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NONPOLAR COMPOUNDS AND FREE FATTY ACIDS FROM MARINE FUNGI *Aspergillus ustus*

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Marine mycelial fungi are known to produce biologically active compounds with unusual chemical structures [1, 2]. Fungi synthesize such compounds because they must adapt to specific marine habitats. One of the consequences of such adaptation is the unusual composition of cellular and extracellular nonpolar compounds and fatty acids isolated from extracts of fungal marine isolates. It was shown that marine fungi produce such important acids as 16:0, 18:0, 18:1n9, 18:2n6, 18:3n3, and 20:4n6 [3]. An unusually high percentage of branched and unsaturated acids was noted in them [4]. Extracts of fungal marine isolates also contained phthalates, which inhibit the catepsin B proteinase enzyme [5].

In continuation of a study of secondary metabolites from marine fungi-micromycetes, we isolated two strains of *Aspergillus ustus* from sediment collected on the Okhotsk Sea shelf (27 m depth) of Sakhalin Island.

The fungus strains were cultivated in standard wort agar-agar medium in seawater for 14 d [6]. Cultures were extracted with EtOAc. The resulting extracts were concentrated at reduced pressure (10 mm Hg). The dry residue was dissolved in aqueous EtOH (10%) and extracted successively with hexane and EtOAc. The hexane fractions were concentrated at reduced pressure (10 mm Hg) and analyzed by GC–MS by comparing the results with mass-spectrometric fragmentation of standards using the NIST98 database. Table 1 presents the results.

The hexane fractions of the studied marine fungi contained linear saturated hydrocarbons, linear hydrocarbons with a terminal double bond, linear hydrocarbons from C16 to C24 with a terminal cyclohexane group for strain 1 and from C14 to C26 for strain 2. Both fractions also contained diene hydrocarbons with C18, C20, and C24. The hexane extract of culture 2 also contained squalene. Both fractions contained di(2-ethylhexyl)phthalate.

The EtOAc fractions of each culture were chromatographed over a column of silica gel using a gradient of hexane:EtOAc (100:0→0:100) to isolate fractions of free fatty acids. The resulting total acids were analyzed as methyl esters (methylation by diazomethane in ether) and pyrrolidides [7] using GC–MS. The derivatives were identified by comparing their mass spectra with those of standards using the NIST98 database. The results are given below:

Compound	*Aspergillus ustus* strain		*Compound*	*Aspergillus ustus* strain	
	1	*2*		*1*	*2*
14:0		0.51	18:0	2.15	10.84
16:1 n 9		0.68	Diisobutylphthalate	13.69	2.12
16:0	15.72	29.85	Dibutylphthalate	8.02	9.07
18:2 n 9,12		36.57	Di(2-ethylhexyl)phthalate	20.05	5.72.
18:1 n 9	36.37	2.64			

Both strains produced linear acids that contained one or two double bonds and esters of phthalic acid.

The qualitative and quantitative compositions of nonpolar compounds in marine isolates of *A. ustus* fungi were not previously determined. Our data differed from results obtained for marine isolates of other fungus species [8].

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TABLE 1. Composition of Hexane Fractions of Facultative Marine Fungi *Aspergillus ustus*, %

Compound	*Aspergillus ustus* fungus strain		Compound	*Aspergillus ustus* fungus strain	
	1	2		1	2
Tetradecene		1.10	1-Cyclohexyltetradecane	3.50	3.43
Tetradecane		0.95	Docosene	6.28	3.95
1-Cyclohexyloctane		1.60	Docosane	2.13	1.61
Hexadecene	7.19	9.13	1-Cyclohexylhexadecane	2.50	2.28
Hexadecane	2.83	2.82	Tetracosene	3.36	2.36
1-Cyclohexyldecane	2.75	3.34	Tetracosane	0.84	1.60
Octadecene	13.83	9.75	Tetracosadiene	0.83	0.90
Octadecane	4.59	2.84	1-Cyclohexyloctadecane	1.38	1.23
Eicosadiene	1.86	1.60	Pentacosane		1.47
1-Cyclohexyldodecane	3.94	3.45	Hexacosene		1.03
Dioctylketone	12.10		Hexacosane		2.03
Eicosene	13.24	7.66	Squalene		15.57
Eicosane	3.18	2.56	Di(2-ethylhexyl)phthalate	6.25	3.03
Docosadiene	1.56	1.71			

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