GLISOPRENINS, NEW INHIBITORS OF ACYL-CoA: CHOLESTEROL ACYLTRANSFERASE PRODUCED BY *Gliocladium* sp. FO-1513

II. STRUCTURE ELUCIDATION OF GLISOPRENINS A AND B

HIROYUKI NISHIDA†, XIN-HUI HUANG, HIROSHI TOMODA and SATOSHI ŌMURA*

Research Center for Biological Function, The Kitasato Institute, Minato-ku, Tokyo 108, Japan

(Received for publication April 27, 1992)

The structure of glisoprenins A and B, novel acyl-CoA: cholesterol acyltransferase (ACAT) inhibitors, was determined by spectroscopic analyses, mainly ^{1}H and ^{13}C NMR and MS. Glisoprenin A was deduced to be a tetrahydroxynonaprenol and glisoprenin B to be an oxidative modification of glisoprenin A.

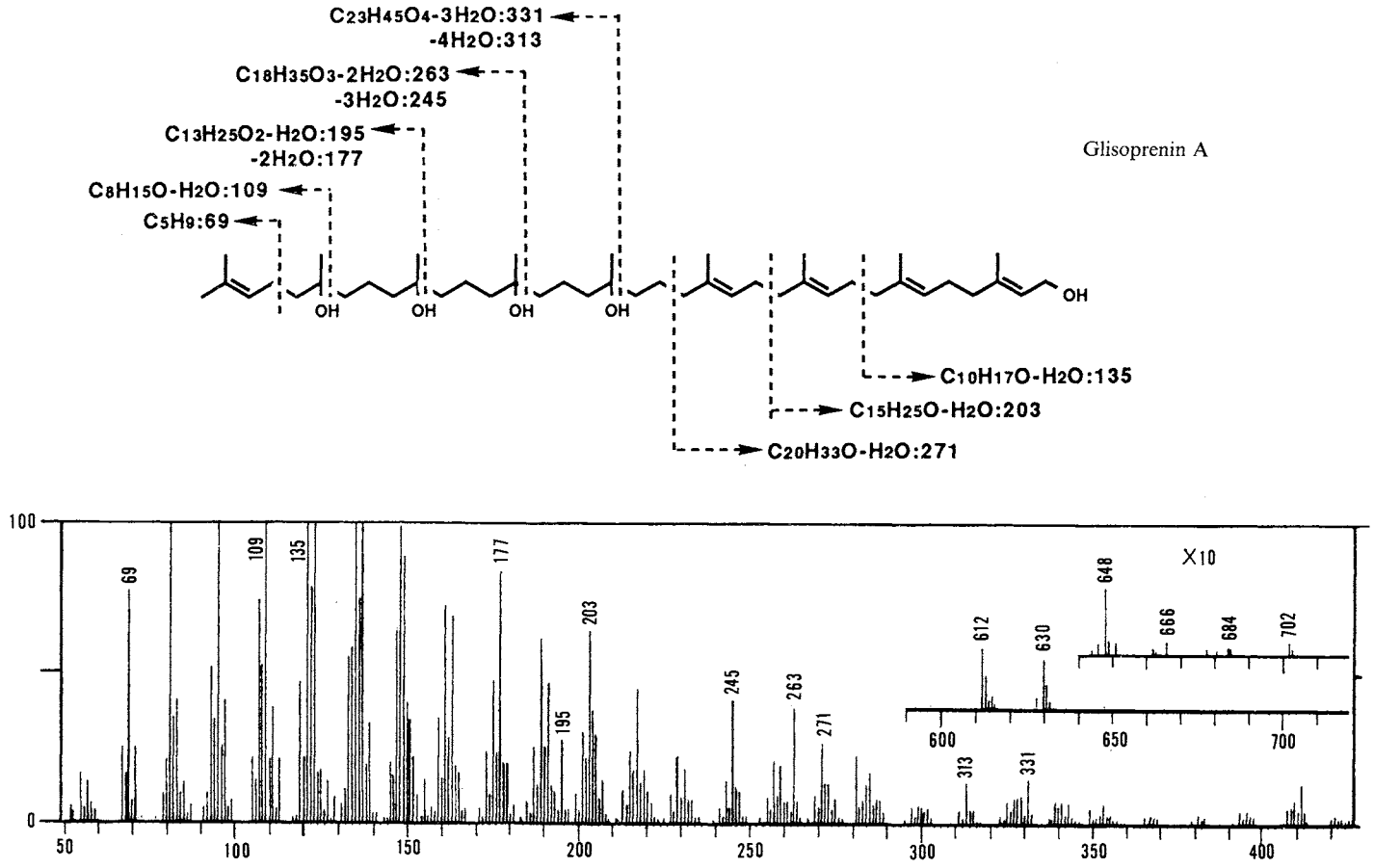
In the course of our screening for acyl-CoA: cholesterol acyltransferase (ACAT) inhibitors, glisoprenins A and B have been isolated from the fermentation broth of *Gliocladium* sp. FO-1513. The taxonomy of the producing organism, fermentation, isolation, physico-chemical properties and biological activity were reported in the preceding paper[1]. In this report, we describe the structure elucidation of glisoprenins A and B.

Structure Elucidation of Glisoprenins A (**1**) and B (**2**)

The MW and molecular formula of glisoprenin A (**1**) were determined to be 702 and $C_{45}H_{82}O_5$ by the analyses of the FAB-MS and HREI-MS spectra, respectively. Because of the existence of dehydration ion peaks at *m/z* 684 ($M-H_2O$), 666 ($M-2H_2O$), 648 ($M-3H_2O$), 630 ($M-4H_2O$) and 612 ($M-5H_2O$) observed in the EI-MS spectrum (Fig. 1), **1** contains 5 hydroxy residues. The ^{13}C NMR (no NOE decoupling) and ^{13}C-^{1}H COSY spectra revealed the presence of 45 carbons, which were assigned as shown in Table 1. The ^{1}H and ^{13}C NMR (Figs. 2 and 3) data suggested that **1** possesses 5 isoprene units and may be a polyprenol-related compound. The structural study of **1** was done in comparison with the ^{1}H and ^{13}C chemical shifts of monoterpenes (Fig. 4), which had been studied in detail in references 2 and 3. The ^{1}H NMR spectrum of **1** indicated the presence of six allylic methyls, one allylic hydroxymethylene (=CH–CH_2–OH), and four vinyl protons. The allylic methyl signals at δ 1.60 (3H) and δ 1.67 (3H), δ 1.58 (9H) and δ 1.66 (3H) in the ^{1}H NMR spectrum are assignable to methyl groups of the ω-terminal isoprene (45-H), three internal *E*-isoprene (38-H, 39-H and 40-H) and the α-terminal *E*-isoprene (37-H) residues, respectively. On the other hand, the methyl signals at δ 1.14 (3H) and δ 1.15 (9H) in the ^{1}H NMR spectrum and at δ 26.71, 26.77 and 26.89 (2C) in the ^{13}C NMR spectrum, and of quarternary oxycarbons at δ 72.7 (4C) and of methylene carbons at δ 41.64, 41.73, 42.24, 42.34 (3C) and 42.43 (2C), and δ 18.07 and 18.14 (2C) in the ^{13}C NMR spectrum indicated that four internal isoprene units are hydroxylated at C-19, -23, -27 and C-31 positions [–CH_2–C(OH)CH_3–CH_2–CH_2–]$_4$ as shown in Fig. 4C and E. By careful analyses of the ^{1}H and ^{13}C NMR chemical shifts of **1**, all the carbons and protons are

† Present address: Pfizer Central Research, Nagoya, Pfizer Pharmaceuticals Inc., 5-2 Taketoyo, Aichi 470-23, Japan.

Fig. 1. EI-MS of glisoprenins A and B.



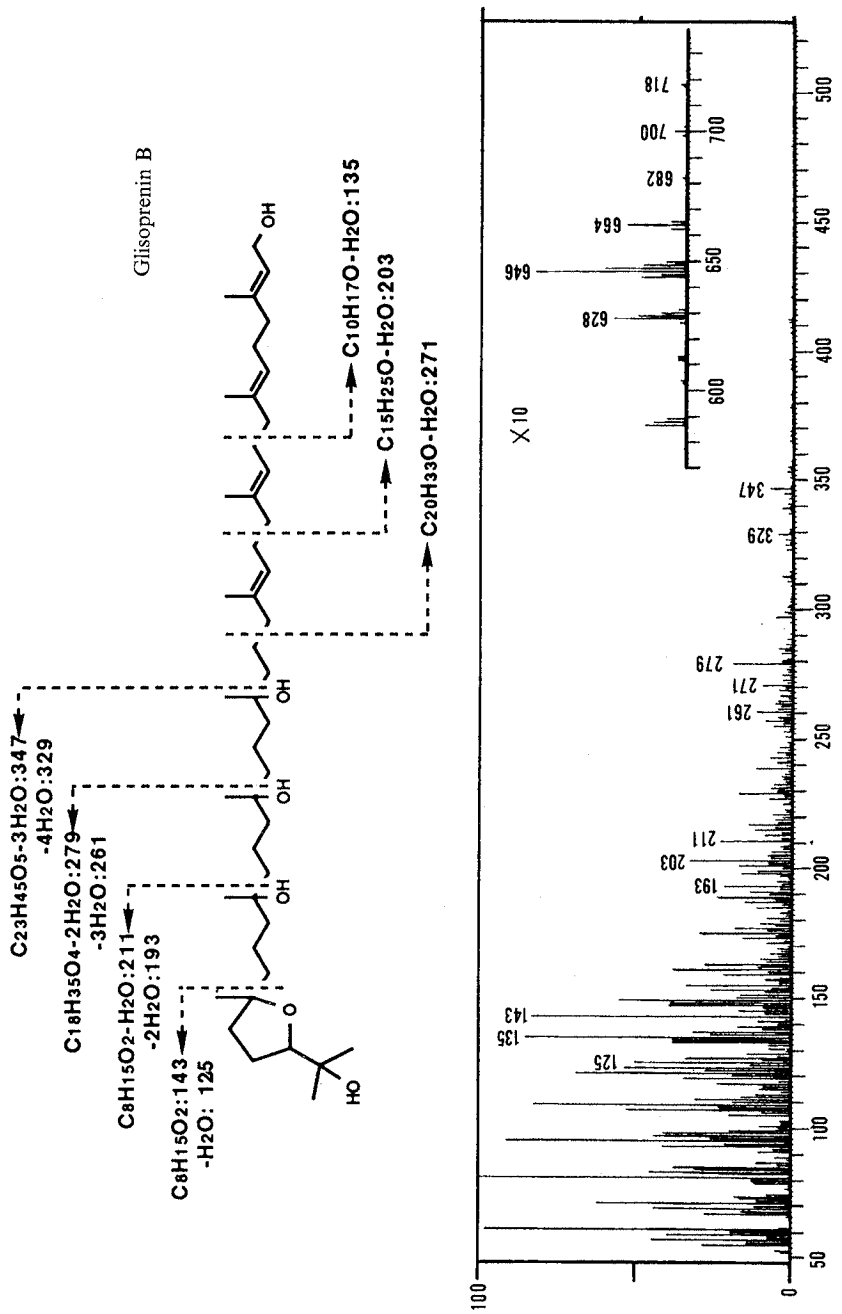

Glisoprenin B
C23H45O5-3H2O:347
-4H2O:329
C18H35O4-2H2O:279
-3H2O:261
C8H15O2-H2O:211
-2H2O:193
C8H15O2:143
-H2O: 125
C10H17O-H2O:135
C15H25O-H2O:203
C20H33O-H2O:271
HO
OH
O
X10
718
700
682
664
646
628
600
650
347
329
279
271
261
211
203
193
143
135
125
100
50
0
150
200
250
300
350
400
450
500

Table 1. ^{1}H and ^{13}C chemical shifts of glisoprenins A and B in $CDCl_3$.

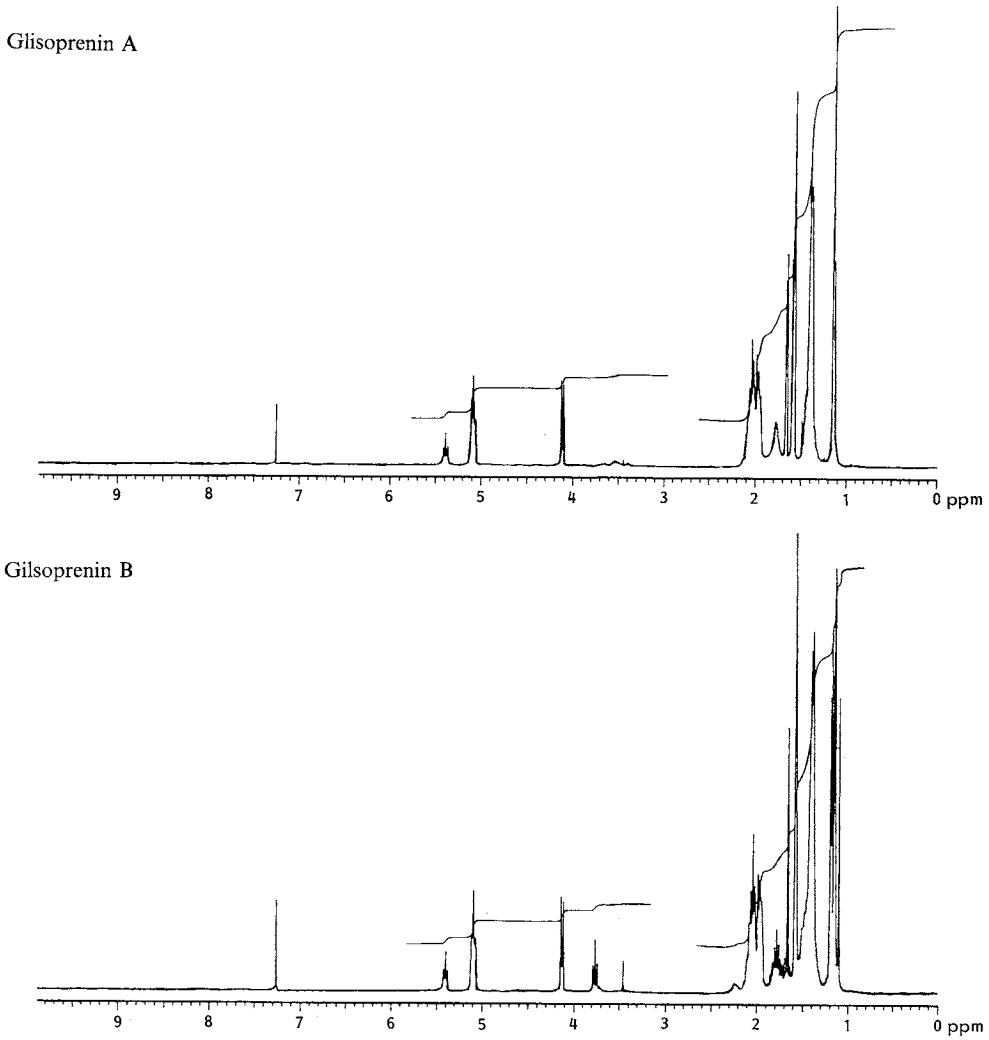
No.	Glisoprenin A			Glisoprenin B		
	^{13}C-ppm		^{1}H-ppm (m)[x]	^{13}C-ppm		^{1}H-ppm (m)[x]
1	59.20	CH_2–O	4.14 (d, J=7.0 Hz)	59.28	CH_2–O	4.14 (d, J=7.0 Hz)
2	123.47	CH=	5.40 (br t)	123.44	CH=	5.40 (br t)
3	139.38	C=		139.48	C=	
4	39.51[c]	CH_2	1.92~2.01 (m)	39.53[k]	CH_2	1.92~2.01 (m)
5	26.25[d]	CH_2	2.01~2.14 (m)	26.26[l]	CH_2	2.01~2.14 (m)
6	123.81[a]	CH=	5.10 (br t)	123.82[i]	CH=	5.10 (br t)
7	134.70[b]	C=		134.73[j]	C=	
8	39.65[c]	CH_2	1.92~2.01 (m)	39.68[k]	CH_2	1.92~2.01 (m)
9	26.51[d]	CH_2	2.01~2.14 (m)	26.54[l]	CH_2	2.01~2.14 (m)
10	124.16[a]	CH=	5.10 (br t)	124.18[i]	CH=	5.10 (br t)
11	134.84[b]	C=		134.88[j]	C=	
12	39.67[c]	CH_2	1.92~2.01 (m)	39.70[k]	CH_2	1.92~2.01 (m)
13	26.56[d]	CH_2	2.01~2.14 (m)	26.58[l]	CH_2	2.01~2.14 (m)
14	124.45[a]	CH=	5.10 (br t)	124.48[i]	CH=	5.10 (br t)
15	135.22[b]	C=		135.27[j]	C=	
16	40.04	CH_2	1.92~2.01 (m)	40.05	CH_2	1.92~2.01 (m)
17	22.23	CH_2	1.30~1.50 (m)	22.23	CH_2	1.30~1.50 (m)
18	41.73[e]	CH_2	1.30~1.50 (m)	41.58	CH_2	1.30~1.50 (m)
19	72.73	C–O		72.72[m]	C–O	
20	42.24[e]	CH_2	1.30~1.50 (m)	42.28	CH_2	1.30~1.50 (m)
21	18.07[f]	CH_2	1.30~1.50 (m)	18.14[m]	CH_2	1.30~1.50 (m)
22	42.34[e]	CH_2	1.30~1.50 (m)	42.35[n]	CH_2	1.30~1.50 (m)
23	72.73	C–O		72.72[m]	C–O	
24	42.34[e]	CH_2	1.30~1.50 (m)	42.37	CH_2	1.30~1.50 (m)
25	18.14[f]	CH_2	1.30~1.50 (m)	18.19[m]	CH_2	1.30~1.50 (m)
26	42.34[e]	CH_2	1.30~1.50 (m)	42.43[n]	CH_2	1.30~1.50 (m)
27	72.73	C–O		72.72[m]	C–O	
28	42.43[e]	CH_2	1.30~1.50 (m)	42.43	CH_2	1.30~1.50 (m)
29	18.14[f]	CH_2	1.30~1.50 (m)	18.97[m]	CH_2	1.30~1.50 (m)
30	42.43[e]	CH_2	1.30~1.50 (m)	42.15[n]	CH_2	1.30~1.50 (m)
31	72.73	C–O		83.17[m]	C–O	
32	41.64	CH_2	1.30~1.50 (m)	37.10	CH_2	1.78 (m)
33	22.68	CH_2	2.01~2.14 (m)	26.49	CH_2	1.80 (m)
34	124.45	CH=	5.10 (br t)	84.67	CH	3.77 (t, J=7.2 Hz)
35	131.59	C=		71.17	C–O	
36	25.66	CH_3	1.67 (s)	24.18	CH_3	1.10 (s)
37	16.24	CH_3	1.66 (s)	16.27	CH_3	1.63 (s)
38	15.82[g]	CH_3	1.58 (s)	15.85[o]	CH_3	1.59 (s)
39	15.96[g]	CH_3	1.58 (s)	16.00[o]	CH_3	1.59 (s)
40	15.96[g]	CH_3	1.58 (s)	16.00[o]	CH_3	1.59 (s)
41	26.71[h]	CH_3	1.14 (s)	26.87[p]	CH_3	1.17 (s)
42	26.77[h]	CH_3	1.16 (s)	26.92[p]	CH_3	1.16 (s)
43	26.89[h]	CH_3	1.16 (s)	26.98[p]	CH_3	1.15 (s)
44	26.89[h]	CH_3	1.16 (s)	25.64	CH_3	1.19 (s)
45	17.62	CH_3	1.60 (s)	27.48	CH_3	1.20 (s)

[a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p] May be reversed.

[x] Multiplicity.

assignable as shown in Fig. 5 and Table 1. The EI-MS spectrum of **1** (Fig. 1) supports the structure because of the presence of the typical fragment ion peaks at m/z 69 (cleavage from the ω-terminal isoprene), 109, 177, 195, 245, 263, 313 and 331 (cleavage from the internal hydroxy isoprenes), and 135, 203 and 271 (cleavage from the internal E-isoprenes and the α-terminal E-isoprene)[4].

Fig. 2. ^{1}H NMR spectra of glisoprenins A and B (400 MHz, $CDCl_3$).



The MW and molecular formula of glisoprenin B (**2**) were determined to be 718 and $C_{45}H_{82}O_6$ by analyses of the FAB-MS and HREI-MS spectra, respectively. The dehydration ion peaks at *m/z* 700 ($M-H_2O$), 682 ($M-2H_2O$), 664 ($M-3H_2O$), 646 ($M-4H_2O$) and 628 ($M-5H_2O$) (Fig. 1) in the EI-MS spectrum indicated that **2** possesses 5 hydroxy residues in the molecule as was found for **1**. The ^{13}C NMR (no NOE decoupling) and ^{13}C-^{1}H COSY spectra revealed the presence of 45 carbons, which were also assigned as shown in Table 1. The ^{1}H and ^{13}C NMR chemical shifts of **2** showed a good coincidence with those of **1** except for the ω-terminal isoprene residue (Figs. 2 and 3 and Table 1). These facts indicated that **2** is an oxidative modification of **1** at the ω-terminal isoprene residue. The structure of the ω-terminal residue of **2** was determined by the ^{1}H-^{1}H COSY and HMBC experiments. As shown in Fig. 6, the long-range couplings from 45-H (δ 1.20) to C-36 (δ 24.18) and C-34 (δ 84.67), and from 36-H (δ 1.10) to C-45 (δ 27.48) and C-34 (δ 84.67) supported the sequence from C-34 to C-36 and C-45. Furthermore, the long-range couplings from 44-H (δ 1.19) to C-32 (δ 37.10) and C-30 (δ 42.15), and from 34-H (δ 3.77) to

Fig. 3. ^{13}C NMR spectra of glisoprenins A and B (100 MHz, $CDCl_3$).

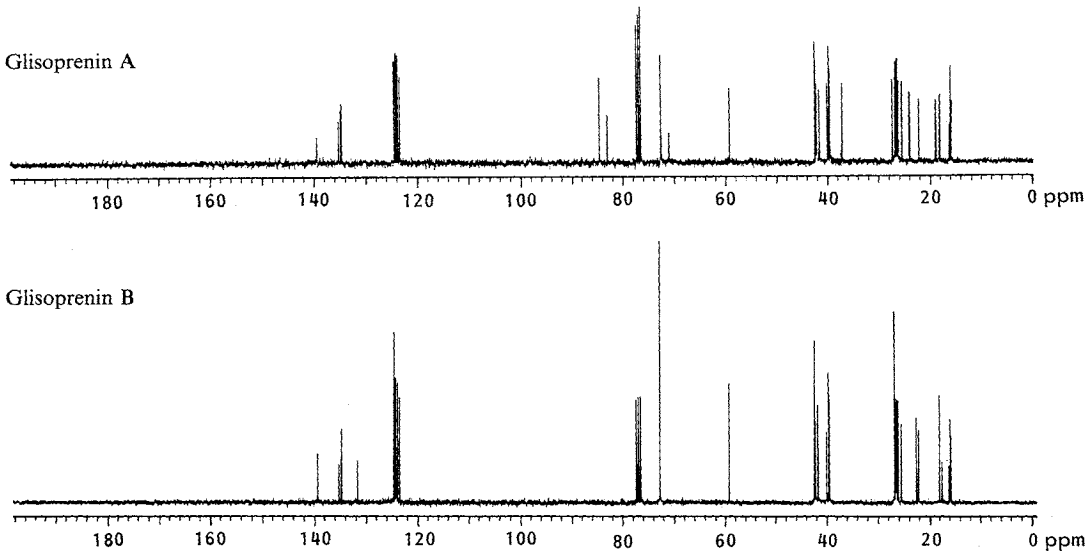
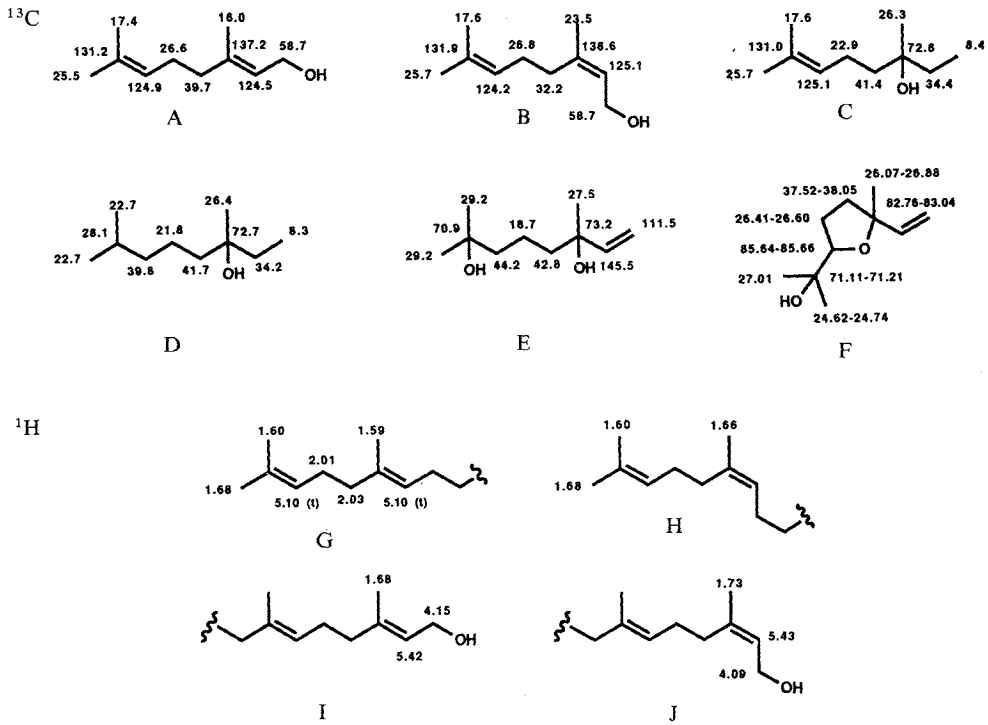
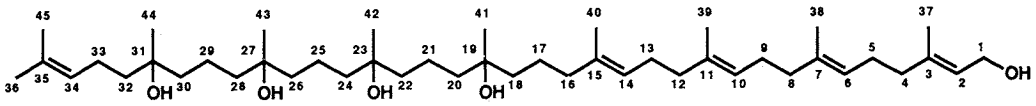


Fig. 4. ^{13}C and ^{1}H NMR chemical shifts of monoterpenes[2,3].

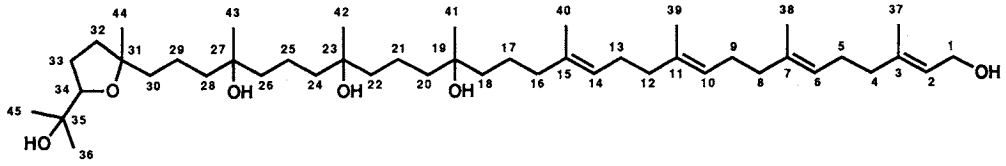


C-32 (δ 37.10), and from 32-H (δ 1.78) to C-34 (δ 84.67) exhibited the presence of (C-30)–(C-31)–(C-32 and -44)–(C-33)–(C-34)–(C-35)–(C-36 and -45) sequence. These results indicated the presence of 2,4-disubstituted tetrahydrofuran moiety as the ω-terminal structure and exhibited a good agreement of

Fig. 5. Structures of glisoprenins A and B.

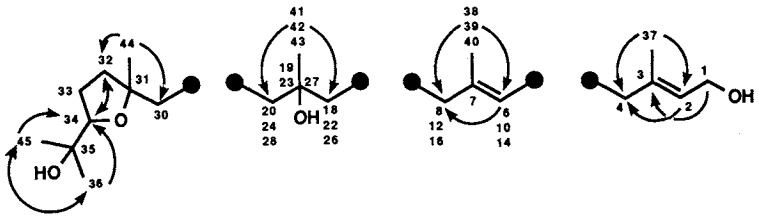


Glisoprenin A



Glisoprenin B

Fig. 6. Long-range couplings of **2** observed by HMBC experiment.



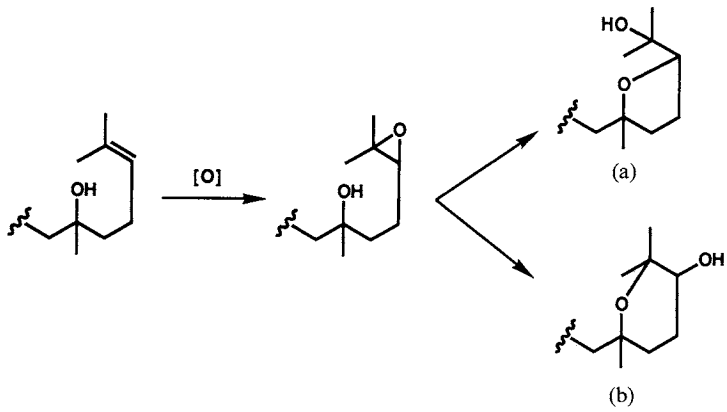
the ^{13}C NMR spectrum with linalyl oxide[2,3] (Fig. 4F). From the above data, all the carbons and protons are assigned as shown in Fig. 5. The EI-MS of **2** (Fig. 1) supports this structure because of the presence of the typical fragment ion peaks at *m/z* 125, 143, 193, 211, 261, 279, 329, 347 (cleavage from the internal hydroxy isoprenes), 135, 203 and 271 (cleavage from the internal *E*-isoprenes and the α-terminal *E*-isoprene)[4].

Consequently, the structure of **1** was determined to be tetrahydroxynonaprenol, which is composed of an ω-terminal isoprene, a geranylgeraniol moiety (all *E*-form) as the α-terminal residue and four internal hydroxy isoprenes. On the other hand, **2** is an oxidative modification of **1**, which is composed of a linalyl oxide moiety as the ω-terminal residue, a geranylgeraniol moiety (all *E*-form) as the α-terminal residue and three internal hydroxy isoprenes.

Discussion

Chemical structures of glisoprenins A (**1**) and B (**2**) were elucidated mainly by analyzing NMR and MS data. Glisoprenins are polyprenol-related compounds. The structure of **1** was deduced to be a tetrahydroxynonaprenol and the structure of **2** was deduced to be an oxidative modification of **1**. The stereochemistry of the isoprene (CH_2–C(CH_3)=CH–CH_2–) residues was determined to be all *E*-form by the ^1H NMR chemical shifts of the allylic methyl signals. Glisoprenins A (**1**) and B (**2**) have interesting structures which are composed of *E*-isoprene and hydroxy isoprene [CH_2–C(OH)CH_3–CH_2–CH_2–] units. In general, the hydration of the isoprene is done through the epoxidation of an olefinic site. Therefore, it is likely that **2** is synthesized from **1** through the epoxidation and cyclization of the ω-terminal isoprene residue. In the cyclization of **1**, two structures are possible. One is a tetrahydrofuran (Scheme 1a) and the

Scheme 1. Epoxidation and cyclization of the ω-terminal isoprene residue of **1** to **2**.



other is a tetrahydropyran (Scheme 1b). In the HMBC experiment on **2**, there was no observation of long-range coupling between 34-H and C-31, but the ^{13}C NMR chemical shifts of **2** (C-31 to C-36, C-44 and C-45) showed a good agreement with those of linalyl oxide (Fig. 4F), suggesting the presence of the tetrahydrofuran structure. Furthermore, the fact that $J_{H34\text{-}33\alpha}$ is the same as $J_{H34\text{-}33\beta}$ (7.2 Hz each) also supports a tetrahydrofuran rather than a tetrahydropyran structure.

Experimental

UV spectra were recorded on a Shimadzu model UV-200S spectrophotometer. IR spectra were recorded on a Jasco model A-102 infrared spectrophotometer. MS were obtained with a Jeol model DX-300 mass spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Varian XL-400 instrument. HMBC spectra were recorded on a Jeol GX-500 instrument.

Acknowledgment

The authors wish to thank Dr. K. SHIOMI for his critical comments on this manuscript and Ms. N. SATO, Kitasato University for the measurement of NMR spectra. The authors are grateful to Dr. H. NOGUCHI, Tokyo University for the measurement of HMBC spectra and for his kind suggestions in the NMR area. The authors are grateful to Dr. S. URANO, Tokyo Metropolitan Institute of Gerontology for his kind suggestions in the NMR area. This work was supported in part by grants from the Ministry of Education, Science and Culture of Japan and from Japan Keirin Association.

References

1) TOMODA, H.; H. NISHIDA, X.-H. HUANG, R. MASUMA, Y. K. KIM & S. ŌMURA: Glisoprenins, new inhibitors of acyl-CoA: cholesterol acyltransferase produced by *Gliocladium* sp. FO-1513. I. Taxonomy, fermentation, isolation, physico-chemical properties and biological activity. J. Antibiotics 45: 1202~1206, 1992
2) KALINOWSKI, H.-O.; S. BERGER & S. BRAUN (*Ed.*): Carbon-13 NMR Spectroscopy. pp. 425~443, John Wiley & Sons, 1988
3) ZECHMEISTER, L.; W. HERZ, H. GRISEBACH & G. W. KIRBY (*Ed.*): Progress in the Chemistry of Organic Natural Products. pp. 23~35, Springer-Verlag, 1979
4) SUGA, T. & T. SHISHIBORI: Structure and biosynthesis of cleomeprenols from the leaves of *Cleme spinosa*. J. Chem. Soc. Perkin I 1990: 2098~2103, 1990