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THREE DIELS-ALDER TYPE ADDUCTS, GINSENOYNES L, M, AND N, FROM THE ROOTS OF *PANAX GINSENG*

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Abstract - Three novel acetylenic compounds named ginsenoynes L, M, and N were isolated from the roots of *Panax ginseng*. These structures were elucidated by 3- and 2-D NMR spectra and other spectral methods. The ginsenoynes L, M, and N were Diels-Alder type adducts of an acetylenic compound and sesquiterpene.

Previously, we described acetylenic compounds isolated from the roots of *Panax ginseng*.[1] In our further study of this plant, we isolated three novel acetylenic compounds named ginsenoynes L (**1**), M (**2**), and N (**3**) (Figure 1).

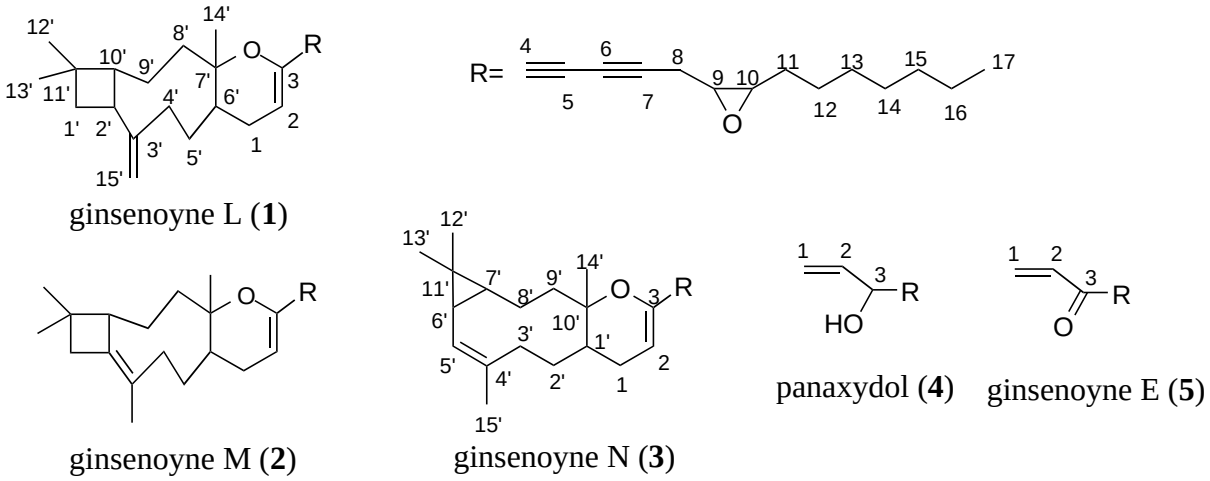


Figure 1. Structures of compounds (**1** ~ **5**)

The dried crushed roots of *Panax ginseng* were extracted with MeOH, and the MeOH solution was concentrated to half volume under reduced pressure, to which H_2O was added. The MeOH-H_2O solution was extracted with petroleum ether. Purification of petroleum ether extract by silica gel column chromatography, followed by preparative HPLC (ODS column) gave three acetylenic compound, ginsenoynes L, M, and N.

Ginsenoyne L (**1**) was obtained as oil and its molecular formula was determinated to be $C_{32}H_{46}O_2$ by HRMS spectrum. The UV spectrum of **1** exhibited typical absorption bands due to a conjugated ene-

diyne chromophore.[2] The IR spectrum of **1** indicated no presence of hydroxyl group. A comparison of the ^{13}C NMR and ^{1}H NMR spectra of **1** with those of panaxydol (**4**) [1a, 3] showed chemical shifts values from C-4 to C-17 and from H-8 to H-17 similar to those of **4** (Tables 1 and 2). These data suggested that **1** had the same straight chain structure bearing diyne and an epoxy ring in its molecule as in **4**. The unknown partial structure ($C_{18}H_{27}O$) of **1** consists of four olefinic carbons, six methylene carbons, three methine carbons, three methyl carbons, one quaternary carbon, and one oxygenated quaternary carbon, as revealed by ^{13}C NMR spectrum. One of the four olefinic carbons was exomethylene, as based on the DEPT method. The important HMBC correlations of the unknown partial structure of **1** are shown in Figure 2. The HMBC correlations of H_2-15' to C-2', C-3' and C-4' showed that the C-15' exomethylene carbon was attached to the C-3'.

The ^{1}H-^{1}H and ^{13}C-^{1}H connectivities in the unknown partial structure were clarified by ^{1}H-^{1}H, ^{13}C-^{1}H, and HMBC spectra (Figure 2, Tables 1 and 2). Detailed analysis of the 2D NMR data indicated that the presence of a three-ring system which consisted of cyclobutane, nine-membered, and heterocyclic six-membered rings for the unknown partial structure. The H_3-12' and H_3-13' showed HMBC correlations to C-11', which indicated that C-12' and C-13' were connected to C-11' as a *gem*-dimethyl group. The HMBC correlations of H_3-14' to C-6', C-7', and C-8' showed that the C-14' methyl group was attached to the C-7'.

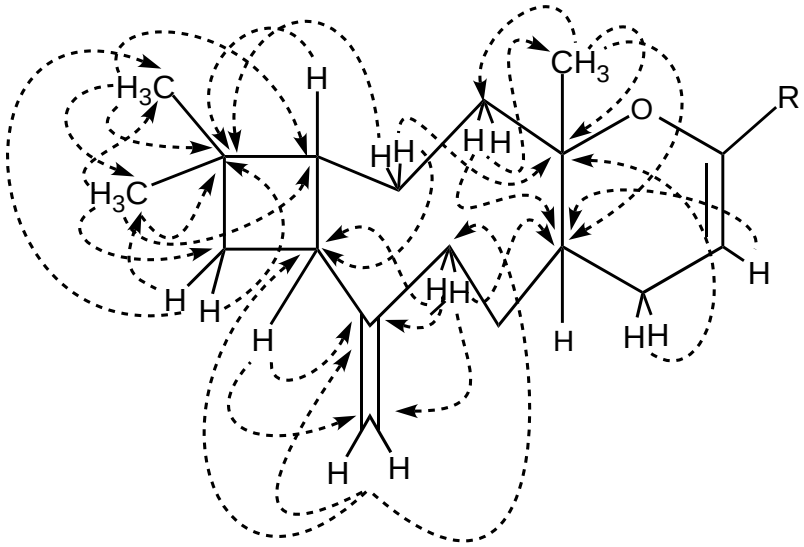


Figure 2. The important HMBC correlations of the unknown partial structure of **1**

These results suggest that the unknown partial structure ($C_{18}H_{27}O$) consists of caryophyllene and pyran ring moieties. The relative configuration of H-2' and H-10' was assumed to be *cis*, and that of the C-14' methyl group and H-6' was assumed to be *trans* through the NOESY. On the other hand, the relative configuration of the epoxy hydrogens (H-9 and H-10) in the straight chain was assumed to be *cis* in terms of the J value (4 Hz) and NOE interaction. Based on these results, we proposed formula (**1**) for the structure of ginsenoyne L.

Ginsenoyne M (**2**) was obtained as oil. Its molecular formula was determined to be $C_{32}H_{46}O_2$ by HRMS spectrum, which was same as **1**. The UV spectrum of **2** was similar to that of **1**. The IR spectrum of **2** indicated no presence of hydroxyl group. The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**, except for the presence of an endocyclic double bond in place of an exocyclic double bond (Tables 1 and 2). The H_3-15' showed the HMBC correlations to C-2', C-3', and C-4', which suggested that the C-15' methyl group was attached to the C-3'. The H-2' proton signal that existed in the ^{1}H NMR of **1** disappeared in that of **2**. These results indicated that **2** was an isomer of **1**

Table 1. ^{1}H NMR and NOE data of compounds (**1**, **2**, **3**, and **4**[1a]) (δ in ppm, J in Hz, $CDCl_3$)

No.	**1**	**2***	**3**	**4**
1a	1.81 ddd, J=2, 12, 18 (H-1b, 2, 14')	1.83 ddd, J=3, 12, 18 (H-1b, 2, 5'a, 5'b, 14)	1.89 ddd, J=3, 11, 19	5.24 ddd, J=1.0, 1.5, 10.1
1b	2.10 dt, J=18, 6 (H-1a, 2, 6')	2.04 dt, J=18, 5 (H-1a, 2, 5'a, 5'b)	2.21 dt, J=19, 6	5.46 ddd, J=1.0, 1.5, 17.1
2	5.29 dd, J=2, 6 (H-1a, 1b)	5.27 dd, J=3, 5 (H-1a, 1b)	5.21 dd,J=3, 6	5.94 ddd, J=5.4, 10.1, 17.1
8a	2.73 dd, J=5, 18 (H-8b, 9, 11)	2.73 dd, J=5, 18 (H-8b, 9, 11)	2.73 dd, J=5, 18	2.70 ddd, J=1.0, 5.4, 17.7
8b	2.39 dd, J=7, 18 (H-8a, 9, 11)	2.38 dd, J=7, 18 (H-8a, 9, 11)	2.38 dd, J=7, 18	2.39 ddd, J=1.0, 7.1, 17.1
9	3.13 ddd, J=4, 5, 7 (H-8a, 8b, 10)	3.12 ddd, J=4, 5, 7 (H-8a, 8b, 10)	3.12 ddd, J=4, 5, 7	3.15 ddd, J=4.1, 5.5, 7.1
10	2.94 br td, J=6, 4 (H-9, 11)	2.94 br td, J=6, 4 (H-9, 11)	2.94 br td, J=6, 4	2.94 br td, J=6.0, 4.1
11	1.45~1.60 m	1.43~1.55 m	1.45~1.55 m	1.45~1.55 m
12				
13	1.25~1.39 m	1.27~1.39 m	1.26~1.39 m	1.25~1.40 m
14				
15				
16				
17	0.89 br t, J=7	0.89 br t, J=7	0.89 br t J=7	0.89 br t, J=7.0
1'a	1.55 m (H-1'b, 2a', 13')	2.22 m (H-12', 13', 15')	1.63 m	
1'b	1.91 t, J=10 (H-1'a, 12', 15'a)		-	(H-3 : 4.92 br d, J=5)
2'a	2.91 br dt, J=10, 8 (H-1'a, 4'a, 10', 13')	-	1.10 m	
2'b	-	-	1.30 m	
3'a	-	-	1.91 td, J=12, 2	
3'b	-	-	2.09 br dd, J=6, 12	
4'a	1.90 m (H-2a', 4'b, 10')	1.98 br ddd, J=1, 7, 14	-	
4'b	2.54 br dt, J=14, 4 (H-4'a, 15'b)	2.10 br t, J=14	-	
5'a	1.35 m	1.10 m (H-1a, 1b, 15')	4.97 br d, J=9	
5'b	1.45 m	1.35 m (H-1a, 1b)	-	
6'	1.88 m (H-1'b)	1.95 m	1.24 t, J=9	
7'	-	-	0.62 ddd, J=3, 9, 12	
8'a	1.55 m (H-8'b, 9'b, 14')	1.86 dd, J=5, 8	0.81 m	
8'b	2.14 ddd, J=7, 12, 15 (H-8'a)		1.60 m	
9'a	1.59 m (H-9'b)	1.59 dtd, J=3, 8, 16 (H-9'b, 10', 12')	1.10 m	
9'b	1.72 m (H-8'a, 9'a, 12')	1.73 dtd, J=8, 5, 16 (H-4'a, 9'a, 10')	1.63 m	
10'	2.31 m (H-2'a, 4'a, 13')	2.81 m (H-4'a, 9'a, 9'b, 13')	-	
C-12'Me	0.90 s (H-1'b, 9'b, 13')	0.97 s (H-1', 8', 9'a, 13')	1.04 s	
C-13'Me	1.19 s (H-1'a, 2'a, 10', 12')	1.10 s (H-1', 10', 12')	1.12 s	
C-14'Me	0.98 s (H-1a, 8'a)	1.02 s (H-1a, 8')	1.03 s	
C-15'Me	-	1.51 br s (H-1', 4'b, 5'a)	1.69 br s	
H-15'a	4.93 br s (H-1'b)	-	-	
H-15'b	4.91 br s (H-4'b)	-	-	

The assignments were based on ^{1}H-^{1}H, ^{13}C-^{1}H COSY, and NOESY.

The NOE interactions in parentheses.

*The NOE interactions were measured by 3D NOESY-HSQC and NOESY.

Table 2. ^{13}C NMR spectra and HMBC data of compounds (**1**, **2**, **3**, and **4**[1a])(δ in ppm, $CDCl_3$)

No.	**1**	**2**	**3**	**4**
1	29.0	29.3	29.0	116.4
2	111.2(H-1a, 1b)	111.2(H-1a)	109.6(H-1a, 1b)	136.2
3	135.1(H-1a, 1b, 2)	135.3(H-1a, 2)	134.9(H-1b, 2)	63.4
4	72.2(H-1a, 2, 8a, 8b)	71.9(H-2)	72.0	75.2
5	71.2(H-8a, 8b)	71.5(H-8a, 8b)	71.3(H-8a, 8b)	70.7
6	67.0(H-8a, 8b)	67.0(H-8a, 8b)	66.9(H-8a, 8b)	66.4
7	78.9(H-8a, 8b)	78.9(H-8a, 8b)	78.9(H-8a, 8b)	76.6
8	19.7(H-9)	19.7(H-9, 10)	19.7(H-9)	19.5
9	54.4(H-8a, 8b)	54.4(H-8a, 8b)	54.4(H-8a, 8b)	54.4
10	56.9(H-8a)	56.9(H-8a)	56.9(H-8a)	57.1
11	27.5(H-10)	27.5(H-10)	27.5(H-10)	27.5
12	26.5	26.5	26.5	26.5
13	29.4	29.4	29.4	29.4
14	29.2	29.2	29.2	29.2
15	31.8(H-17)	31.8(H-17)	31.8(H-17)	31.7
16	22.6(H-17)	22.6(H-17)	22.6(H-17)	22.6
17	14.1	14.1	14.1	14.1
1'	35.3(H-2a', 13')	42.1(H-12', 13')	34.4(H-2, 3'a, 3'b)	
2'	40.3(H-4'b, 9'b, 15'a, 15'b)	131.3(H-1', 4'a, 4'b, 9'a, 10', 15')	32.1	
3'	151.3(H-2a', 4'a, 4'b, 15'b)	129.0(H-1', 4'a, 4'b, 15')	40.3(H-5', 15')	
4'	40.2(H-15'a, 15'b)	34.6(H-15')	136.1(H-3'a, 3'b, 15')	
5'	33.6(H-6', 4'b)	28.7(H-4'a, 4'b, 6')	121.4(H-3'a, 3'b)	
6'	37.2(H-1a, 1b, 2, 4'b, 8'a, 14')	39.8(H-1a, 1b, 2, 4'a, 4'b, 8')	24.6(H-12', 13')	
7'	80.6(H-1a, 1b, 9'a, 14')	82.0(H-1a, 1b, 8', 9'b, 14')	27.4(H-12', 13')	
8'	33.6(H-14')	35.6(H-10', 14')	19.2	
9'	21.9(H-2a', 8'b, 10')	20.1(H-8', 10')	35.6(H-14')	
10'	46.1(H-2a', 9'a, 9'b, 12', 13')	51.2(H-1', 8', 9'a, 9'b, 12', 13')	81.7(H-1b, 14')	
11'	32.9(H-1'a, 1'b, 9'b, 10', 12', 13')	33.4(H-1', 9'a, 9'b, 10', 12', 13')	20.3(H-5', 12', 13')	
12'	23.7(H-1'b, 13')	24.2(H-1', 13')	28.9(H-13')	
13'	29.7(H-1'a, 12')	31.1(H-1', 10', 12')	15.2(H-12')	
14'	19.6(H-8'a)	18.9(H-6', 8')	20.7(H-9'a)	
15'	112.3(H-2a', 4'a, 4'b)	17.8(H-4'a, 4'b)	16.9(H-3'a, 5')	

The assignments were based on DEPT, ^{13}C-^{1}H COSY and HMBC experiments.
The HMBC correlations in parentheses.

on the position of the double bond. The (*Z*)-geometry for the double bond was presumed in terms of the NOE interactions between H-4'a and H-9'b, H_3-15' and H-1'. The relative configuration of the C-14' methyl group and H-6' was assumed to be *trans* through the NOESY, and that of the epoxy hydrogens (H-9 and H-10) was assumed to be *cis* by means of the J value (4 Hz) and NOE interaction. Based on these results, we proposed formula (**2)** for the structure of ginsenoyne M.

Ginsenoyne N (**3**) was obtained as oil and its molecular formula was determined to be $C_{32}H_{46}O_2$ by HRMS spectrum. The UV spectrum of **3** was similar to those of **1** and **2**. A comparison of ^{1}H and ^{13}C NMR spectra with those of **1** and **2** suggested that **3** had the same straight chain and pyran ring moieties in its molecule as in **1** and **2** (Tables 1 and 2). In the ^{1}H NMR spectrum of **3**, the H-7' signal was observed at a high field (δ_H 0.62), which suggested that the unknown partial structure of **3** had a cyclopropane ring in its molecule. The HMBC correlations indicated that the cyclopropane ring consisted of C-6', C-7' and C-11' with a *gem*-dimethyl group (Figure 3).

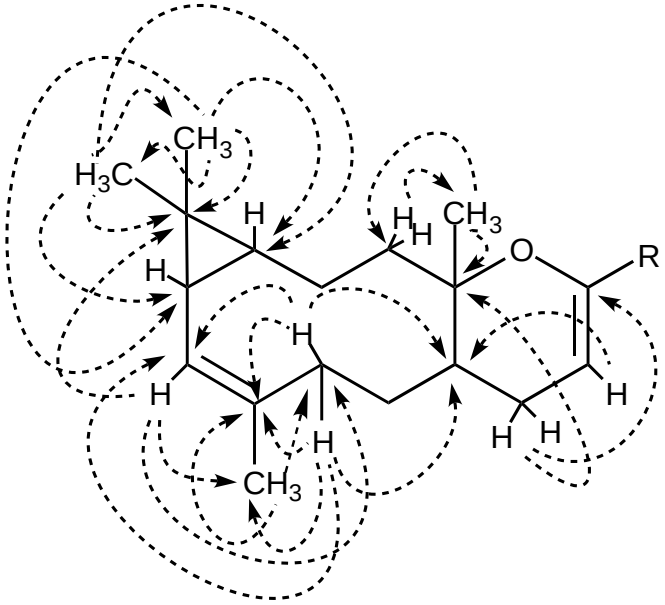


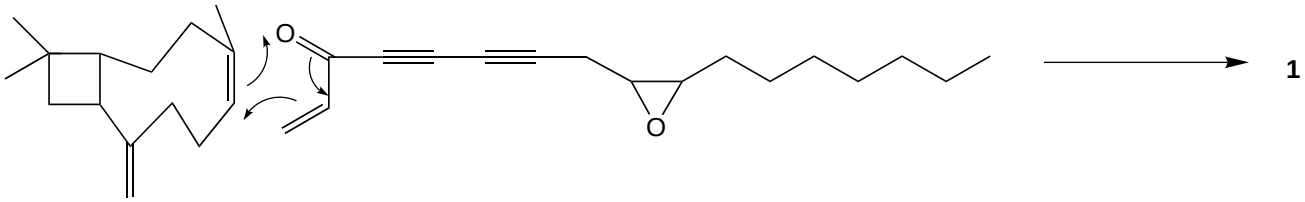
Figure 3. The important HMBC correlations of unknown partial structure of **3**

The ^{1}H-^{1}H and ^{13}C-^{1}H connectivities in the unknown partial structure were clarified by ^{1}H-^{1}H, ^{13}C-^{1}H, and HMBC spectra (Figure 3, Tables 1 and 2). Detailed analysis of the 2D NMR data indicated that the presence of cyclopropane and ten-membered rings for the unknown partial structure. The H_3-14' showed the HMBC correlations to C-10' and C-9', which suggested that the C-14' methyl group was connected to the C-10'. The HMBC correlations of H_3-15' to C-3' and C-4' indicated that the C-15' methyl group was attached to the C-4'. The (*Z*)-geometry for the double bond was deduced from the ^{13}C NMR chemical shifts (δ_C 16.9)[4] of C-15'. These results indicated that **3** consisted of bicyclogermacrene, pyran ring, and straight chain moieties. The relative configuration of H-6' and H-7' was deduced to be *cis* based on the J value (9 Hz), as was that of the epoxy hydrogens (H-9 and H-10) by the J value (4 Hz).

Thus, the structure of ginsenoyne N (**3**) was concluded to be represented by formula (**3**).

From a biogenetic point of view, ginsenoynes L (**1**) and M (**2**) seem to be Diels-Alder type adducts derived from caryophyllene as dieneophile and ginsenoyne E (**5**) as diene (Chart 1). The other, ginsenoyne N (**3**) seems to be derived from bicyclogermacrene and **5**. These precursors have already been reported as components of the roots.[1a, 5] Although many Diels-Alder type adducts have been isolated in nature,[4, 6] ginsenoynes L, M, and N are the first examples of the [4+2] cycloadduct of

acetylenic compound and sesquiterpene found in nature.



caryophyllene ginsenoyne E (**5**)

Chart 1. Diels-Alder type adduction of caryophyllene and **5**

EXPERIMENTAL

General Methods

IR spectra were measured using a HITACHI 270-30 Infrared spectrophotometer, while UV spectra were measured using a HITACHI U-3200 spectrophotometer. Low and high-resolution EIMS spectra were obtained using on KRATOS CONCEPT 1S spectrometer. NMR spectra were recorded on a BRUKER DRX-400, AM-500, and DRX-600. For the NMR data, chemical shifts are expressed in δ ppm from tetramethylsilane (TMS) as an internal standard, and coupling constants (J) are given in Hz. Specific rotations were recorded on a JASCO DIP-360 polarimeter. Silica gel 60 F_{254}, 0.25 mm (Merck) was used for TLC. Preparative HPLC employed a CIG column system (22 ϕ x 100 mm or 45 ϕ x 500 mm, Kusano Scientific Co., Tokyo) and the silica gel (50 μm) was used for the stationary phase. Preparative reversed phase HPLC used a 20 ϕ x 300 mm packed column YMC S-343 (Yamamura Chemical Laboratories, Co., Ltd., Kyoto).

Plant material

The roots of *Panax ginseng* were collected in Gunma prefecture, Japan, and were cultivated.

Isolation of ginsenoynes L (1), M (2), and N (3)

Dried and crushed roots (60 kg) were divided into 10 parts and each part was extracted three times by refluxing with MeOH (100 L) for 1 h. Each MeOH solution was concentrated under reduced pressure, until reduced by about half and H_2O (6 L) was added. Each MeOH-H_2O solution was extracted four times with petroleum ether (24 L). Each petroleum ether solution was collected and concentrated under reduced pressure to give an extract (1.4 kg). The extract was chromatographed on a silica gel column (4 kg) eluted with hexane-EtOAc mixture increasing polarity. The eluates were divided into 7 fractions: F-1 (119.8 g), F-2 (87.6 g), F-3 (166.6 g), F-4 (229.4 g), F-5 (121.0 g), F-6 (29.2 g), and F-7 (14.8 g). The fraction F-2 was separated by preparative HPLC with hexane-EtOAc (18 : 1), hexane-C_6H_6 (1 : 2), and hexane-Et_2O-acetone (70 : 0.5 : 1) successively to give mixture of **1**, **2**, and **3**. The mixture was rechromatographed on preparative reversed phase HPLC, eluted with MeCN-THF (20 : 1) to give **1** (41 mg, 6.8 x 10^{-5} %), **2** (31 mg, 5.2 x 10^{-5} %), and **3** (88 mg, 1.5 x 10^{-4} %).

Ginsenoyne L (1)

The compound (**1**) was obtained as oil. $[\alpha]_D$-37.3˚ (*c* 3.3 $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 2928, 1632, 1450, 1102; UV λ_{max}^{EtOH} nm (log ε) : 212 (4.25), 240 (3.72), 253 (3.95), 267 (4.15), 283(4.10); EIMS *m/z* : 462 $[M]^+$, 203, 161; HREIMS *m/z* : 462.3500 (Calcd for $C_{32}H_{46}O_2$ $[M]^+$: 462.3498); ^1H NMR : see Table 1; ^{13}C NMR :

see Table 2.

Ginsenoyne M (2)

The compound (**2**) was obtained as oil. $[\alpha]_D$-58.6˚(*c* 2.1 $CHCl_3$); IR$\nu_{max}^{CHCl_3}$ cm^{-1} : 2928, 1630, 1462, 1100; UVλ_{max}^{EtOH} nm (log ε) : 212 (4.35), 241 (3.79), 253 (4.01), 267 (4.22), 283 (4.17); EIMS *m/z* : 462 $[M]^+$, 203, 161; HREIMS *m/z* 462.3498 (Calcd for $C_{32}H_{46}O_2$ $[M]^+$: 462.3498); ^{1}H NMR : see Table 1; ^{13}C NMR : see Table 2.

Ginsenoyne N (3)

The compound (**3**) was obtained as oil. $[\alpha]_D$+10.3˚ (*c* 1.7 $CHCl_3$); IR$\nu_{max}^{CHCl_3}$ cm^{-1}: 2950, 1620, 1454, 1102; UVλ_{max}^{EtOH} nm (log ε) : 212 (4.33), 240 (3.77), 253 (3.95), 267 (4.15), 283 (4.11); EIMS *m/z* : 462 $[M]^+$, 203, 161; HREIMS *m/z* : 462.3501(Calcd for $C_{32}H_{46}O_2$ $[M]^+$: 462.3498); ^{1}H NMR : see Table 1; ^{13}C NMR : see Table 2.

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