STUDIES ON CELL GROWTH STIMULATING SUBSTANCES OF LOW MOLECULAR WEIGHT

PART 4. MISAKIMYCIN, A MAMMALIAN CELL GROWTH STIMULATING SUBSTANCE PRODUCED BY *Streptomyces misakiensis*†

Sir:

In the course of our screening program for mammalian cell growth stimulating substances of low molecular weight, we isolated lavanducyanin[1], exfoliazone[2] and resorcinin[3]. Further screening resulted in the isolation of misakimycin from *Streptomyces misakiensis* BP-3621, which was isolated from a soil sample collected at Hachioji, Tokyo, Japan. It stimulated the growth of NIH 3T3 mouse fibroblast cells in the presence of only 1% of fetal calf serum (FCS). Without addition of this active material, the cells could not grow under the same condition.

In this communication, we report the isolation, characterization and structural elucidation of misakimycin.

S. misakiensis BP-3621 was cultivated at 27°C in a 60-liter jar fermentor with an agitation rate of 300 rpm and an air flow of 30 liters/minute. The medium consisted of glucose 2.5%, soybean meal 1.5%, dry yeast 0.2% and $CaCO_3$ 0.4% (pH 7.0).

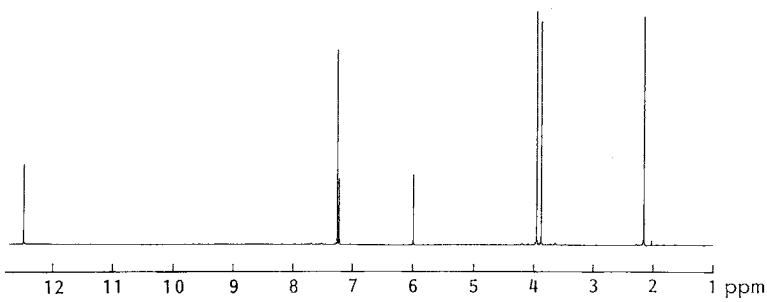
After a fermentation period of 72 hours, the mycerial cake collected by centrifugation from 30 liters of the fermentation broth was extracted twice with each 2.5 liters of acetone. The solvent extract was concentrated to a small volume and the aqueous residue was extracted three times with each one liter of EtOAc. The solvent layer was evaporated and the residue was chromatographed on a silica gel column ($CHCl_3$ - MeOH, 100 : 1). The active fraction was evaporated and further subjected to silica gel column chromatography (EtOAc - MeOH, 4 : 1). The active fraction was evaporated, and misakimycin was obtained as orange prism crystals from MeOH (30 mg).

The physico-chemical properties of misakimycin were as follows: MP 210~212°C; UV λ_{max} nm (ε) 218 (27,700), 254 (22,200), 258 (22,000), 303 (13,800), 421 (6,000) in MeOH, 218 (28,000), 254 (19,300), 258 (19,300), 299 (12,800), 421 (5,200), 535 (2,100) in alkaline MeOH; IR (KBr) cm^{-1} 3430, 2930, 1680, 1630, 1600, 1485, 1460, 1420, 1370, 1310, 1265, 1210, 1140, 1115, 1005, 980, 955, 920, 870, 860, 790, 705. HRFAB-MS of misakimycin showed the molecular ion at m/z $(M+H)^+$ 249.0787 corresponding to the molecular formula $C_{13}H_{12}O_5$ (calcd 249.0763). The UV absorption maxima of misakimycin at 258, 303 and 421 nm closely resembled to those of 5-hydroxy-3,7-dimethoxy-1,4-naphthoquinone[4] indicating the presence of a similar naphthoquinone moiety in misakimycin.

As shown in Fig. 1, the 500 MHz ^1H NMR spectrum of misakimycin taken in $CDCl_3$ revealed the presence of one methyl (2.170 ppm, 3H, s), two methoxys (3.880 ppm and 3.965 ppm, each 3H, s), two aromatic methines (5.980 ppm and 7.220 ppm, each 1H, s) and one hydrogen-bonded hydroxyl function (12.470 ppm, s). The UV and ^1H NMR spectral data suggested misakimycin to be a hydroxynaphthoquinone derivative with one methyl and two methoxy substituents.

These functional groups were arranged on the naphthoquinone ring as shown in Fig. 2 through analysis of ^{13}C-^1H long range couplings observed in a heteronuclear multiple-bond correlation (HBMC)[5] spectrum. The observed relations were as follows; from 3-H (5.980 ppm) to C-1 (179.7

Fig. 1. 500 MHz ^1H NMR spectrum of misakimycin in $CDCl_3$.



† For part 3[3].

Fig. 2. Structure of misakimycin and ^{13}C-^{1}H long range couplings.

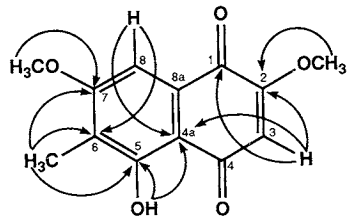


Table 1. ^{1}H and ^{13}C NMR assignments of misakimycin (500 MHz and 125 MHz, respectively, in $CDCl_3$).

	δ_H	δ_C
1	—	179.7
2	—	160.7[a]
2-OCH_3	3.880	56.5
3	5.980	102.6
4	—	190.0
4a	—	108.9
5	—	160.7[a]
5-OH	12.470	—
6	—	121.7
6-CH_3	2.170	8.2
7	—	162.7
7-OCH_3	3.965	56.1
8	7.220	109.3
8a	—	129.8

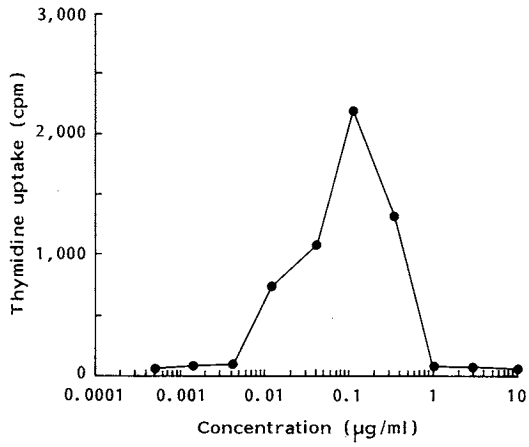
[a] C-2 and C-5 were observed as overlapping signals.

ppm), C-2 (160.7 ppm) and C-4a (108.9 ppm), from 5-OH (12.470 ppm) to C-4a and C-5 (160.7 ppm), from 6-CH_3 (2.170 ppm) to C-5, C-6 (121.7 ppm) and C-7 (162.7 ppm) and from 8-H (7.220 ppm) to C-4a and C-6. The ^{1}H and ^{13}C NMR assignments of misakimycin are summarized in Table 1. Thus, the structure of misakimycin was established as 5-hydroxy-2,7-dimethoxy-6-methyl-1,4-naphthoquinone. This compound had been synthesized by OHTA *et al.* in the course of the structural elucidation of fibrostatins[6,7], inhibitors of prolyl hydroxylase produced by *Streptomyces catenulae*. The good agreement of the physico-chemical properties of natural and synthetic samples corroborated the structure of misakimycin.

In the presence of 1% FCS, misakimycin stimulated the proliferation of NIH 3T3 cells at low doses ranging from 0.04 to 1 μg/ml. This stimulative activity of misakimycin was measured in the same way as detailed in former paper[3].

The proliferation activity determinated by the [^{3}H]thymidine uptake is shown in Fig. 3.

Fig. 3. Growth stimulative activity of misakimycin against NIH 3T3.



Misakimycin stimulated the growth of NIH 3T3 cells at very low dose. Addition of 0.1 μg/ml of misakimycin to the cells increased the [^{3}H]thymidine uptake by 35 times.

Although many kinds of substituted 1,4-naphthoquinone derivatives are known to be widespread in nature including plants[8~11], fungi[12~14], yeast[15] and insect[16] as antimicrobial compounds or pigments, mammalian cell growth regulating activities of these compounds have never been reported so far. It is, therefore, interesting to investigate the mammalian cell growth stimulating activities of quinone derivatives.

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