Arch. Pharm. Res. Vol.19, No.5, pp. 406-410, 1996

Isolation of Handelin from *Chrysanthemum boreale*

Sam Sik Kang, Ju Sun Kim, Kun Ho Son[1], Chong Ock Lee[2] and Young Hee Kim[3]

Natural Products Research Institute, Seoul National University, Seoul 110-460, [1]Department of Food and Nutrition, Andong National University, Andong 760-749, [2]Pharmaceutical Screening Lab., Korea Research Institute of Chemical Technology, P.O. Box 107, Daedeog-Danji, Taejeon 305-606 and [3]College of Natural Sciences, Sangji University, Wonju 220-702, Korea

(Received May 30, 1996)

The flowers of *Chrysanthemum boreale* afforded handelin, a unique guaianolide dimer and a mixture of *n*-hydrocarbons and *n*-hydrocarbon alcohols in addition to β-sitosterol and β-sitosterol glucoside. Detailed analysis of the ^{1}H- and ^{13}C-NMR spectra of handelin was carried out by the application of two-dimensional ^{1}H- ^{1}H-COSY and ^{1}H- ^{13}C multiple-bond, multiple-quantum spectroscopic correlation techniques. Handelin was inactive in the *in vitro* anti-tumor activity.

Key words : *Chrysanthemum boreale*, Compositae, Guaianolide dimer, Handelin, NMR assignment, Anti-tumor activity

INTRODUCTION

The dried flower and petal of *Chrysanthemum boreale* Mak. (Compositae) and other related species such as *C. indicum* L. and *C. lavandulaefolium* Mak. have been used in China to relieve certain hypertensive symptoms (Huang, 1993). And the flowers of *C. boreale* have been used as an antipyretic, and also to treat vertigo and red eyes (Perry, 1980). The isolation of flavonoids and polyacetylenes from this plant has been reported (He *et al.*, 1982; Bohlmann *et al.*, 1960, 1964; Matsuo *et al.*, 1974), but there are no reports on the pharmacological work of this plant. Recently we reported that flavonoids from this plant showed strong aldose reductase inhibitory activity (Shin *et al.*, 1995). Further investigation has led to the isolation of a unique dimeric guaianolide, handelin together with *n*-hydrocarbons and *n*-hydrocarbon alcohols. In this paper, we report the isolation, structure elucidation and cytotoxic activity of handelin.

MATERIALS AND METHODS

The general experimental methods and plant material are the same as those described in Shin *et al.* (1995). GC/MS was measured on a Hewlett-Packard 5988 MS. GC was done on a Hewlett-Packard 5890 II gas chromatograph [Conditions : column, ULTRA-1 50 m × 0.2 mm, column temperature, 40°C (4 min) to 300°C with 10°C/min, carrier gas, He (0.5 ml/min), detector : FID]. NMR spectra were measured on either a Varian Gemini 2000 (300 MHz), a Bruker AM-300 (300 MHz), or a Bruker AMX-500 (500 MHz) spectrometer in pyridine-d_5.

Correspondence to: Sam Sik Kang, Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea

Isolation

The dried flowers (2.15 kg) were extracted with hot MeOH in a water bath three times, which was again separated into CH_2Cl_2, EtOAc and *n*-BuOH soluble fractions. The CH_2Cl_2 fraction (143.9 g) was subjected to SiO_2 column chromatography with *n*-hexane-EtOAc gradient system (5 : 1 to 5 : 5 and then EtOAc) to give 17 subfractions. Recrystallization of subfractions 2, 4, 5, 14 and 17 afforded compounds I (**1**), II (**2**), III (**3**), IV (**4**) and V (**5**), respectively. A further chromatography of subfraction 3 with *n*-hexane-EtOAc yielded compound III (**3**).

n-Hydrocarbons (1)

Semisolid from MeOH (1.85 g), IR ν_{max} (KBr) cm^{-1} 2957, 2918, 2849 (CH), 1473, 1464 (CH_2), 1379 (CH_3), 729, 720 [$(CH_2)n$]. GC/MS t_R 28.45 min [n-$CH_3(CH_2)_{19}CH_3$] : *m/z* 57, 71, 85, 113, 141, 169, 225, 239, 296 $[M]^+$; t_R 30.56 min [n-$CH_3(CH_2)_{21}CH_3$] : *m/z* 57, 71, 85, 113, 155, 183, 225, 267, 324 $[M]^+$; t_R 31.60 min [n-$CH_3(CH_2)_{22}CH_3$] : *m/z* 57, 71, 85, 113, 155, 207, 253, 281, 338 $[M]^+$; t_R 32.86 min [n-$CH_3(CH_2)_{23}CH_3$] : *m/z* 57, 71, 85, 127, 155, 197, 239, 281, 352 $[M]^+$; t_R 34.16 min [n-$CH_3(CH_2)_{24}CH_3$] : *m/z* 57, 71,

85, 127, 183, 207, 281, 309, 366 $[M]^+$; t_R 31.60 min [*n*-$CH_3(CH_2)_{25}CH_3$] : *m/z* 57, 71, 85, 127, 169, 211, 281, 309, 380 $[M]^+$; t_R 37.53 min [*n*-$CH_3(CH_2)_{26}CH_3$] : *m/z* 57, 71, 85, 127, 207, 281, 309, 394 $[M]^+$; t_R 39.56 min [*n*-$CH_3(CH_2)_{27}CH_3$] : *m/z* 57, 71, 85, 155, 183, 225, 281, 323, 408 $[M]^+$; t_R 45.35 min [*n*-$CH_3(CH_2)_{29}CH_3$] : *m/z* 57, 71, 85, 141, 211, 239, 323, 436 $[M]^+$.

n-Hydrocarbon alcohols (2)

Amorphous from MeOH (214 mg), IR ν_{max} (KBr) cm^{-1} 3306 (OH), 2918, 2849 (CH), 1473, 1462 (CH_2), 1063 (C-O), 729, 720 [(CH_2)n]. GC/MS t_R 32.56 min [*n*-$CH_3(CH_2)_{20}CH_2OH$] : *m/z* 55, 83, 111, 139, 195, 209, 252, 280, 308 $[M-H_2O]^+$; t_R 33.91 min [*n*-$CH_3(CH_2)_{21}CH_2OH$] : *m/z* 43, 69, 111, 153, 182, 223, 282, 322 $[M-H_2O]^+$; t_R 35.56 min [*n*-$CH_3(CH_2)_{22}CH_2OH$] : *m/z* 43, 83, 111, 153, 195, 237, 280, 336 $[M-H_2O]^+$; t_R 35.71 min [*n*-$CH_3(CH_2)_{23}CH_2OH$] : *m/z* 43, 83, 111, 125, 153, 209, 250, 279, 322, 350 $[M-H_2O]^+$; t_R 39.73 min [*n*-$CH_3(CH_2)_{24}CH_2OH$] : *m/z* 57, 83, 125, 167, 208, 252, 294, 336, 364 $[M-H_2O]^+$; t_R 42.00 min [*n*-$CH_3(CH_2)_{25}CH_2OH$] : *m/z* 43, 83, 125, 167, 208, 265, 378 $[M-H_2O]^+$; t_R 45.45 min [*n*-$CH_3(CH_2)_{26}CH_2OH$] : *m/z* 55, 83, 125, 167, 237, 264, 306, 392$[M-H_2O]^+$.

β-Sitosterol (3)

Amorphous from MeOH (54 mg), mp 131~2°, IR ν_{max} (KBr) cm^{-1} 3424 (OH), 2961, 2936, 2868 (CH), 1638 (C=C), 1460 (CH_2), 1381(CH_3), 1055 (C-O), 839 (C=CH). It was identified as β-sitosterol by direct comparison with an authentic sample.

Handelin (4)

Amorphous from MeOH (540 mg), mp 235~7°, UV λ_{max} nm 204 (end absorption); IR ν_{max} (KBr) cm^{-1} 3463 (OH), 2975, 2942, 2868 (CH), 1753, 1745 (lactone), 1725,1248 (OAc), 1453, 1152, 1024, 988, 930, 910, 814, 799; EI-MS (70 eV), *rel. int.* (%), *m/z* 456 $[M-2H_2O-HOAc]^+$ (0.5), 288 $[C_{17}H_{22}O_5-H_2O]^+$ (1.7), 246 $[C_{15}H_{18}O_3]^+$ (33.9), 228 $[C_{17}H_{22}O_5-H_2O-HOAc]^+$ (100), 213 (45.3), 203 (36.4), 200 (31.5); ^{1}H- and ^{13}C-NMR :

Table 1. NMR chemical shifts and correlations of handelin (**4**) in pyridine-d_5

Carbon No.	δ_H	δ_C	DEPT	HMBC
C-1	ca 2.75 (m)	55.97	CH	
C-2	ca 2.06 (m)	33.56	CH_2	
C-3	5.53 (brs)	126.25	CH	C-1, C-2, C-4, C-5
C-4	-	144.71	C	
C-5	3.03 (brt, 9.2)	54.70	CH	C-6
C-6	4.09 (t, 9.2)	79.41	CH	C-8
C-7	4.13 (m)	47.79	CH	C-8
C-8	5.62 (m)	71.26	CH	C-6
C-9	2.13 (brs)	39.00	CH_2	C-8, C-10
C-10	-	72.10	C	
C-11	-	59.80	C	
C-12	-	178.45	C	
C-13	1.71(d,12.1); 2.72 (d,12.1)	37.67	CH_2	C-7, C-12, C-2', C-5'
C-14	1.29 (s)	33.31	CH_3	C-1, C-9, C-10
C-15	2.01 (brs)	18.48	CH_3	C-3, C-4
C-1'	-	65.66	C	
C-2'	6.07 (d, 5.4)	134.99	CH	C-1', C-4', C-5', C-11
C-3'	6.30 (d, 5.4)	140.08	CH	C-11, C-4', C-5'
C-4'	-	57.59	C	
C-5'	2.67 (d, 9.9)	66.78	CH	C-2', C-3', C-6'
C-6'	4.16 (t, 9.9)	80.37	CH	C-8'
C-7'	3.61 (m, $W_{1/2}$=19.5 Hz)	43.82	CH	
C-8'	ca 1.40 (m); ca 2.33 (m)	24.38	CH_2	C-7'
C-9'	1.84 (brt, 7.2, 8.8)	35.67	CH_2	
C-10'	-	71.74	C	
C-11'	-	142.52	C	
C-12'	-	170.78*	C	
C-13'	5.29 (d, 2.8); 6.18 (d, 3.3)	117.96	CH_2	C-7', C-12'
C-14'	1.38 (s)	29.61	CH_3	C-1', C-9', C-10'
C-15'	1.70 (s)	15.80	CH_3	C-3', C-4', C-5', C-11
OAc	1.99 (s)	22.00		
		170.66*		
OH	6.23 (s), 5.85 (brs)			

*may be interchangeable.

see Table I.

Daucosterin (5)

Amorphous from MeOH (590 mg), mp 285~7°, IR ν_{max} (KBr) cm^{-1} 3410, 2959, 2936, 2870, 2730, 1638, 1464, 1379, 1368, 1163, 1105, 1074, 1024, 841, 801. It was identified as daucosterin (=β-sitosterol 3-O-β-D-glucoside) by direct comparison with an authentic sample.

Test for the cytotoxicity *in vitro*

The *in vitro* cytotoxic activity test against cultured human tumor cell lines including A-549, SKOV-3, SKMEL-2, XF-498 and HCT-15 was performed according to Lee *et al.* (1995).

RESULTS AND DISCUSSION

The IR spectrum **1** showed typical absorption bands for *n*-hydrocarbons. It exhibited more than nine peaks in its GC chromatogram, among which the major peaks were identified as *n*-heneicosane ($C_{21}H_{44}$), *n*-tricosane ($C_{23}H_{48}$), *n*-tetracosane ($C_{24}H_{50}$), *n*-pentacosane ($C_{25}H_{52}$), *n*-hexacosane ($C_{26}H_{54}$), *n*-heptacosane ($C_{27}H_{56}$), *n*-octacosane ($C_{28}H_{58}$), *n*-nonacosane ($C_{29}H_{60}$) and *n*-hentriacontane ($C_{31}H_{64}$) by analyses of GC/MS spectra. The odd number of *n*-hydrocarbons are abundant than even ones. The IR spectrum **2** exhibited typical absorption bands for *n*-hydrocarbon alcohols. In a similar manner, **2** was identified as a mixture of C_{22} to C_{28} *n*-hydrocarbon alcohols. The most abundant components are *n*-hexacosanol and *n*-octacosanol. **3** and **5** were identified as β-sitosterol and its 3-O-glucoside, daucosterin by direct comparisons with authentic samples.

The IR spectrum of **4** showed absorptions at 3463 cm^{-1} (OH), 1753, 1745 (lactone) and 1725, 1248 (OAc), and appeared only end absorption in its UV spectrum. The EI-MS spectrum exhibited a fragment ion at *m/z* 456 $[M-2H_2O-HOAc]^+$ in the high mass region followed by other important fragment ions at *m/z* 288 $[C_{17}H_{22}O_5-H_2O]^+$, 246 $[C_{15}H_{18}O_3]^+$ and 228 $[C_{17}H_{22}O_5-H_2O-HOAc]^+$. These results strongly suggested that **4** seems to be a dimeric sesquiterpene lactone. The ^{1}H-NMR spectrum exhibited two doublet signals at δ 5.29 (1H, *J*=2.8 Hz) and 6.18 (1H, *J*=3.3 Hz), which are characteristic of exocyclic α-methylene-γ-lactone (Samek, 1970; Asada *et al.*, 1984), as well as five methyl signals at δ 1.29 (3H, s), 1.38 (3H, s), 1.70 (3H, s), 1.99 (3H, s, OAc) and 2.01 (3H, brs) and three olefinic protons at δ 5.53 (1H, brs), 6.07

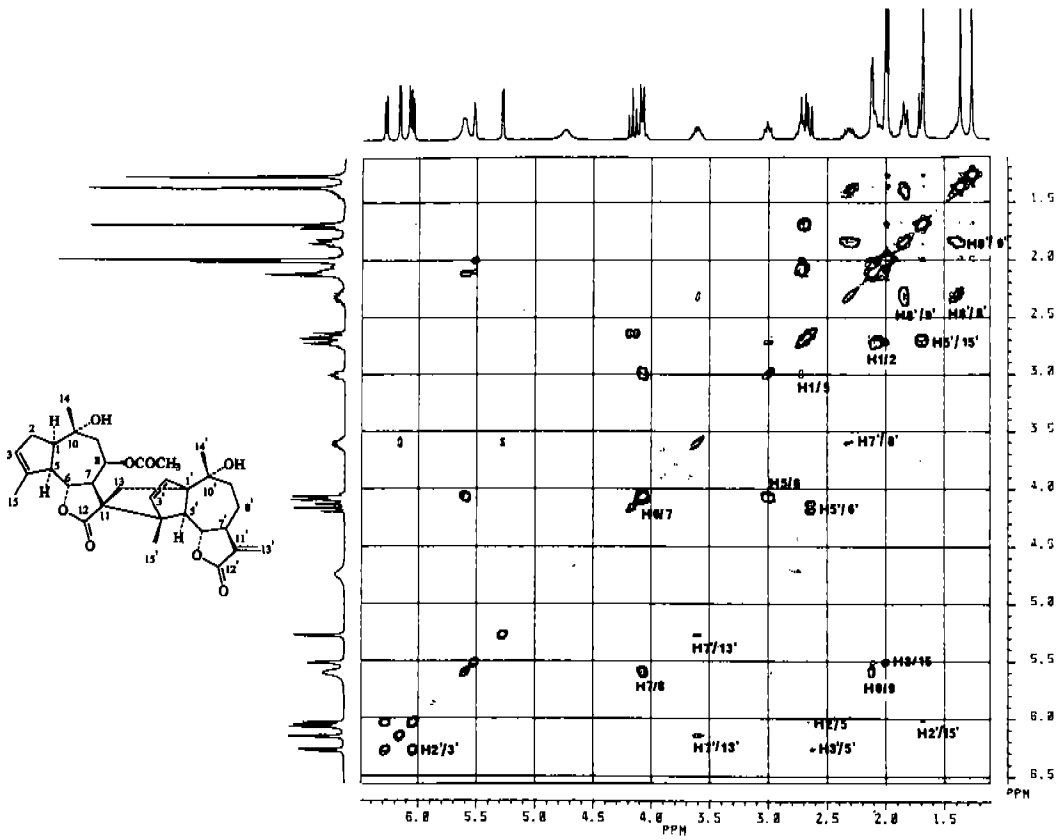

Fig. 1. ^{1}H-^{1}H COSY-45 spectrum (300 MHz) for handelin (**4**) in pyridine-d_5

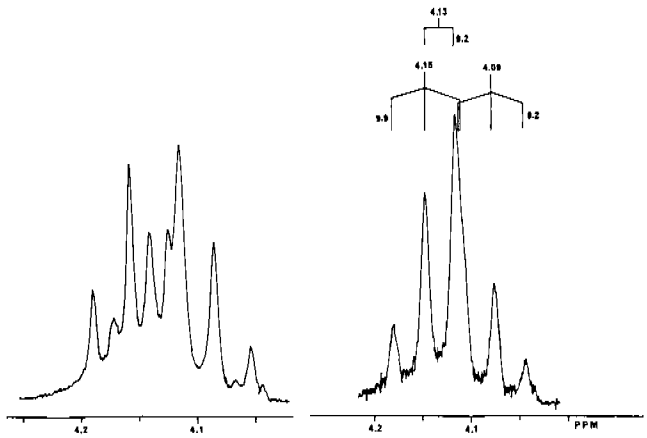

Fig. 2. Normal ^{1}H-NMR spectrum (300 MHz) for handelin (**4**) in 4.05~4.20 ppm region (left) and decoupled ^{1}H-NMR spectrum for handelin (**4**) of the same region obtained by irradiation of H-8 signal at 5.62 ppm (right) in pyridine-d_5

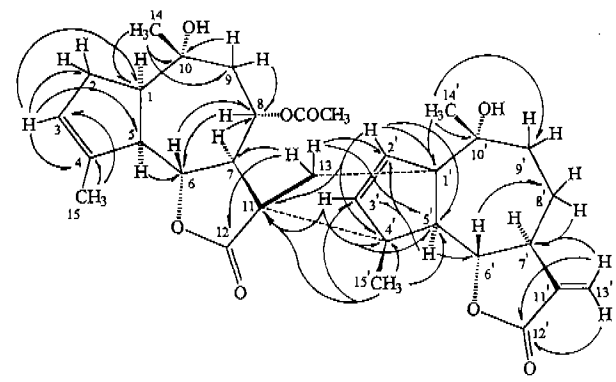

Fig. 3. Structure and CH correlations (HMBC) for handelin (**4**)

(1H, d, *J*=5.4 Hz), 6.30 (1H, d, *J*=5.4 Hz). The olefinic proton signal at δ 5.53 was allylicly coupled with a broad singlet methyl signal at δ 2.01. The remaining two olefinic protons at δ 6.07 and 6.30 were coupled each other which were in turn long-range coupled with a methine proton at δ 2.67 (1H, d, *J*=9.9 Hz), proton at δ 6.07 was homoallylicly coupled with a methyl signal at δ 1.70 in the ^{1}H-^{1}H-COSY-45 spectrum (Fig. 1). It also showed two triplets at δ 4.09 (1H, t, *J*=9.2 Hz) and 4.16 (1H, t, *J*=9.9 Hz) assignable H-6 and H-6' of 6,7-*trans*-lactonized guaianolides (Massanet *et al.*, 1984; Miyase *et al.*, 1985), in addition to a multiplet at δ 4.13 (1H) and a methine signal at δ 3.61(1H, m, $W_{1/2}$=19.5 Hz). These assignments were supported by irradiation of the H-8 proton signal at δ 5.62 ppm, which resulted in the collapse of the multiplet near 4.05~4.18 ppm to two triplets [δ 4.09 (1H, t, *J*=9.2 Hz); 4.16 (1H, t, *J*=9.9 Hz)] and a doublet at δ 4.13 (1H, d, *J*=9.2 Hz) as shown in Fig. 2 and by HMQC spectrum. The ^{1}H-^{1}H-COSY-45 and HMQC spectra along with the above data suggested that compound **4** seems to be an unique dimeric sesquiterpene lactone, handelin. ^{13}C-NMR spectrum showed an acetoxyl (δ_C 22.0, 170.66), four methyls (δ_C 33.31, 18.48, 29.61, 15.80), an exocyclic γ-lactone carbonyl (δ_C 170.78), and other γ-lactone carbonyl resonated at δ_C 178.45, indicating an 11,13-dihydrolactone (Asada *et al.*, 1984). Among four methyl groups, two methyl groups (δ_C 33.31, 29.61) were attached at the oxygenated quaternary carbons at δ_C 72.10 and 71.74. The long-range couplings from methyl protons at δ 2.01 to C-3 and C-4 carbons were observed in the HMBC spectrum. Other long-range couplings from the olefinic proton at δ 5.53 to C-1, C-2, C-4 and C-5 carbons were also appeared. C-13 methylene protons showed long-range couplings with C-7, C-2', C-5' and 11,13-dihydrolactonic carbon. The C-2' and C-3' olefinic protons and C-15' methyl protons also showed cross peaks with C-11. In addition to establishing the assignments of quternary carbons as well as of fragment connectivities, cross peaks in the HMBC spectrum unambiguously supported the connectivities between two sesquiterpene lactone moieties from three-bond correlations as mentioned above. All the observed HMBC connectivities are given in Fig. 3 and Table I. From the above data **4** was identified as handelin (= yejuhua lactone). While handelin (**4**) has already been isolated from *Handelia trichophylla* (Tarasov *et al.*, 1976), *C. indicum* (Chen and Xu, 1987) and *C. ornatum* var. *spontaneum* (Haruna *et al.*, 1981), this is the first isolation from this plant species and the NMR data were fully assigned by the multiple-bond and multiple-quantum spectroscopic correlation techniques.

A number of sesquiterpene lactones have previously been shown to possess cytotoxic activities. The α-methylene-γ-lactone moiety of this chemical class may play a significant role to exert their biological activities (Kupchan *et al.*, 1970; Willuhn, 1987). Handelin was evaluated for *in vitro* cytotoxic activity against cultured human tumor cell lines including A-549, SK-OV-3, SK-MEL-2, XF-498 and HCT-15. However, it was found to have no inhibitory activity against these tumor cell lines (ED_{50} : >400, 313, 192, 289 and 174 μg/ml, respectively).

REFERENCES CITED

Asada, H., Miyase, T. and Fukushima, S., Sesquiterpene lactones from *Ixeris tamagawaensis* Kitam. II, *Chem. Pharm. Bull.*, 32, 3036-3042 (1984).

Bohlmann, F., Arnot, C. and Bornowski, H., Polyacetylene compounds. Part 28. Several polyacetylenes from the tribe anthemideae L., *Chem. Ber.*, 93, 1937-1944 (1960).

Bohlmann, F., Arnot, C., Bornowski, H., Kleine, K.M. and Herbst, P., Polyacetylene compounds (LVI).

New acetylene derivatives from *Chrysanthemum* species, *Chem. Ber.*, 97, 1179-1192 (1964).

Chen, Z.-N. and Xu, P.-J., Structural determination of yejuhua lactone, isolated from *Chrysanthemum indicum* L., *Acta Pharm. Sinica*, 22, 67-69 (1987).

Haruna, M., Kato, M., Ito, K., Nikai, T., Sugihara, H. and Murata, H., Angeloylcumabrin-B, an antimicrobial sesquiterpene lactone from *Chrysanthemum ornatum* var. *spontaneum, Phytochemistry*, 20, 2583-2584 (1981).

He, Y.Q., Li, R.Z. and Shen, L., Separation and identification of flavonoids from the flower of *Chrysanthemum indicum, Peiching Hsueh Yuan Hsueh Pao*, 14, 259-261 (1982).

Huang, K.C., *The pharmacology of Chinese herbs*, CRC press, Boca Raton, 1993, p. 74.

Kupchan, S.M., Fessler, D.C., Eakin, M.A. and Giacobbe, T.J., Reactions of alpha methylene lactone tumor inhibitors with model biological nucleophiles, *Science*, 168, 376-378 (1970).

Lee, S.-H., Ryu, S.Y., Choi, S.U., No, Z.S., Kim, S.K., Lee, C.O. and Ahn, J.-W., Antitumor agent from the rhizomes of *Anemarrhena asphodeloides, Kor. J. Pharmacogn.*, 26, 47-50 (1995).

Massanet, G.M., Collado, I.G., Macias, F.A., Luis, F.R. and Vergara, C., Integrifolin, a guaianolide from *Andrayala integrifolia, Phytochemistry*, 23, 912-913 (1984).

Matsuo, A., Uchio, Y., Nakayama, M. and Hayashi, S., Two new acetylenic compounds from *Chrysanthemum boreale, Tetrahedron Lett.*, 1885-1888 (1974).

Miyase, T., Kuroyanagi, M., Noro, T., Ueno, A. and Fukushim, S., Studies on sesquiterpenes from *Macroclinidium trilobum* Makino. II, *Chem. Pharm. Bull.*, 33, 4445-4450 (1985).

Perry, L.M., *Medicinal plants of East and Southeast Asia: Attributed properties and uses*, The MIT press, Cambridge, 1980, p. 90.

Samek, Z., The determination of the stereochemistry of five-membered α,β-unsaturated lactones with an exomethylene double bond based on the allylic long-range couplings of exomethylene protons, *Tetrahedron Lett.*, 671-676 (1970).

Shin, K.H., Kang, S.S., Seo, E.A. and Shin, S.W., Isolation of aldose reductase inhibitors from the flowers of *Chrysanthemum boreale, Arch. Pharm. Res.*, 18, 65-68 (1995).

Tarasov, V.A., Abdullaev, N.D., Kasymov, Sh.Z., Sidyakin, G.P. and Yagudaev, M.R., Structure of handelin, a new diguaianolide from *Handelia trichophylla, Khim. Prir. Soedin.*, 6, 745-752 (1976).

Willuhn, G., Sesquiterpenlactone, potentielle Leitsubstanzen fuer die Arzneistoffindung, *Deutsche Apotheker Zeitung*, 127, 2511-2517 (1987).