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MK800-62F1, a New Inhibitor of Apoptotic Cell Death, from *Streptomyces diastatochromogenes* MK800-62F1

II. Structure Elucidation

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A new compound, MK800-62F1, was isolated from a cultured broth of *Streptomyces diastatochromogenes* MK800-62F1. The structure was determined by NMR analysis and degradation experiments.

MK800-62F1 (**1**) has been isolated from a cultured broth of *Streptomyces diastatochromogenes* MK800-62F1. Compound **1** showed inhibitory activity of H_2O_2-induced as well as anticancer drug-induced apoptotic cell death. The taxonomy of the producing strain, fermentation, isolation, physico-chemical properties and biological activities of **1** were described in the previous paper[1]. This paper deals with structure elucidation of **1** (Fig. 1).

Result and Discussion

Structure Elucidation

The molecular formula of MK800-62F1 (**1**) was established as $C_{35}H_{58}O_8$ on the basis of HRFAB-MS and NMR spectral analysis. The multiplicities of carbon signals were determined by DEPT experiments. All bond correspondings between ^1H and ^{13}C signals were determined by DEPT and HMQC experiments. The ^{13}C NMR spectrum of **1** showed 35 carbon signals. The DEPT and HMQC experiments revealed the presence of seven methyls, ten methylenes, ten methines, one olefinic methine, six sp^3 quaternary carbons and one olefinic quaternary carbon. The ^1H and ^{13}C NMR data of **1** were similar to those of soyasaponins[2] or kudzusaponins[3] except for NMR data of the sugar moiety. The ^1H and ^{13}C NMR spectral data of **1** are summarized in Table 1.

The ^1H-^1H COSY and HMBC spectra of **1** suggested the aglycone moiety of **1** as shown in Fig. 2. A hydroxy group at C-3 was supported by HMBC correlations from 3-H to C-1 and C-5. Hydroxy groups at C-21 and C-24 were also supported by HMBC correlations from 21-H to C-19 and C-17, from 24-H to C-3, C-4, C-5 and C-23, respectively.

The relative configuration of the aglycone moiety of **1**

Fig. 1. Structure of MK800-62F1.

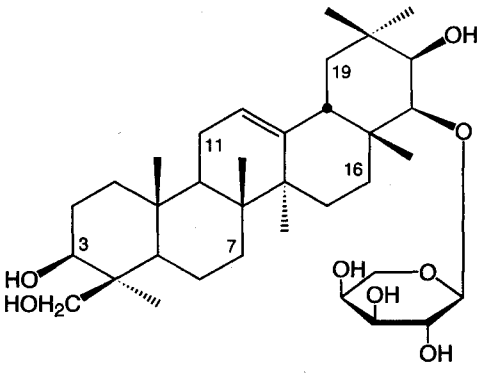
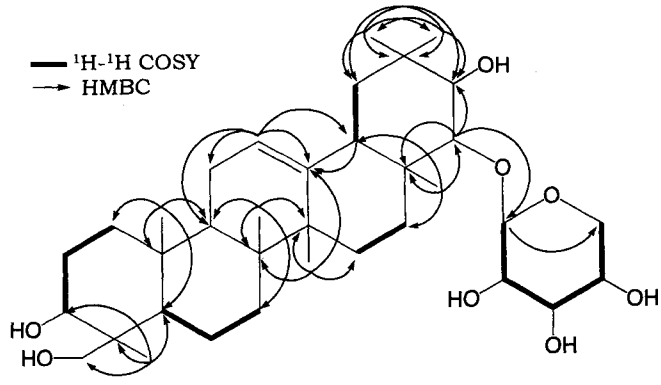


Table 1. ^{13}C and ^{1}H NMR assignments of MK800-62F1 in pyridine-d_5.

Position	δ_C (ppm)	δ_H (ppm)
1	38.9	0.97 (m), 1.57 (m)
2	28.4	1.90 (m), 2.00 (m)
3	80.1	3.63 (br dd, 11.2, 4.0)
3-OH		6.67 (br s)
4	43.2	
5	56.3	0.97 (dd)
6	19.1	1.42 (m), 1.71 (m)
7	33.1	1.27 (m), 1.51 (m)
8	40.3	
9	48.1	1.65 (dd, 10.8, 7.0)
10	36.9	
11	24.2	1.89 (m), 1.89 (m)
12	122.5	5.36 (dd, 3.5, 3.4)
13	144.4	
14	41.8	
15	26.7	0.97 (m), 1.87 (m)
16	27.9	1.11 (m), 2.01 (m)
17	39.3	
18	44.5	2.50 (dd, 13.8, 4.1)
19	47.3	1.37 (dd, 13.8, 4.1)
		2.10 (dd, 13.8, 13.8)
20	37.0	
21	75.8	3.91 (dd, 10.7, 3.4)
21-OH		6.13 (d, 10.7)
22	92.7	3.77 (d, 3.4)
23	23.6	1.56 (s)
24	64.6	3.71 (d,11.6)
		4.51 (d,11.6)
24-OH		5.37 (br s)
25	16.2	0.91 (s)
26	16.8	0.96 (s)
27	26.7	1.26 (s)
28	23.2	1.39 (s)
29	31.5	1.23 (s)
30	21.4	1.42 (s)
1'	108.8	4.89 (d, 7.9)
2'	73.9	4.51 (dd, 9.1, 7.9)
3'	75.4	4.09 (dd, 9.1, 3.8)
4'	70.0	4.21
		(br ddd, 3.8, 2.1, 2.0)
5'	67.7	3.71 (dd, 12.2, 2.0)
		4.29 (dd, 12.2, 2.1)

Chemical shifts in ppm from TMS as internal standard
Hydroxyl protons of sugar were not observed

Fig. 2. Selected HMBC and ^{1}H-^{1}H COSY correlations for MK800-62F1.



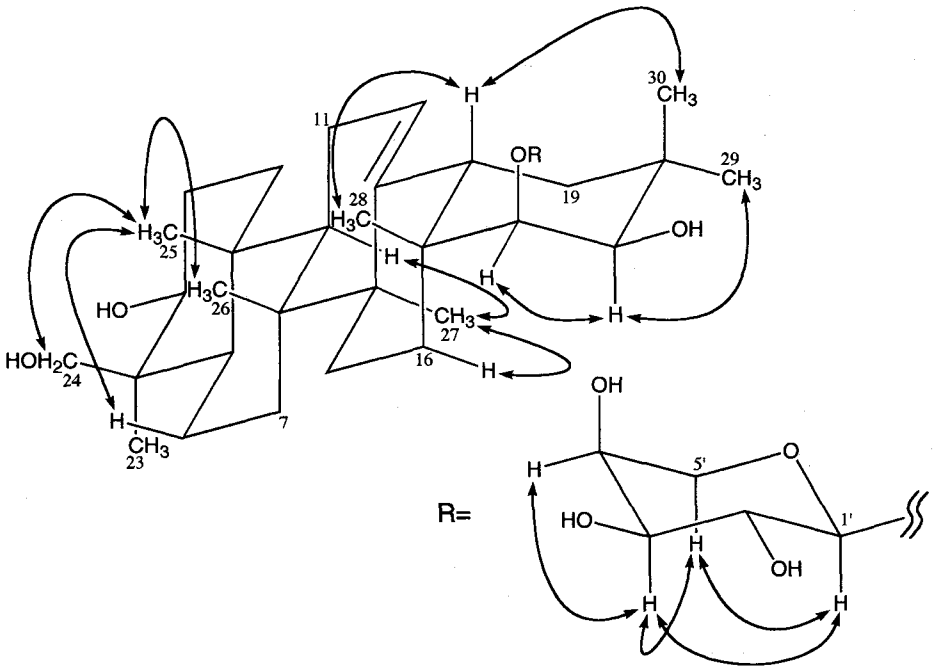
was determined by the NOESY spectrum, coupling constant values and comparison of the ^{13}C NMR values of **1** with those of a related compound. As shown in Fig. 3, NOESY correlations were seen from 25-H_3 to 6-H_{ax}, 24-H_2 and 26-H_3, and also from 28-H_3 to 9-H and 16-H_{ax}, signifying the axial nature of these protons. Cross peaks were also observed from 18-H to 27-H_3 and 30-H_3. Consequently, it was suggested the oleanane-type skeleton had *trans* A/B, B/C and C/D and *cis* D/E ring junctures.

A coupling constant of 11.2 Hz between 2-H_{ax} and 3-H_{ax} supported the diaxial orientation between these protons, and indicated the 3β-hydroxy group. A coupling constant of 3.4 Hz between 21-H and 22-H supported the *cis* orientation between these protons. No NOESY correlations from 30-H_3 to 21-H and 22-H were observed, suggesting 21α- and 22α- orientations of these protons. Thus, the presence of a 21β-hydroxy group and 22β-glycosidic linkage were concluded. The relative configuration of the aglycone moiety of **1** was the same as that of soyasapogenol A, and the ^{13}C NMR spectral data of **1** were in accordance with those of kudzusaponin SA_4, 3-*O*-β-D-glucurono-pyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol A 22-*O*-α-L-arabinopyranoside[3].

The sugar moiety of MK800-62F1 (**1**) was shown to be of the arabinopyranose configuration by its coupling constants and ^{1}H-^{1}H COSY data. The absolute configuration of the sugar moiety of **1** was determined according to a procedure developed by HARA *et al.*[4]. A thiazolidine derivative of the sugar component from **1** was prepared for GLC analysis. The sugar derivative of **1** gave a single peak, no interfering peak was observed, and the retention time was 21.2 minutes. Standard sugar samples were prepared, and checked by GLC in the same way. The retention times of the peaks for the thiazolidine derivative of L-arabinose and D-arabinose were clearly separated at 21.2 minutes and 24.4 minutes, respectively. By comparison with the retention times of the standard samples, the sugar moiety of **1** was determined to be L-arabinose.

The position of the glycosidic linkage was revealed by the HMBC spectrum as shown in Fig. 2. The anomeric

Fig. 3. Selected NOESY correlations for MK800-62F1.



proton 1′-H (δ_H 4.89) was coupled to C-22 (δ_C 92.7) and the methine proton 22-H (δ_H 3.77) was also coupled to the anomeric carbon C-1′ (δ_C 108.8).

The configuration of the anomeric center (C-1′) of **1** was determined to be α by the large coupling constant (J=7.9 Hz) between the anomeric proton 1′-H and the adjacent axial methine proton 2′-H (δ_H 4.51). Thus, the structure of **1** was elucidated as shown in Fig. 1.

Experimental

Spectral Analysis

Optical rotation was measured with a Perkin-Elmer model 241 polarimeter. UV spectra were recorded with a Hitachi U-3210 spectrophotometer. IR spectrum was recorded with a Horiba FT-210 fourier transform infrared spectrometer. The ^1H and ^{13}C NMR spectra were measured with a JEOL JNM-A500 spectrometer. The mass spectra were recorded with a JEOL JMS-SX102 mass spectrometer.

Determination of Absolute Configuration of the Sugar

A small amount of **1** was warmed in 1N HCl/H_2O at 90°C for 2 hours. The solution was neutralized with Ag_2CO_3, and washed with chloroform. The aqueous layer was concentrated to give a crude sugar. The sugar was dissolved in pyridine. The pyridine solution and L-cysteine methyl ester hydrochloride were mixed, and warmed at 60°C for 1 hour. The trimethylsilylation reagent (HMDS-TMCS, Sigma) was added, and the warming at 60°C was continued for another 30 minutes. The precipitate was centrifuged off, and the supernatant was subjected to GLC analysis. GLC analysis was performed with GL Science GL353 gas chromatograph equipped with an H_2 flame ionization detector. GLC conditions: column, TC-17 (30 m×0.25 mm); column bath temperature, 200°C; injection temperature, 250°C; carrier gas, He (1 ml/minute).

Acknowledgements

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