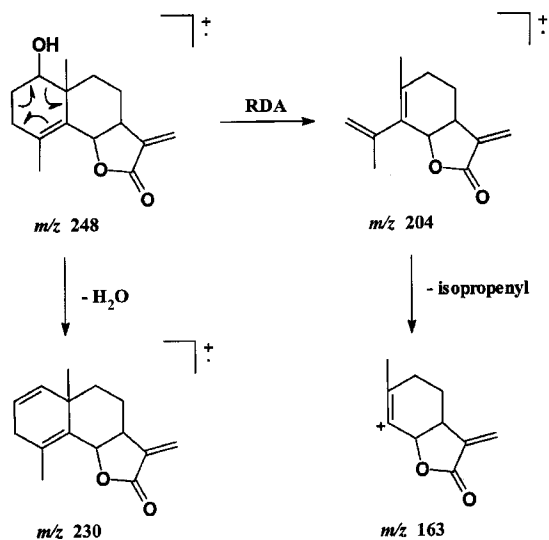


**Fig. 4.** Mass spectral fragmentation of **1**.

under the name artesin from *Artemisia barrelieri* and *Artemisia herba-alba subsp. Herba-alba*, but has not been reported from Cichorium species. Therfore, the guaianolide suggested by El-Masry *et al.* (1984) should be revised to artesin. $^{13}$C-NMR data of **2** were in good agreement with the result of $^{13}$C-NMR assignment of artesin suggested by Marco (1989) and Villar *et al.* (1983). In addition, the $^{13}$C-NMR spectral data of 11β,13-dihydrolactucin (**3**) were assigned for the first time by 2D-NMR spectroscopy.

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