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Chemical Characterization and Biological Activities of Antioxidants from *Streptomyces* sp. AO-0511

Hung Bae Chang[1]* and Jae-Heon Kim[2]

[1]*Department of BioQuality Control, Korea Biopolytechnic College,* [2]*Department of Microbiology, Dankook University*

We isolated a streptomycete strain, AO-0511 producing antioxidants which decolorize the stable blue free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH). The cell wall type of the strain was determined as Type Ⅰ, containing LL-diaminopimelic acid (LL-DAP). The major fatty acids were anteisopentadecanoic acid (anteiso 15:0) and isopalmitic acid (iso 16:0), and the major menaquinones were MK-9(H_4), MK-9(H_6) and MK-9(H_8), respectively.

From the culture filtrate, two active substances were purified by solvent extraction and silica gel column chromatography. Based on spectroscopic measurements including UV, IR, NMR and MS, one antioxidant having benzoquinoid group was determined as herbimycin A and the other antioxidant having hydroquinoid group as dihydroherbimycin A, respectively. The DPPH scavenging activity of dihydroherbimycin A (IC_{50} 1.3 μM) was much stronger than that of herbimycin A (IC_{50} >1.0 mM), and 2-3 folds stronger than ascorbic acid or α-tocopherol. Only weak antibiotic activities were observed for both herbimycin A and dihydroherbimycin A against *Bacillus subtilus* and *Micrococcus luteus*, while their anti-microbial activities against the other Gram-positive bacteria as well as fungi and yeasts were negligible. In anticancer activities compared with the authentic anticancer agent, comptothecin, herbimycin A and dihydroherbimycin A showed similar inhibitory activities on HT-29 (colon cancer, IC_{50}s 5.6 μM and 4.2 μM) but 2-3.5 times higher activities on HEC-1-B (endometrial cancer, IC_{50}s 41 μM and 26 μM), 4-6 times higher activities on A-549 (lung cancer, IC_{50}s 4.5 μM and 7.6 μM), and, most strikingly, 1,000 times higher activities on HL-60 (leukemia cells, IC_{50}s 17 nM and 17 nM). In immunosuppressive activities tested by Con A and MLR assays, the activities of herbimycin A (IC_{50}s 7.1 nM and 3.9 nM) showing stronger than dihydroherbimycin A (IC_{50}s 27 nM and 17 nM) were similar to those of the well known immunosuppressant cyclosporin A but 100 times weaker than tacrolimus (FK-506). In the cytotoxicity test using L5178Y and P388 cell lines, herbimycin A (IC_{50}s 0.1 μM and 1.7 μM) and dihydroherbimycin A (IC_{50}s 1.0 μM and 14.5 μM) showed lower cytotoxicities than camptothecin (IC_{50}s 0.05 μM and 0.4 μM) and higher cytotoxicities than cyclosporin A (IC_{50}s 14.6 μM and 5.6 μM).

Therefore, dihydroherbimycin A as well as herbimycin A could be good candidates for the development of new anticancer drugs and immunosuppressive agents. This is the first report that describes the antioxidant, anticancer, and immunosuppressive activities of dihydroherbimycin A and herbimycin A.

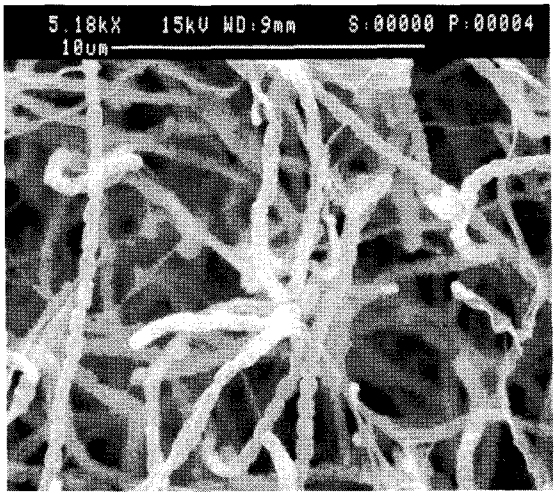

Fig. Scanning electron micrograph of the strain AO-0511

Table. DPPH scavenging activity of antioxidants including herbimycin A, dihydroherbimycin A and known antibiotics

Compound	IC_{50}(μM)
Ascorbic acid	4.2
α-Tocopherol	2.7
Novobiocin	14
Spectinomycin	ND
Erythromycin	ND
Rifampicin	25.3
Herbimycin A	>100
Dihydroherbimycin A	1.3

Table. Inhibition of lipid peroxidation by α-tocopherol, herbimycin A and dihydroherbimycin A (100㎍/㎖) in rat liver microsome

Compound	Inhibitory activity (%)
α-Tocopherol	93
Herbimycin A	61
Dihydroherbimycin A	72

Table. Anticancer activity of camptothecin, herbimycin A and dihydroherbimycin A against human cancer line (IC_{50}, μM)

Compound	Human cancer cell line			
	A 549	HEC-1-B	HL-60	HT-29
Camptothecin	26.5	98.4	22	4.4
Herbimycin A	4.5	41	<0.017	5.6
Dihydroherbimycin A	7.6	26	<0.017	4.2

Table. Cytotoxicity of herbimycin A, dihydroherbimycin A and known antifungal compounds.

Compound	$IC_{50}(\mu M)$	
	L5178Y	P388
Antimycin A	0.5	0.3
Tunicamycin	0.04	1.8
Toyocamycin	0.01	0.003
Cycloheximide	0.03	0.1
Cyclosporin A	14.6	5.6
Lenoremycin	0.3	0.04
Camptothecin	0.05	0.4
Herbimycin A	0.1	1.7
Dihydroherbimycin A	1.0	14.5

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