ELSEVIER

www.elsevier.nl/locate/carres

Carbohydrate Research 323 (2000) 57–62

CARBOHYDRATE RESEARCH

A new bioactive steroidal saponin, furcreastatin, from the plant *Furcraea foetida*

Masaki Itabashi [a], Kaoru Segawa [b], Yoko Ikeda [c], Shinichi Kondo [c], Hiroshi Naganawa [c], Takashi Koyano [d], Kazuo Umezawa [a,*]

[a] *Department of Applied Chemistry, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-0061, Japan*

[b] *Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan*

[c] *Institute of Microbial Chemistry, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141-0021, Japan*

[d] *Temko Corporation, 4-27-4 Honcho, Nakano-ku, Tokyo 164-0012, Japan*

Received 24 May 1999; accepted 20 August 1999

Abstract

Microbial and plant secondary metabolites were screened for compounds that are selectively cytotoxic to mutant p53-expressing mouse fibroblasts. As a result, furcreastatin, a novel steroidal saponin, was isolated from an EtOH extract of the leaves of *Furcraea foetida*. Furcreastatin consisted of hecogenin as the aglycone and a hexasaccharide containing D-galactose, L-rhamnose and four D-glucose residues. The structure was determined to be (3β,5α,25*R*)-3-hydroxyspirostan-12-one 3-*O*-[α-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→3)-{β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→2)}-β-D-Glc*p*-(1→4)-β-D-Gal*p*] by extensive NMR spectroscopic studies. Furcreastatin decreased the viability of mutant p53-overexpressing cells with an ED_{50} of 4.0 μg/mL, and decreased that of the parental cell-line with an ED_{50} of 9.6 μg/mL.

Keywords: *Furcraea foetida*; Furcreastatin; Steroidal saponin; Hecogenin hexasaccharide; p53

1. Introduction

Human carcinogenesis can now be explained by the activation of oncogenes and the mutation or deletion of tumor-suppressor genes. Inhibitors of the functions of oncogene products, such as protein tyrosine kinase inhibitors and anti-Ras compounds, have been extensively studied [1]. However, modification of tumor-suppressor gene products has been poorly studied. Recovery of lost tumor-suppressor gene functions may become a new field of cancer chemotherapy.

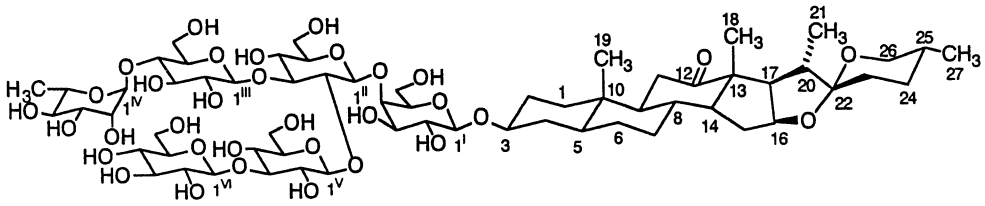
The tumor-suppressor gene p53 is mutated in about 70% of human neoplasias. The wild-type p53 protein induces a G1 block in the cell cycle and enhances apoptosis [2]. However, mutation of p53 can diminish these cellular activities. Therefore, we searched for microbial and plant secondary metabolites that induce selective cell death in mutated p53-expressing fibroblasts. As a result we isolated a novel steroidal saponin, furcreastatin, from the plant *Furcraea foetida*.

2. Results and discussion

Mutant p53-overexpressing Balb/c-3T3-10(1) mouse fibroblasts (10(1)-mp53 cells)

* Corresponding author. Fax: +81-45-562-7625.
E-mail address: umezawa@applc.keio.ac.jp (K. Umezawa)

0008-6215/00/$ - see front matter
PII: S0008-6215(99)00255-4



Furcreastatin (**1**)

were prepared by transfection of Balb/c-3T3-10(1) cells with mutant p53 DNA (see Section 3). After screening of microbial culture broths and plant extracts, one of the plant extracts induced cell death in 10(1)-mp53 cells at lower doses than in the parental cell line. The plant was identified as *F. foetida*.

Extraction of the dried leaves (15.64 g) of *F. foetida* with 80% aqueous EtOH, followed by concentration and extraction with BuOH, gave a crude material that was purified by column chromatography on silica gel, and then by gel filtration to yield furcreastatin (**1**, 211 mg) as colorless needles.

Table 1
NMR data for the aglycone of furcreastatin (**1**) in 10:1 pyridine-d_5–MeOH-d_4

Position	^{13}C, δ	^{1}H, δ (J, Hz)	
1	36.7	0.72m	1.33m
2	29.7	1.57m	1.98m
3	77.3	3.87m	
4	34.7	1.33m	1.78m
5	44.6	0.86m	
6	28.7	1.14m	
7	31.8 [a]	0.80m	1.58m
8	34.4	1.78m	
9	55.6	0.91m	
10	36.4		
11	38.1	2.23dd (5, 14)	2.39dd (13, 14)
12	213.0		
13	55.4		
14	56.0	1.38m	
15	31.5	1.58m	2.10ddd (6, 7, 13)
16	79.8	4.47m	
17	54.4	2.74dd (8, 9)	
18	16.1	1.08s	
19	11.8	0.69s	
20	42.7	1.92dq (7, 8)	
21	13.9	1.34d (7)	
22	109.4		
23	31.9 [a]	1.70m	
24	29.3	1.58m	
25	30.6	1.58m	
26	67.0	3.48dd (11, 12)	3.58dd (4, 12)
27	17.3	0.71d (6.4)	

[a] Interchangeable.

The IR spectrum of **1** exhibited typical absorption bands of alcoholic C–O at 1000–1200 cm^{-1}, suggesting the presence of saccharide components. In the positive- and negative-ion FAB mass spectra, the molecular ion peaks of **1** appeared at m/z 1409 and 1385, indicating $[M + Na]^+$ and $[M - H]^-$, respectively. HRFABMS (neg.) showed m/z 1385.6266 $[M - H]^-$ ($C_{63}H_{101}O_{33}$ requires 1385.6225). Furthermore, fragment-ion peaks at m/z 1239 [M – deoxyhexosyl]$^-$, 1223 [M – hexosyl]$^-$, 1077 [M – deoxyhexosyl – hexosyl]$^-$ and 1061 [M – hexosyl – hexosyl]$^-$ were observed in FABMS (neg.). These results indicated the presence of two terminal disaccharide units, deoxyhexosyl–hexose and hexosyl–hexose in the branched saccharide chain of **1**.

As shown in Tables 1 and 2, ^{1}H and ^{13}C chemical shift assignments were made by standard 1D and 2D NMR techniques. By 2D NMR studies, the structure of **1** was proposed to be a steroidal saponin having a new hexasaccharide chain. The gross structure of the aglycone with 27 carbons was elucidated to be 3-hydroxyspirostan-12-one by conducting ^{1}H–^{1}H-COSY, HMBC, and NOESY experiments (Table 1). Acid hydrolysis of **1** gave the aglycone and three monosaccharides. The aglycone was identical to authentic hecogenin, (3β,5α,25*R*)-3-hydroxyspirostan-12-one [3], in all respects.

The three monosaccharides were determined to be D-glucose, D-galactose and L-rhamnose, respectively, by measuring their optical rotation values.

^{1}H and ^{13}C chemical shifts of the hexasaccharide consisting of 4 mol of glucopyranose, and 1 mol each of galactopyranose and rhamnopyranose, were assigned by DEPT, ^{1}H–^{1}H-COSY, HOHAHA, NOESY, HMQC and HMBC (Table 2). Although the H-4^{I}

Table 2
NMR data for the hexasaccharide of furcreastatin (**1**) in 10:1 pyridine-d_5–MeOH-d_4

Position	^{13}C, δ	^{1}H, δ (J, Hz)	^{1}H–^{1}H-COSY [a]	HMBC	^{1}H–^{1}H-NOESY
$(3\rightarrow1^{I})$-Gal-$(4^{I}\rightarrow$					
1^{I}	102.5	4.79d (8.2)	H-2^{I}	C-3	H-3^{I}, H-5^{I}
2^{I}	73.0	4.34m	H-1^{I}, H-3^{I}	C-1^{I}, C-3^{I}, C-4^{I}	
3^{I}	75.3	4.08m	H-2^{I}, H-4^{I}	C-2^{I}	H-1^{I}, H-4^{I}
4^{I}	80.1	4.56m	H-3^{I}	C-2^{I}, C-1^{II}	H-3^{I}, H-5^{I}, H-1^{II}
5^{I}	75.3	3.97m	H-6b^{I}	C-4^{I}	H-1^{I}, H-4^{I}
6a^{I}	60.6	4.19m	H-6b^{I}	C-4^{I}	
6b^{I}		4.61dd (10, 11)	H-5^{I}, H-6a^{I}	C-4^{I}	
$(4^{I}\rightarrow1^{II})$-Glc-$(3^{II}\rightarrow$					
1^{II}	104.8	5.10d (7.8)	H-2^{II}	C-4^{I}	H-4^{I}, H-5^{II}
2^{II}	80.8	4.27m	H-1^{II}, H-3^{II}	C-3^{II}, C-1^{V}	H-4^{II}, H-1^{V}
3^{II}	88.1	4.16m	H-2^{II}, H-4^{II}		H-1^{III}
4^{II}	70.4	3.73dd (9, 10)	H-3^{II}	C-5^{II}	
5^{II}	77.5	3.79m	H-6a^{II}, H-6b^{II}	C-4^{II}	H-2^{II}
6a^{II}	62.8	3.97m	H-5^{II}, H-6b^{II}		
6b^{II}		4.38m	H-5^{II}, H-6a^{II}		
$(3^{II}\rightarrow1^{III})$-Glc-$(4^{III}\rightarrow$					
1^{III}	104.1	5.18d (8.2)	H-2^{III}	C-3^{II}	H-3^{II}, H-3^{III}, H-5^{III}
2^{III}	75.3	3.93dd (8.2, 10)	H-1^{III}, H-3^{III}	C-1^{III}, C-3^{III}	H-4^{III}
3^{III}	76.4	4.04m	H-2^{III}, H-4^{III}		H-1^{III}
4^{III}	78.1	4.28m	H-3^{III}	C-3^{III}, C-5^{III}, C-1^{IV}	H-2^{III}, H-1^{IV}
5^{III}	77.2	3.76m	H-6a^{III}, H-6b^{III}		H-1^{III}
6a^{III}	61.9	4.25m	H-5^{III}, H-6b^{III}		
6b^{III}		4.37m	H-5^{III}, H-6a^{III}		
$(4^{III}\rightarrow1^{IV})$-Rha					
1^{IV}	102.6	5.69s	H-2^{IV}	C-4^{III}, C-2^{IV}, C-5^{IV}	H-4^{III}, H-2^{IV}
2^{IV}	72.4	4.55m	H-1^{IV}, H-3^{IV}	C-3^{IV},C-4^{IV}	H-1^{IV}
3^{IV}	72.5	4.45m	H-2^{IV}, H-4^{IV}		
4^{IV}	73.8	4.25m	H-3^{IV}, H-5^{IV}	C-3^{IV}, C-5^{IV}	H-6^{IV}
5^{IV}	70.4	4.85dq (6.4, 11)	H-4^{IV}, H-6^{IV}	C-4^{IV}	H-6^{IV}
6^{IV}	18.5	1.66d (6.4)	H-5^{IV}		H-4^{IV}, H-5^{IV}
$(2^{II}\rightarrow1^{V})$-Glc-$(3^{V}\rightarrow$					
1^{V}	103.9	5.54d (7.8)	H-2^{V}	C-2^{II}	H-2^{II}, H-3^{V}, H-5^{V}
2^{V}	74.6	4.07m	H-1^{V}		
3^{V}	88.0	4.06m		C-2^{V}, C-4^{V}	H-1^{V}, H-1^{VI}
4^{V}	69.2	4.10m	H-5^{V}		
5^{V}	77.8	3.76m	H-4^{V}, H-6a^{V}		H-1^{V}
6a^{V}	61.0	3.99m	H-5^{V}	C-5^{V}	
6b^{V}		4.18m			
$(3^{V}\rightarrow1^{VI})$-Glc					
1^{VI}	105.5	5.05d (8.2)	H-2^{VI}	C-3^{V}	H-3^{V}, H-3^{VI}, H-5^{VI}
2^{VI}	75.5	4.00m	H-1^{VI}	C-1^{VI}	
3^{VI}	78.0	4.12dd (10, 10)		C-4^{VI}	H-1^{VI}
4^{VI}	71.5	4.07m	H-5^{VI}		
5^{VI}	78.4	3.87m	H-4^{VI}, H-6a^{VI}, H-6b^{VI}		H-1^{VI}
6a^{VI}		4.22m	H-5^{VI}, H-6b^{VI}		
6b^{VI}	62.4	4.46m	H-5^{VI}, H-6a^{VI}	C-4^{VI}	

[a] Assignments are supported by HOHAHA and HMQC experiments.

signal (δ 4.56) overlapped with that of H-2^{IV} (δ 4.55), these signals had only small couplings. Then, the saccharide glycosidically linked to the 3-OH group of the aglycone was decided to be a 4-*O*-substituted Gal. The Glc glycosidically linked to the 4-OH of Gal was also glycosylated by Glc groups at 2-OH and 3-OH. Therefore, two terminal disaccharides detected by FABMS fragmentation peaks were assigned to Rha-Glc and Glc-Glc. The β anomeric configurations for Glc and Gal were assigned based on their large coupling constants ($J_{1,2}$ 7.8–8.2 Hz). The anomeric configuration of Rha was determined to be α,

by comparison of NMR chemical shift data of H-1 (lower-field shift in the α anomer than in the β), C-3 and C-5 (higher-field shift in the α anomer than in the β) [4–6]. The conformations of glucopyranose ($^4C_1$), galactopyranose ($^4C_1$) and rhamnopyranose ($^1C_4$) were also confirmed by ^{1}H–^{1}H-NOESY. Five NOEs between anomeric protons and glycosylated saccharide ring-protons were observed at δ 5.10 (H-1^{II}) and 4.56 (H-4^{I}), at δ 5.18 (H-1^{III}) and 4.16 (H-3^{II}), at δ 5.69 (H-1^{IV}) and 4.28 (H-4^{III}), at δ 5.54 (H-1^{V}) and 4.27 (H-2^{II}) and at δ 5.05 (H-1^{VI}) and 4.06 (H-3^{V}). Thus, the hexasaccharide sequence was established, and the structure of **1** was decided to be (3β,5α,25*R*)-3-hydroxyspirostan-12-one-3-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-{β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2)}-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside].

Among the known steroidal saponins, two hecogenin saccharides having an α-L-rhamnopyranosyl residue(s), agavesaponins D and E, were isolated from *Agave americana* [7–9]. The former has a branched pentasaccharide composed of Rha-(1→3)-[Xyl-(1→2)]-Glc-(1→4)-Glc-(1→4)-Gal-; and the latter, a branched hexasaccharide, Rha-(1→4)-Rha-(1→3)-[Xyl-(1→2)]-Glc-(1→4)-Glc-(1→4)-Gal-. Most recently, terrestrosin D, a hecogenin tetrasaccharide having a Gal-(1→2)-[Xyl-(1→3)]-Glc-(1→4)-Gal- chain was found in the fruits of *Tribulus terrestris* [10]. However, **1** is significantly different from the others in having a previously unreported saccharide chain.

As shown in Table 3, **1** showed cytotoxicity toward 10(1)-mp53 cells at lower concentrations than in 10(1) cells. The EC_{50} values were 4.0 and 9.6 μg/mL in 10(1)-mp53 and 10(1) cells, respectively. It also showed a cytotoxic effect on human carcinoma cells. Its cytotoxic activities are shown in Table 3 for HSC-3 (mutant p53), Ca9-22 (mutant p53), SK-Br-3 (mutant p53) and BT549 (mutant p53) cells.

Table 3
Cytotoxic (EC_{50}) activities of furcreastatin (**1**) on human carcinoma cell lines

Cells	EC_{50} (μg/mL) [a]
10(1)	9.6
10(1)-mp53	4.0
HSC-3 (oral squamous)	2.6
Ca9-22 (oral squamous)	3.2
SK-Br-3 (breast)	6.3
BT549 (breast)	2.6

[a] The viability was assayed by the Trypan Blue dye-exclusion method.

3. Experimental

General.—Melting points were determined with a Yanagimoto apparatus and are uncorrected. Optical rotations were taken on a Perkin–Elmer 241 polarimeter. UV spectra were recorded on a Hitachi U-3210 spectrophotometer. IR spectra were taken on a Horiba FT210 infrared spectrometer. Mass spectra were measured on Jeol JMS-SX102 (FAB mode) and Jeol JMS-700 (HRFABMS) mass spectrometers. NMR spectra were measured in a mixture of pyridine-d_5–MeOH-d_4 (14.5 mg of **1** in 0.6 mL) with a Jeol JNM-A500 spectrometer, with Me_4Si ($\delta = 0$) used as the internal standard. ^{1}H NMR spectra were recorded at 500 MHz and ^{13}C NMR spectra at 125 MHz. The 2D experiments were carried out with the following parameters. (a) ^{1}H–^{1}H-COSY: spectral width 4271 Hz, and data matrix size 1024 × 1024; (b) ^{1}H–^{1}H-NOESY: spectral width 4246 Hz, data matrix size 512 × 512 and mixing time 500 ms; (c) HOHAHA: spectral width 3305 Hz, data matrix size 1024 × 1024 and mixing time 80 ms; (d) HMQC (pulsed field gradient mode): ^{1}H spectral width 4231 Hz, ^{13}C spectral width 19,033 Hz and data matrix size 1024 × 512; (e) HMBC (pulsed field gradient mode): ^{1}H spectral width 4083 Hz, ^{13}C spectral width 26,357 Hz and data matrix size 1024 × 512, and a delay of 60 ms. TLC was performed on silica gel plates (Kieselgel $60F_{254}$, E. Merck Art.5715), and stained with phosphomolybdic acid–H_2SO_4 and triphenyltetrazolium chloride reagents. PC was performed on filter paper (Toyo No. 51A) by the ascending technique.

Plant material.—Leaves of *F. foetida* were collected in Thailand near Khon Kaen in April and July, 1998. The plant was identified by Professor T. Kowithayakorn, Plant Science Department, Faculty of Agriculture, Khon Kaen University, where a voucher specimen is maintained.

Extraction and isolation.—Extraction of the dried leaves (15.64 g) of *F. foetida* with 80% aq EtOH (600 mL), followed by concentration to 120 mL and extraction with an equal volume of BuOH gave crude material, which was dissolved in water (200 mL) and washed with EtOAc (200 mL) to yield a crude solid (810 mg). Further purification by chromatography on a silica gel column (100 mL) eluted with 20:2:3 BuOH–MeOH–H_2O (240 mL) and by gel-filtration with Toyopearl HW40 column (Tosoh, 200 mL) developed with MeOH yielded **1** (211 mg) as colorless needles.

Furcreastatin (**1**).—Colorless needles; mp > 280 °C; $[\alpha]_D^{25} - 42°$ (*c* 0.4, MeOH); UV: λ_{max}^{MeOH} (ε) 232 nm (600); IR ν_{max} (KBr): 3431, 2929, 2873, 1705, 1637, 1456, 1427, 1375, 1348, 1308, 1244, 1159, 1074, 1039, 1018, 980, 899, 611 cm^{-1}; FABMS (pos.), *m/z* 1409 $[M + Na]^+$; FABMS (neg.), *m/z* 1385 $[M - H]^-$, 1239 [M − rhamnosyl]$^-$, 1223 [M − glucosyl]$^-$, 1077 [M − rhamnosyl − glucosyl]$^-$, 1061 [M − glucosyl − glucosyl]$^-$; HRFABMS (neg.), *m/z* 1385.6266 $[M - H]^-$ ($C_{63}H_{101}O_{33}$ requires 1385.6225); ^1H and ^{13}C NMR data, see Tables 1 and 2.

Acid hydrolysis of **1**.—Compound **1** (69.3 mg) was hydrolyzed with 1:1 2 M HCl–1,4-dioxane (10 mL) in a sealed tube for 3 h at 100 °C, and the reaction mixture was extracted with EtOAc (50 mL, twice). The solvent extract was purified by preparative TLC on silica gel plates (19:1 CH_2Cl_2–MeOH, R_f 0.53) to afford the aglycone (11.8 mg) as colorless crystals: mp 263–265 °C, $[\alpha]_D^{24} + 7.2°$ (*c* 0.8, $CHCl_3$) (lit. mp 263–265 °C, $[\alpha]_D + 6°$ [11]). The aglycone was identical to authentic hecogenin (Sigma) in all respects including NMR data [12–14].

D *and* L *configurations of component saccharides.*—Evaporation of the aq layer of the aforementioned hydrolyzate gave the residue (55 mg) containing monosaccharides, which was separated into rhamnose (5.8 mg, R_f 0.68), glucose (20.8 mg, R_f 0.46), and galactose (8.1 mg, R_f 0.40) by preparative PC (6:4:3 BuOH–pyridine–H_2O). The optical rotation values of the isolated monosaccharides showed these sugars to be L-rhamnose ($[\alpha]_D^{25} + 6°$, lit. + 8.2° [15]), D-glucose ($[\alpha]_D^{25} +$ 53°, lit. + 52.7° [15]) and D-galactose ($[\alpha]_D^{24} +$ 67°, lit. + 80.2° [15]), respectively.

Preparation of mutant p53-expressing mouse fibroblast cells.—Balb/c-3T3-10(1)-mp53 cells were prepared by transfection of 10(1) cells (p53 null) with pSV-p53c17 plasmids (p53: 168G/234I) [16] by the calcium phosphate precipitation method [17]. The cells were incubated with Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 100 units/mL penicillin G, and 100 μg/mL kanamycin.

Screening method.—Balb/c-3T3-10(1)-mp53 or 10(1) cells were seeded at 7.0×10^3 cells in a 96-well plastic plate. After 24 h the test samples were added to the cells. Then, the cells were further incubated for 24 h and subjected to the MTT assay, which measures the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to its insoluble formazan.

Cell viability assay.—Balb/c-3T3-10(1), 10(1)-mp53, HSC-3, Ca9-22, SK-Br-3 and BT-549 cells were seeded at 3.5×10^4 cells in 1 mL of fresh medium in 12-well plates. After 24 h **1** was added, then after 24 h the cells were harvested. The viable cells were counted by the Trypan Blue dye-exclusion method.

Acknowledgements

This work was financially supported in part by a grant from the Ministry of Science, Education, Culture and Sports of Japan and the Science Research Promotion Fund of the Promotion and Mutual Aid Corporation for Private Schools of Japan.

References

[1] K. Umezawa, in S. Pandalai (Ed.), *Research Developments in Agricultural and Food Chemistry*, Research Signpost, Trivandum, 1997, pp. 277–287.
[2] D.P. Lane, *Nature*, 358 (1992) 15–16.
[3] M. Soriano-Garcia, R.A. Toscano, I. Lopez, A. Hernandez, P. Enriquez, *Acta Crystallogr. Sect. C*, 40 (1984) 2116–2118.
[4] R. Kasai, M. Okihara, J. Asakawa, K. Mizutani, O. Tanaka, *Tetrahedron*, 35 (1979) 1427–1432.
[5] D. Zhang, T. Miyase, M. Kuroyanagi, K. Umehara, A. Ueno, *Chem. Pharm. Bull.*, 43 (1995) 115–120.
[6] T. Inoue, Y. Mimaki, Y. Sashida, T. Nikaido, T. Ohmoto, *Phytochemistry*, 39 (1995) 1103–1110.
[7] G.V. Lazurevskii, V.A. Bobeiko, P.K. Kintya, *Dokl. Akad. Nauk SSSR*, 224 (6) (1975) 1442–1444.

[8] V.A. Bobeiko, P.K. Kintya, *Khim. Prir. Soedin.*, 11 (1975) 751–754.
[9] P.K. Kintya, V.A. Bobeiko, in V.A. Rasskazov (Ed.), Tezisy Dokl.-Vses. Simp. Bioorg. Khim.; Akad. Nauk SSSR: Vladivostok, USSR, 1975, p. 20.
[10] W. Yan, K. Ohtani, R. Kasai, K. Yamasaki, *Phytochemistry*, 42 (1996) 1417–1422.
[11] Y. Mazur, N. Danieli, F. Sondheimer, *J. Am. Chem. Soc.*, 82 (1960) 5889–5908.
[12] P.K. Agrawal, D.C. Jain, R.K. Gupta, R.S. Thakur, *Phytochemistry*, 24 (1985) 2479–2496.
[13] J.A.R. Garcia, H.T.V. Castro, *Magn. Reson. Chem.*, 25 (1987) 831–834.
[14] D.N. Kirk, H.C. Toms, C. Douglas, K.A. White, *J. Chem. Soc., Perkin Trans. 2*, (1990) 1567–1594.
[15] R. Schaffer, in W. Pigman, D. Horton (Eds.), *The Carbohydrates. Chemistry and Biochemistry*, Vol. 1A, second ed., Academic Press, New York, London, 1972, pp. 69–111.
[16] D. Eliyahu, N. Goldfinger, O. Pinhasi-Kimhi, G. Shaulsky, Y. Skurnik, N. Arai, V. Rotter, M. Oren, *Oncogene*, 3 (1988) 313–321.
[17] Y.X. Zeng, K. Somasundaram, W.S. El-Deiry, *Nat. Genet.*, 15 (1997) 78–82.