Glisoprenins C, D and E, New Inhibitors of Appressorium Formation in *Magnaporthe grisea*, from Cultures of *Gliocladium roseum*

2. Structure Determination

OLOV STERNER*

Department of Organic Chemistry 2, Lund University, P.O. Box 124, S-22100 Lund, Sweden

ECKHARD THINES, FRANK EILBERT and HEIDRUN ANKE

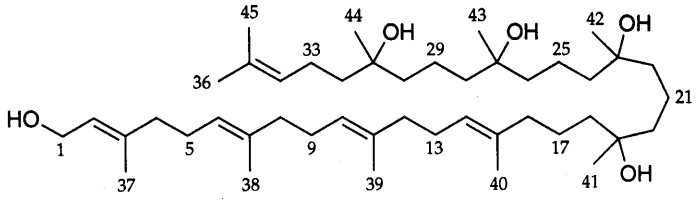
Lehrbereich Biotechnologie, University of Kaiserslautern, D-67663 Kaiserslautern, Germany

(Received for publication October 28, 1997)

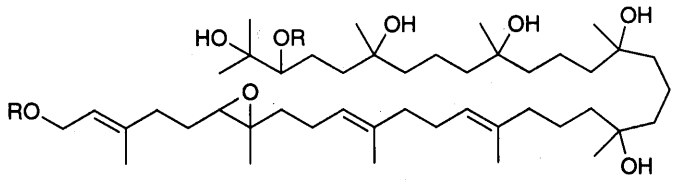
The structures of three new polyterpenoids, glisoprenins C (**2a**), D (**3**) and E (**4**), isolated from submerged cultures of the deuteromycete *Gliocladium roseum* HA190-95 as inhibitors of appressorium formation in *Magnaporthe grisea*, were determined by spectroscopic techniques. They are oxidised derivatives of glisoprenin A (**1**), which also was isolated in this investigation.

During a screening of fungal extracts for metabolites that inhibit the formation of appressoria in the plant pathogenic ascomycete *Magnaporthe grisea*, the extracts of submerged cultures of the deuteromycete *Gliocladium roseum* HA190-95 were found to be active. The preceding paper accounts for the isolation of the active metabolites by bioactive-guided fractionation and their biological activities[1], while this part describes the determination of their structures by NMR spectroscopy and mass spectrometry. By comparison of the spectroscopic data[2], one of the isolated compounds could be shown to be identical to glisoprenin A (**1**) (see Figure 1 for structures),

Fig. 1.

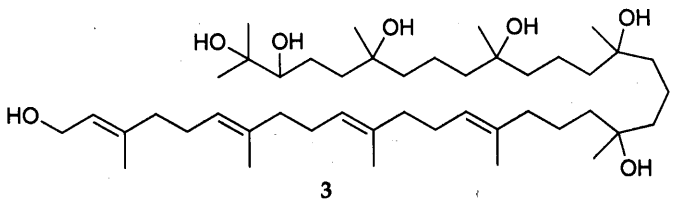


1

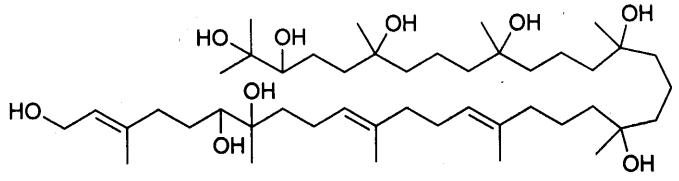


2

a: R = H; b: R = Ac.



3



4

previously isolated from a *Gliocladium* species together with glisoprenin B as an inhibitor of acyl-CoA : cholesterol acyltransferase[3]. In order to facilitate the comparison of the spectroscopic data of the new compounds with glisoprenin A (**1**), the same atom numbering of the terpene skeleton is used here[2]. The spectral data of the new compounds are presented in Tables 1, 2 and 3.

Structure Determination of Glisoprenin C (**2a**)

The NMR spectra of glisoprenin C (**2a**) suggested that it is related to glisoprenin A (**1**), although several distinct differences could be noted. Especially noteworthy is the appearance of two new signals in the ^{1}H NMR spectrum of **2a** (see Table 2), a double doublet at δ 3.32

Table 1. Physico-chemical properties of glisoprenins C (**2a**), D (**3**) and E (**4**).

	2a	**3**	**4**
Appearance	Colourless oil	Colourless oil	Colourless oil
$[\alpha]_D^{22}$	$-13°$ (c 1.8 in $CHCl_3$)	$+5°$ (c 0.3 in $CHCl_3$)	$\pm 0°$ (c 0.1 in $CHCl_3$: CH_3OH 20 : 1)
Molecular formula	$C_{45}H_{84}O_8$	$C_{45}H_{84}O_7$	$C_{45}H_{86}O_9$
HRFAB-MS			
Observed	753.6211 $M+H^+$ 775.6039 $M+Na^+$	759.6093 $M+Na^+$ 775.5866 $M+K^+$	771.6354 $M+H^+$
Calculated	753.6244 for $C_{45}H_{85}O_8$ 775.6064 for $C_{45}H_{84}O_8Na$	759.6114 for $C_{45}H_{84}O_7Na$ 775.5854 for $C_{45}H_{84}O_7K$	771.6350 for $C_{45}H_{87}O_9$
EI-MS (m/z)		646 (7%), 644 (5%), 628 (5%), 626 (3%), 279 (10%), 271 (6%), 203 (19%), 149 (32%), 135 (54%), 121 (48%), 95 (50%), 81 (100%)	
UV (MeOH)	No maxima above 210 nm		
IR (KBr) cm^{-1}	3425, 2940, 1635, 1465, 1385, 1170, 1075, 915 and 585	3425, 2940, 1635, 1465, 1385, 1170, 1075, 915 and 585	3425, 2940, 1635, 1465, 1385, 1170, 1075, 915 and 585

Table 2. ^{1}H (500 MHz) NMR data (δ; multiplicity; J) for glisoprenin C (**2a**), D (**3**) and E (**4**), in $CDCl_3$ (compounds **2a** and **3**) or $CDCl_3$ with 5% CD_3OD (compound **4**) with the $CHCl_3$ signal (7.26 ppm) as reference. The coupling constants J are given in Hz.

H	**2a**	**3**	**4**	H	**2a**	**3**	**4**
1	4.12; d; 6.8	4.14; d; 6.8	4.05; d; 6.8	26	1.35~1.45; m	1.35~1.50; m	1.25~1.40; m
2	5.43; t; 6.8	5.40; t; 6.8	5.35; t; 6.8	28	1.35~1.45; m	1.35~1.50; m	1.25~1.40; m
4	2.16/2.12; m	2.00~2.10; m	2.03/2.20; m	29	1.35~1.45; m	1.35~1.50; m	1.25~1.40; m
5	1.64; m	1.95~2.10; m	1.37/1.57; m	30	1.35~1.45; m	1.35~1.50; m	1.25~1.40; m
6	2.70; t; 6.2	5.10; m	3.24; m	32	1.35~1.45; m	1.35~1.50; m	1.25~1.40; m
8	1.65; m	1.95~2.10; m	1.51; m	33	1.33/1.55; m	1.32/1.1.55; m	1.27/1.51; m
9	2.06; m	1.95~2.10; m	2.01; m	34	3.32; dd; 1.5, 10.3	3.36; dd; 1, 10	3.22; m
10	5.07; t; 6.9	5.10; m	5.01; t; 6.8				
12	1.98; m	1.95~2.10; m	1.90~2.10; m	36	1.20[a]; s	1.22[b]; s	1.12[e]; s
13	2.06; m	1.95~2.10; m	1.97/2.05; m	37	1.67; s	1.67; s	1.60; s
14	5.07; t; 6.9	5.10; m	5.05; t; 6.8	38	1.25; s	1.59[c]; s	1.08; s
16	1.94; m	1.95~2.10; m	1.90~2.10; m	39	1.59; s	1.59[c]; s	1.51; s
17	1.35~1.45; m	1.35~1.50; m	1.25~1.40; m	40	1.57; s	1.58[c]; s	1.54; s
18	1.35~1.45; m	1.35~1.50; m	1.25~1.40; m	41	1.14; s	1.15[d]; s	1.08[f]; s
20	1.35~1.45; m	1.35~1.50; m	1.25~1.40; m	42	1.16; s	1.17[d]; s	1.09[f]; s
21	1.35~1.45; m	1.35~1.50; m	1.25~1.40; m	43	1.16; s	1.17[d]; s	1.09[f]; s
22	1.35~1.45; m	1.35~1.50; m	1.25~1.40; m	44	1.17; s	1.19[d]; s	1.10[f]; s
24	1.35~1.45; m	1.35~1.50; m	1.25~1.40; m	45	1.14[a]; s	1.16[b]; s	1.09[e]; s
25	1.35~1.45; m	1.35~1.50; m	1.25~1.40; m				

[a,b,c,d,e,f] Interchangable.

and a triplet at δ 2.70, both integrating for one proton. Compared to glisoprenin A (**1**), the signal at δ 5.07 integrates only for two protons, indicating that glisoprenin C (**2a**) contains two olefinic protons less. The high resolution mass spectrometry data (see Table 1) suggest that the elemental composition of glisoprenin C (**2a**) is $C_{45}H_{84}O_8$, with an unsaturation index of 4. As the ^{13}C NMR data (see Table 3) show that glisoprenin C (**2a**) contains three double bonds, it must consequently contain one ring. Acetylation of **2a** yielded the diacetate **2b** (see Experimental for data), and the two signals that are shifted downfield in the ^1H NMR spectrum of **2b** integrate for one and two protons, respectively, showing that glisoprenin C (**2a**) contains one primary and one secondary alcohol function. The NMR shifts for 6-H, C-6 and C-7, as well as the fact that the shift for 6-H is identical in **2a** and **2b**, support the suggestion that C-6 and C-7 are part of an epoxide ring. The remaining 5 oxygens are thereby part of tertiary alcohol functions, which is in agreement with the ^{13}C NMR data. The relative positioning of the different isoprene units in glisoprenin C (**2a**) is based on the long range ^1H-^{13}C correlations observed in the HMBC spectrum, especially between the methyl protons and the neighbouring carbons (summarised in Fig. 2). The first unit (containing the primary alcohol function on C-1), the third and the fourth contain double bonds, while the epoxide function is present in the second. The presence of two vicinal hydroxyl groups in the terminal isoprene unit could also be established by the 2D NMR data. The last four hydroxlated units are chemically and spectroscopically almost identical, and it is hazardous to assign the various 2D correlations between the overlapping signals. However, the fact that the chemical shifts are so similar, and also in agreement with those reported for glisoprenin A (**1**)[2], establishes the structure of glisoprenin C (**2a**) as shown in Fig. 1.

Table 3. ^{13}C (125 MHz) NMR data (δ; multiplicity) for glisoprenin C (**2a**) and D (**3**) in $CDCl_3$, with the solvent signal (77.0 ppm) as reference.

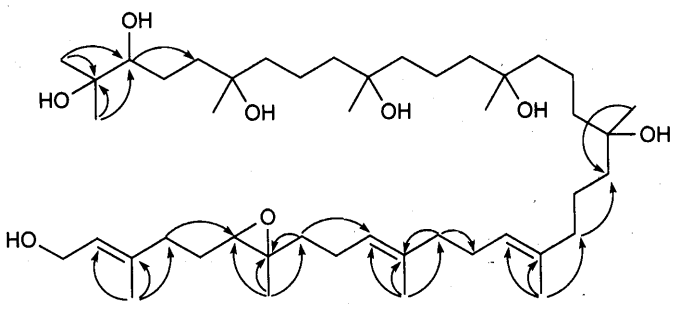
C	2a	3	C	2a	3
1	59.1; t	59.3; t	24	42.2; t	42.2[c]; t
2	124.2; d	123.4; d	25	18.1; t	18.1[d]; t
3	138.2; s	139.4; s	26	42.4; t	42.3[c]; t
4	36.2; t	39.5; t	27	72.8; s	72.8[b]; s
5	27.0[a]; t	26.3; t	28	42.4; t	42.3[c]; t
6	63.3; d	123.8; d	29	18.1; t	18.2[d]; t
7	60.9; s	134.7; s	30	42.5; t	42.4[c]; t
8	38.8; t	39.7; t	31	72.8; s	72.8[b]; s
9	23.8; t	26.5; t	32	38.8; t	38.7; t
10	123.6; d	124.2; d	33	25.8; t	25.8; t
11	135.4; s	134.9; s	34	78.9; d	78.9; d
12	39.6; t	39.7; t	35	73.1; s	73.1; s
13	26.4; t	26.3; t	36	26.4; q	26.6; q
14	124.3; d	124.5; d	37	16.2; q	16.3; q
15	134.9; s	135.3; s	38	16.5; q	15.9; q
16	40.0; t	40.0; t	39	15.9; q	16.0; q
17	22.2; t	22.3; t	40	15.8; q	16.0; q
18	41.7; t	41.7; t	41	26.8; q	26.8; q
19	72.6; s	72.7[b]; s	42	26.9[a]; q	27.0; q
20	42.2; t	42.3[c]; t	43	27.0[a]; q	27.2; q
21	18.1; t	18.2[d]; t	44	27.2; q	27.2; q
22	42.2; t	42.2[c]; t	45	23.6; q	23.5; q
23	72.8; s	72.8[b]; s			

[a,b,c,d] Interchangable.

Structure Determination of Glisoprenins D (3) and E (4)

The NMR data of glisoprenin D (**3**) show substantial similarities both with those of glisoprenin A (**1**) and glisoprenin C (**2a**). It contains the same vicinal dihydroxy/geminal dimethyl group as **2a**, although it lacks the epoxide function, and has four double bonds. The high resolution mass spectrometry data show that it contains one oxygen less compared to glisoprenin C (**2a**), and there is consequently no ring in glisoprenin D (**3**). The positioning of the double bonds in the first four

Fig. 2. Pertinent HMBC correlations observed with glisoprenin C (**2a**).



isoprene units of glisoprenin D (**3**) is supported by the EI-MS data (see Table 1), as ions of the fragments obtained after the cleavage between C-8 and C-9 (*m/z* 135 after loss of H_2O), between C-12 and C-13 (*m/z* 203 after loss of H_2O), and between C-16 and C-17 (*m/z* 271 after loss of H_2O) can be observed. This is in agreement with the EI-MS data reported for glisoprenin A (**1**)[2]. The amounts of glisoprenin E (**4**) obtained were insufficient for ^{13}C NMR spectroscopy, and the structure determination is based on ^1H NMR data, COSY correlations and FAB-MS data. Compared to glisoprenin C (**2a**) it is hydrated, and all three unsaturations are accounted for by double bonds. The suggestion that the additional molecule of water comes from the hydrolysis of the epoxide function of glisoprenin C (**2a**) is supported by the proton signal at δ 3.24 (6-H), which *via* 5-H_2 can be correlated to 4-H_2. The latter is correlated to 1-H_2 by long range ^1H-^1H couplings.

The parent compound of the glisoprenins is obviously glisoprenin A (**1**), which after epoxidation of C-34/C-35 can form glisoprenin B or be hydrolysed to glisoprenin D (**3**). The subsequent epoxidation of C-6/C-7 of 3 yields glisoprenin C (**2a**), which gives glisoprenin E (**4**) after hydrolysis. Only few polyterpenoids, composed of more than 8 isoprene units, have so far been isolated from natural sources, and the glisoprenins are rare examples of bioactive polyterpenoids[4].

Experimental

General

UV spectra were obtained with a Perkin Elmer λ 16 spectrometer, and IR spectra with a Bruker IFS 48. The optical rotation was measured with a Perkin Elmer 1541 polarimeter with a cell path of 10 cm. EI-MS (direct inlet, 70 eV) and FAB-MS spectra (direct inlet, positive ions) were recorded with a Jeol JMS-SX102 spectrometer. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temperature with a Bruker ARX 500 spectrometer with an inverse 5 mm probe equipped with a shielded gradient coil. COSY, HMQC and HMBC experiments were performed with gradient enhancements using sine shaped gradient pulses, and for the 2D heteronuclear correlation spectroscopy the refocusing delays were optimised for $^1J_{CH}=145$ Hz and $^2J_{CH}=10$ Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker UXNMR software (rev. 941001).

Diacetylglisoprenin C (**2b**)

Compound **2b** was obtained as colourless oil as the only product of acetylation of glisoprenin C (**2a**) (2 mg) with acetic anhydride (0.5 ml) in pyridine (1 ml). $[\alpha]_D^{22}$ $-10°$ (*c* 0.2 in $CHCl_3$). UV (MeOH) λ_{max} (ε): No maxima above 210 nm. IR (KBr): 3400, 2950, and 1750 cm^{-1}. ^1H NMR (500 MHz in $CDCl_3$), δ, multiplicity, *J* (Hz): 5.38, t, $J_{1\sim2}=7.1$, 2-H; 5.09, t, $J=7$, 10-H and 14-H; 4.79, dd, $J_{33a\sim34}=10.2$, $J_{33b\sim34}=2.6$, 34-H; 4.58, d, $J_{1\sim2}=7.1$, 1-H_2; 2.70, t, $J_{5\sim6}=6.3$, 6-H; 2.20, m, 4-Ha; 2.14, m, 4-Hb; 2.11, s, Ac; 2.04, s, Ac; 1.90~2.10, m, 9-H_2, 12-H_2, 13-H_2 and 16-H_2; 1.60~1.80, m, 5-H_2 and 8-H_2; 1.72, s, 37-H_3; 1.60; s, 39-H_3; 1.58, s, 40-H_3; 1.35~1.50, m, 17-H_2, 18-H_2, 20-H_2, 21-H_2, 22-H_2, 24-H_2, 25-H_2, 26-H_2, 28-H_2, 29-H_2, 30-H_2, 32-H_2 and 33-H_2; 1.25, s, 38-H_3; 1.20, s, 36-H_3; 1.19, s, 44-H_3; 1.17, s, 42-H_3; 1.17, s, 43-H_3; 1.15, s, 41-H_3; 1.15, s, 45-H_3. MS (FAB), *m/z*: 859.6304 ($M+Na^+$, $C_{49}H_{88}O_{10}Na$ requires 859.6275).

Acknowledgment

This work was supported financially by the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie, the BASF AG, Ludwigshafen, and the Swedish Natural Science Research Council. We thank SARAH MENSCH and RALPH REISS for expert technical assitance.

References

1) THINES, E.; F. EILBERT, H. ANKE & O. STERNER: Glisoprenins C, D and E, new inhibitors of appressorium formation in *Magnaporthe grisea*, from cultures of *Gliocladium roseum*. 1. Production and biological activities. J. Antibiotics 51: 117~122, 1998
2) NISHIDA, H.; X.-H. HUANG, H. TOMODA & S. OMURA: Glisoprenins, new inhibitors of acyl-CoA : cholesterol acyltransferase produced by *Gliocladium* sp. FO-1513. II. Structure elucidation of glisoprenins A and B. J. Antibiotics 45: 1669~1676, 1992
3) TOMODA, H.; X.-H. HUANG, H. NISHIDA, R. MASUMA, Y. K. KIM & S. OMURA: Glisoprenins, new inhibitors of acyl-CoA : cholesterol acyltransferase produced by *Gliocladium* sp. FO-1513. I. Production, isolation and physico-chemical and biological properties. J. Antibiotics 45: 1202~1206, 1992
4) CONNOLLY, J. D. & R. A. HILL: Dictionary of Terpenoids. Volume 2. Di- and higher terpenoids. pp. 1459~1460, Chapman and Hall, London, 1991