Chinese Chemical Letters Vol. 12, No. 7, pp 613 – 616, 2001

A New Spirostanol Saponin from *Dioscorea futshauensis*

Hong Wei LIU[1], Hisayoshi KOBAYASHI[2], Ge Xia QU[1], Xin Sheng YAO[1*]

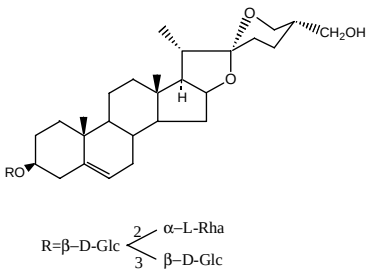
[1]Department of Natural Products Chemistry, Shenyang Pharmaceutical University, Shenyang 110015
[2]Institute of Molecular & Cellular Biosciences, The University of Tokyo, Tokyo, Japan 113

Abstract: A new spirostanol saponin presenting strong activity of inducing morphological deformation of *Pyricularia oryzae* mycelia was isolated from *Dioscorea futshauensis* R. Kunth by bioactivity-guided fractionation. The structure was established as (25S)-spirost-5-en-3β, 27-diol-3-O- [α-L-rhamnopyranosyl (1→2)-β-D -glucopyranosyl (1→3)]-β-D-glucopyranoside on the basis of chemical evidences and spectral analysis, especially by 2D-NMR techniques.

Keywords: New spirostanol glycoside, Dioscorea futshauensis, Pyricularia oryzae.

A new screening bioassay detecting deformation of mycelia germinated from conidia of *Pyricularia oryzae* P-2b was first developed for quantitative application to screen antifungal and antineoplastic agents by H. Kobayashi[1,2]. We have introduced and applied this bioassay in the search of bioactive agents from Traditional Chinese Medicine[3,4]. The ethanol extract of *Dioscorea futshauensis* R. Kunth (Dioscoreaceae) showed a strong activity against the growth of *Pyricularia oryzae* P-2b. Its butanol-soluble fraction was subjected to repeated silica gel column chromatography and reversed phase HPLC to afford a novel bioactive compound **1** along with **3** known bioactive spirostanol saponins, dioscin, gracillin and prosapogenin A of dioscin.

Figure 1 The structure of compound **1**



Compound **1** was obtained as white amorphous powder, mp 284-285°C (dec.), $[\alpha]_D^{24}$: -80.3 (pyridine; *c* 0.01), positive to the Libermann-Burchard reaction and Molish reagents. The molecular formula was established as $C_{45}H_{72}O_{18}$ on the basis of

^{13}C-NMR and FAB-MS. The positive FAB-MS gave the quasimolecular ion peak $[M+H]^+$ at *m/z* 901. The IR spectrum showed absorption bands at 3420, 2940, 1639, 1454, 1381, 1046, 912cm^{-1}. The lack of the characteristic normal F-ring spirostene bands at 980, 920, 900 and 880 indicated the change of substitution group in ring F[5]. By comparing the ^{13}C-NMR of compound **1** with that of gracillin, a great similarity was observed in their A-E ring moiety, except for the C-24, C-25, C-26 and C-27 in its F ring[6]. The changes in the chemical shifts of C-26 and C-27 (-2.9, +47 ppm) indicated the substitution of a hydroxyl group at C-27[7]. Due to the quasimolecular ion peak $[M+H]^+$ at *m/z* 885 in positive FAB-MS of gracillin, the difference of *m/z* 16 between quasimolecular ion peak of **1** and that of gracillin was deduced from the presence of an oxygen atom.

The ^{1}H-NMR spectrum of **1** showed the presence of four methyl groups at δ 0.82 (s, Me-18), 1.05 (s, Me-19), 1.15 (s, Me-21), 1.75 (d, 3H, *J*=6.0 Hz, Rha Me-6") instead of five methyl groups in gacillin, three anomeric protons at δ 6.40 (br.s, Rha-1"), 4.93 (d, 1H, *J*=6.8 Hz, Glc-1') and 5.10 (d, 1H, *J*=8.0 Hz, Glc-1'"), and one olefinic proton at δ 5.30 (br. s, H-6). The ^{13}C-NMR assignments of the aglycone of **1** were based mainly on HMQC and HMBC spectra. 27 carbon signals consisting four methyl, eleven methylene, eight methine, and four quaternary carbons were exhibited in its ^{13}C-NMR spectra of **1** (**Table 1**). All the analysis tends to establish the aglycone of compound **1** as (25S) spirost -5-en-3β, 27-diol.

The C-25 configuration of **1** was deduced to be S from its IR and ^{1}H-NMR spectrum. The strong band at 912 cm^{-1} in the IR spectrum corresponded to that at 912 cm^{-1} in isonarthogenin[5], which also suggested the presence of a hydroxyl methyl group on C-25. The 25S-configuration (equatorial orientation of the CH_2OH group) of the molecule was indicated by the ^{1}H-NMR parameters of the C-26 proton (δ 3.86, t, 1H, $J_{26ax,\ 26eq}$ =11.2Hz, $J_{26ax,\ 25ax}$ =11.2Hz, 26α-H and δ 4.13, dd, 1H, $J_{26eq,\ 26ax}$ =11.2Hz, $J_{26eq,\ 25ax}$ =4.0Hz, 26β-H)[7].

On acid hydrolysis, the sugar moieties were detected as glucose, rhamnose by silica gel TLC in comparison with the authentic samples. The positive FAB-MS of **1** also gave three fragments $[M+H - Glc]^+$ at *m/z* 739, $[M+H - Rha - Glc]^+$ at *m/z* 593, $[M+H –Glc×2 - Rha]^+$ at *m/z* 431. By analyzing the ^{1}H- ^{1}H COSY, HMQC, HMBC spectra and comparing with the report for gracillin[6], ^{1}H and ^{13}C NMR signals (**Table 1**) of sugar moiety could be assigned. The linkage sites of sugar moiety on aglycon and inter linkages among sugar were determined by HMBC spectra analysis and comparison with that of gracillin. β-configration at the anomeric position may be inferred from the values of the coupling constants for both glucopyranosyl units (6.8, 8.0 Hz). The α-configuration of the anomeric carbon of the rhamnose was assured by comparison of the chemical shift values of carbons 3" and 5" with those of the corresponding carbons of methyl α- and β-rhamnopyronoside[8]. Therefore, the structure of compound **1** is proposed to be (25S) spirost-5-en-3β, 27-diol-3-O- [α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl (1→3)]-β-D-glucopyranoside on the basis of above evidences.

General procedure for the bioassay

Preparation of conidia suspention: *Pycularia oryzae* P-2b was grown on a slant culture

medium consisting of 0.2% yeast extract, 1% soluble starch and 2% agar at 27°C. The conidia were collected on 8 to 13 days after inoculation by suspending in 10 ml sterilized water. The conidia suspention was filtered to separate from mycelia. The filtrate was added a 2% solution of yeast extract and adjusted to the concentration of 0.02%.

Table 1. ^{13}C NMR data for compound **1** in C_5D_5N (δ values)[a]

Position	**1**	Gracillin	Position	**1**	Gracillin
1	37.5	37.5	3-O-Glc		
2	30.1	30.1	1'	100.0	100.0
3	77.9	77.9	2'	77.0	77.0
4	38.7	38.7	3'	89.6	89.6
5	140.8	140.8	4'	69.6	69.6
6	121.9	121.9	5'	77.9	77.7
7	32.3	32.2	6'	62.4	62.4
8	31.7	31.7	Rha (1→2)		
9	50.3	50.3	1"	102.2	102.2
10	37.1	37.1	2"	72.5	72.5
11	21.1	21.1	3"	72.8	72.8
12	39.9	39.9	4"	74.1	74.1
13	40.5	40.5	5"	69.6	69.6
14	56.6	56.6	6"	18.7	18.7
15	32.3	32.2	Glc (1→3)		
16	81.1	81.1	1'"	104.6	104.6
17	62.9	62.9	2'"	75.0	75.0
18	16.3	16.3	3'"	78.5	78.5
19	19.4	19.4	4'"	71.5	71.5
20	42.0	42.0	5'"	78.6	78.7
21	15.0	15.0	6'"	62.4	62.4
22	109.7	109.7			
23	31.7	31.7			
24	24.0	29.1			
25	39.2	30.6			
26	64.0	66.9			
27	64.4	17.3			

[a] All the signals were assigned by ^1H - ^1H COSY, HMQC and HMBC spectra, recorded on a JNM Alpha-500 (^1H 500 MHz, ^{13}C 125 MHz) spectrometer in C_5D_5N.

Bioassay: A 96-cell microplate with 12 columns was used for the bioassay. The first and last columns were preserved for negative and positive controls (rhizoxin), repectively. 50 μl of conidia suspention was added into each well, and 50 μl of each test sample (1.0 mg/ml, in 10% MeOH) was added into the first well of each column. One column (eight wells) was usually used for one test material with eight different concentrations (from 1.0 mg/ml to 8.0 μg/ml by successive duplicate dilution). The plate was then incubated at 27°C for 16 hr. The morphology of mycelia and conidia was observed in comparition and negative controls under an inverted microsope. For negative control, 50 μl 10% MeOH solution was added into the first well.

The bioactivity of compound **1**, dioscin, gracillin, prosapogenin A of dioscin against *P. oryzae* were listed in **Table 2**.

Table 2 Bioactivity against the growth of *P. oryzae*

Compounds	*P. oryzae* (μM)
dioscin	3.2
gracillin	10.0
prosapogenin A of dioscin	6.1
compound 1	8.6
rhizoxin	0.008

Acknowledgments

We appreciate the kind help of Prof. Qishi Sun in Shenyang Pharmaceutical University for his identification of the plant material. Thanks are also extented to Associate Prof. Naili Wang and doctor Feng Qiu for their advices and assistance.

References

1. H. Kobayashi, R. Sunaga, K. Furihata, N. Morisaki, S. Iwasaki. *J. Antibiotics*, **1995**, *48*(1), 42.
2. H. Kobayashi, M. Namikoshi, T. Yoshimoto, T. Yokochi. *J. Antibiotics*, **1996**, *49*(9), 873.
3. K. Hu, A. J. Dong, X. S. Yao, H. Kobayashi, S. Iwasaki. *Planta Med.*, **1996**, *62*(6), 573.
4. K. Hu, A. J. Dong, X. S. Yao, H. Kobayashi, S. Iwasaki. *Planta Med*., **1997**, *63*(2), 161.
5. K. Takeda, H. Minato, A. Shimaoka, Y. Matsui. *J. Chem. Soc.*, **1963,** 4815.
6. Yang, C. R. Wang Zh.. *Acta Botanica Yunnanica*.**, 1986,** *8*(3), *355*.
7. G. Blunden, A. Patel, T. A. Crabb. *J. Nat. Prod*. **1986,** *49*, 689.
8. P. K. Agrawal, D. C. Jain, P. K. Gupta, R. S. Thakur. *Phytochemistry*, **1985**, *24*(11), 2479.

Received 19 December, 2000