Antimicrobial Activity of Extracts of Chemical Races of the Lichen *Pseudevernia furfuracea* and their Physodic Acid, Chloroatranorin, Atranorin, and Olivetoric Acid Constituents

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The antimicrobial activity and the MIC values of the ethanol, chloroform, diethyl ether, and acetone extracts of the chemical races of *Pseudevernia furfuracea* (var. *furfuracea* and var. *ceratea*) and their physodic acid, chloroatranorin, atranorin, and olivetoric acid constituents have been investigated against some microorganisms. Nearly all extracts of both chemical races showed antimicrobial activity against *Aeromonas hydrophila*, *Bacillus cereus*, *Bacillus subtilis*, *Listeria monocytogenes*, *Proteus vulgaris*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Yersinia enterocolitica*, *Candida albicans*, *Candida glabrata*, *Alternaria alternata*, *Ascochyta rabiei*, *Aspergillus niger*, *Fusarium culmorum*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Fusarium solani*, and *Penicillium notatum*. There was no antimicrobial activity of the extracts against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas syringae*, *Salmonella typhimurium*, *Alternaria citri*, *Alternaria tenuissima*, and *Gaeumannomyces graminis*. Chloroatranorin and olivetoric acid were active against the same microorganisms with few exceptions. Physodic acid was active against about the same bacteria and yeasts and inactive against all of the filamentous fungi tested. Also no activity of atranorin against the filamentous fungi was observed.

Key words: Pseudevernia furfuracea, Antimicrobial Activity, Lichen Compounds

Introduction

Lichens have been used in folk medicine since ancient times and are symbiotic organisms of fungi and algae that produce unique secondary metabolites. Studies in the last two decades proved the antimicrobial, antiviral, antiprotozoal, antipyretic, antitumour, antiproliferative, anti-inflammatory, photoprotective, analgesic, and enzyme inhibitory activities of some of the lichen extracts and compounds. Although about 600 secondary metabolites are known from lichens (Fahselt, 1994) and most of them have been characterized, the biological activities of most of them are yet to be studied. Limited number of investigated lichen compounds and lichens that show antibacterial, antifungal, growth and enzyme inhibitory activities are listed by Huneck (1999, 2001). Furthermore two recent reviews were devoted only to the biological activities of usnic acid, which is the most well-known, widely distributed and extensively investigated lichen compound (Cocchietto *et al.*, 2002; Ingólfsdóttir, 2002). Lauterwein *et al.* (1995) determined *in vitro* activities of (+)-usnic acid, (−)-usnic acid and vulpinic acid against some aerobic and anaerobic microorganisms. Fournet *et al.* (1997) studied the activity of usnic acid, pannarine, and 1′-chloropannarine against promastigote forms of three strains of *Leishmania* spp. Ingólfsdóttir *et al.* (1998) screened *in vitro* activities of (+)-usnic acid, atranorin, lobaric acid, salazinic acid, and (+)-protolichesterinic acid for *Mycobacterium aurum* and found the highest activity for (+)-usnic acid. Protolichesterinic acid has been found to exhibit *in vitro* activity against *Helicobacter pylori* (Ingolfsdottir *et al.*, 1997). Perry and coworkers (1999) screened the antimicrobial, antiviral, and cytotoxic activities of 69 species of New Zealand lichens as well as usnic acid, atranorin, rangiformic acid, stictic acid, 7β-acetoxyhopan-22-ol, and hopan-15α,22-diol. Our group also reported antimicrobial activities of protolichesterinic acid, (+)-usnic acid, (−)-usnic acid, norstictic

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acid, protocetraric acid, atranorin, fumarprotocetraric acid, and 3-hydroxyphysodic acid and the extracts of *Cetraria aculeata*, *Ramalina farinacea*, *Cladonia foliacea*, and *Hypogymnia tubulosa* (Özdemir Türk *et al.*, 2003; Tay *et al.*, 2004; Yılmaz *et al.*, 2004, 2005).

This paper presents the results of antimicrobial activity studies on the extracts of the chemical races of the lichen *Pseudevernia furfuracea* (L.) Zopf [var. *furfuracea* and var. *ceratea* (Arch.) D. Hawksw.] and their physodic acid, chloroatranorin, atranorin and olivetoric acid constituents (Fig. 1). In lichens, variation in chemistry is not unusual within a species and a species could contain two or more 'chemical races' which differ from each other in their main chemical constituents (Orange *et al.*, 2001). Thus the var. *furfuracea* and var. *ceratea* are two chemical races of *P. furfuracea*. In the literature, there are some reports with limited information on the biological activity of *P. furfuracea*, physodic acid, and atranorin. A study carried out by Cosar *et al.* (1988) has shown that two strains of *Staphylococcus aureus* were inhibited by the extracts of *P. furfuracea*. Kırmızıgül *et al.* (2003) also reported the inhibition of the same bacterium by the extract of *P. furfuracea.* In fact *P. furfuracea* is a lichen with an economical value and in 1997, about 1900 tons of *P. furfuracea* were processed in the perfume industry as a fixative (Huneck, 1999). Also an ethnobotanical survey by Agelet and Vallès (2003) indicated that *P. furfuracea* has antiastmatic, anticatarrhal and hypotensive uses in the region of Pallars. Gollapudi *et al.* (1994) have given MIC values of physodic acid as well as (–)-usnic acid and alectosarmentin obtained from the lichen *Alectoria sarmentosa* against seven microorganisms. Physodic acid was found to be active against *S. aureus* and *Mycobacterium smegmatis.* Our group reported the activity of atranorin against some bacteria and yeasts previously (Yılmaz *et al.*, 2004). To the best of our knowledge, no information about the antimicrobial activities of chloroatranorin and olivetoric acid is available in the literature for the last decade.

Experimental

Microorganisms

Staphylococcus aureus (ATCC 6538), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Aspergillus niger* sp. (ATCC 9807) and *Fusarium solani* (ATCC 12820) (from American Type Culture Collection, Manassas, Virginia, USA), *Proteus vulgaris* (NRRL B-123), *Bacillus cereus* (NRRL B-3711), *Bacillus subtilis* (NRRL B-744), *Streptococcus faecalis* (NRRL B-14617), and *Penicillium notatum* (NRRL 807) (from Northern Regional Research Laboratory of the USDA, Peoria, Illinois, USA), *Listeria monocytogenes* (from Ankara University, Faculty of Agriculture, Ankara, Turkey), *Yersinia enterocolitica* and *Aeromonas hydrophila* (from Ankara University, Faculty of Veterinary, Ankara, Turkey), *Pseudomonas syringae* pv. *tomato* (TPPB 4212), *Fusarium moniliforme*, *Fusarium oxysporum,* and *Fusarium culmorum* (from Agriculture Research Center, Eskişehir, Turkey), *Candida albicans* and *Candida glabrata* (from Osmangazi University, Faculty of Medicine, Eskişehir, Turkey), *Alternaria tenuissima*, *Alternaria citri*, and *Alternaria alternata* (from Trakya University, Department of Biology, Edirne, Turkey), *Ascochyta rabiei*, *Gaeumannomyces graminis*, *Klebsiella pneumoniae*, and *Salmonella typhimurium* (available in our department) were used as microorganisms. Bacteria and yeasts were kept on nutrient agar and yeast extract agar plates at 4 °C, respectively. Fungal test cultures were subcultured on potato dextrose agar (PDA) for 5–7 d at 25 °C.

Lichen material

Both chemical races of *P. furfuracea* (var. *furfuracea* and var. *ceratea*) were collected from the trunks of *Pinus nigra* subsp. *pallasiana* in Bozdağ, Eskişehir Province, Turkey at 1200 m. The chemical races, which have the same morphologies, were distinguished using the C-spot test (Orange *et al.*, 2001; Huneck and Yoshimura, 1996) and separated from each other. Vouchers are stored at the Herbarium of Anadolu University in the Department of Biology (ANES).

Extraction

After 10 g air-dried and ground *P. furfuracea* var. *furfuracea* sample had been added to 100 ml of solvents ethanol, acetone, diethyl ether, and chloroform, the mixtures were first sonicated for 1 h, then left at room temperature overnight and filtered. The amounts of the extract residues in the filtrates were determined after removal of the solvents of the filtrates with a rotary evaporator (Table I). The same procedure was used for the extraction of *P. furfuracea* var. *ceratea* with the

Table I. The amounts of the extracts residues and some of the constituents of the chemical races of the lichen *P. furfuracea*[a].

Solvent	var. *furfuracea*				var. *ceratea*			
	E[b]	C[b]	DEE[b]	A[b]	E[b]	C[b]	DEE[b]	A[b]
Extract [mg]	1497	848	502	604	685	738	519	560
Physodic acid [mg]	260	195	96	228	–	–	–	–
Atranorin [mg]	10	25	8	16	20	22	7	20
Chloroatranorin [mg]	10	24	6	30	6	24	6	6
Olivetoric acid [mg]	–	–	–	–	116	100	44	165

[a] The amounts were obtained from 10 g lichen species after extraction with 100 ml solvent.
[b] E, ethanol; C, chloroform; DEE, diethyl ether; A, acetone.

same solvents and determination of the amounts of the extract residues (Table I). The amounts of the physodic acid, atranorin, chloroatranorin, and olivetoric acid constituents in the extract residues determined after their isolations by thin layer chromatography are given in Table I. The isolation procedure of these compounds is described in detail below.

Determination of MIC values of the extracts

The Kirby and Bauer disk diffusion method (National Committee for Clinical Laboratory Standards, 1993) has been used to determine MIC values of the extracts of the chemical races of the lichen *P. furfuracea* against test bacteria and fungi. The preparation of the extract residue containing filter paper disks was carried out the same way as described in our previous works (Yılmaz *et al.*, 2004; Tay *et al.*, 2004). From each extract, ten sets of sterilized disks with varying amounts of extract residue were prepared. Each set contained 60 sterilized disks and the amount of the residue on a disk in a set was twice as much as that of a disk in the next set. The *P. furfuracea* var. *furfuracea* extract residue contents of the disks varied from 6.4 mg/disk to 12.5 μg/disk for the ethanol extract, from 2.56 mg/disk to 5 μg/disk for the acetone extract, from 2.13 mg/disk to 4.16 μg/disk for the diethyl ether extract, and from 3.63 mg/disk to 7.08 μg/disk for the chloroform extract. The *P. furfuracea* var. *ceratea* extract residue contents of the disks varied from 2.92 mg/disk to 5 μg/disk for the ethanol extract, from 2.38 mg/disk to 4 μg/disk for the acetone extract, from 2.21 mg/disk to 4 μg/disk for the diethyl ether extract, and from 3.14 mg/disk to 6 μg/disk for the chloroform extract.

After inoculation of 250 μl (10^8 cells/ml or spores/ml) solutions of the bacteria onto nutrient agar and of the yeasts and filamentous fungi onto potato dextrose agar, an array of the disks containing different amounts of the extract residue from the same chemical race and the same extract was transferred into inoculated microorganism media to determine the MIC value of the extract. Pure solvent treated and dried disks were used as negative control disks. Chloramphenicol and ketoconazole were used as positive control substances. The bacterial plates were incubated for 24–48 h at 35–37 °C and the fungal plates were incubated for 5 d at 20–25 °C. The MIC values were determined by checking the inhibition zones formed. All MIC value determination experiments were done twice.

Isolation and characterization of physodic acid, chloroatranorin, atranorin, and olivetoric acid

Physodic acid, chloroatranorin, and atranorin were isolated from 150 ml acetone extract of 10 g of *P. furfuracea* var. *furfuracea*. After evaporation of the solvent of the extract, the remaining residue dissolved in a small amount of acetone was spotted on preparative TLC plates coated with silica gel 60 F_{254} (20 × 20, Merck) and the plates were developed in solvent system C which is a solvent system used in the TLC of lichen substances and consists of toluene and acetic acid (170:30, v:v) (Orange *et al.*, 2001; Huneck and Yoshimura, 1996). The spots belonging to physodic acid (R_f 0.18), chloroatranorin (R_f 0.79) and atranorin (R_f 0.81) on the developed plates were located using the R_f values of these compounds given in the literature (Orange *et al.*, 2001; Huneck and Yoshimura, 1996). Then physodic acid was recovered pure from the TLC plates. Because of close R_f values of chloroatranorin and atranorin on the plates developed with solvent system C, they were recovered as a mixture. A second preparative TLC sep-

aration was applied to the chloroatranorin and atranorin mixture using solvent system E which is one of the TLC solvent systems for lichen compounds and consists of cyclohexane and ethyl acetate (75 : 25, v : v). The R_f values of chloroatranorin and atranorin on TLC plates developed with this solvent system were 0.30 and 0.57, respectively. The amounts of physodic acid, chloroatranorin, and atranorin were 227 mg, 30 mg, and 16 mg, respectively. Olivetoric acid was isolated from 150 ml acetone extract of 10 g of *P. furfuracea* var. *ceratea*. This chemical race is a control lichen for olivetoric acid (Orange *et al.*, 2001). The TLC plates were prepared with olivetoric acid containing residue as described above and developed with solvent system C. The R_f value of olivetoric acid was 0.25 and 245 mg olivetoric acid were recovered. Besides the R_f values of these substances, their further characterizations were done comparing their melting points and IR spectra with the ones given in the literature (Huneck and Yoshimura, 1996).

Determination of MIC values of chloroatranorin, physodic acid, and olivetoric acid

The MIC value determinations of chloroatranorin, physodic acid, and olivetoric acid against the bacteria, yeasts, and fungi were carried out the same way as described for the determinations of MIC values of the extracts against the microorganisms. First stock solutions of chloroatranorin (25 mg/ml and 15 mg/ml), physodic acid (25 mg/ml) and olivetoric acid (20 mg/ml) in acetone were prepared. Then 1.0 ml of chloroatranorin, 1.0 ml of physodic acid, and 1.25 ml of olivetoric acid solutions were taken from these stock solutions and diluted twofold with acetone 10 times. From the sets of these solutions of chloroatranorin, physodic acid and olivetoric acid, filter paper disks containing these compounds in varying amounts were prepared. The substrate contents of these disks used in the MIC value determinations against the bacteria and yeasts varied from 0.63 mg/disk to 1.22 μg/disk for chloroatranorin and physodic acid and from 0.83 mg/disk to 1.63 μg/disk for olivetoric acid. Those of the ones used in the MIC value determinations against the fungi varied from 0.60 mg/disk to 1.17 μg/disk for chloroatranorin and from 1.0 mg/disk to 1.95 μg/disk for physodic acid and olivetoric acid.

Results and Discussion

In the search for biologically active substances, lichens and lichen metabolites received considerable attention in the last decades. Within our ongoing study on the antimicrobial activities of lichen extracts and compounds, we report here the antimicrobial activity of the extracts of the chemical races of *Pseudevernia furfuracea* (var. *furfuracea* and var. *ceratea*) and their major compounds, namely chloroatranorin, physodic acid, atranorin, and olivetoric acid.

The specimens of both chemical races of *P. furfuracea* used in this study were collected from the trunks of *Pinus nigra* subsp. *pallasiana* at the same location and grow side-by-side. Although var. *ceratea* tends to grow in more continental areas whereas var. *furfuracea* prefers to grow under humid climatic conditions (Martellos, 2003), both chemical races can also co-exist at the same locations and on the same substrates. The separation of both chemical races from each other was carried out by applying the C-spot test on the thalli of the specimens, where a dilute hypochlorite solution was used as a colour-test reagent and the test gives red, rose, orange, or green as positive reactions (Orange *et al.*, 2001; Huneck and Yoshimura, 1996). It is well known that the difference between two chemical races of *P. furfuracea* is due to the presence of olivetoric acid in var. *ceratea* and its absence in var. *furfuracea*. Owing to two free hydroxy groups in *meta*-position, olivetoric acid is one of the lichen substances that gives a positive reaction (red) in the C-spot test.

After separation of both chemical races of *P. furfuracea* and obtaining their extracts in ethanol, chloroform, diethyl ether, and acetone, we screened the antimicrobial activity and determined MIC values of the extracts against thirteen bacteria, two yeasts and eleven filamentous fungi. Furthermore we isolated the compounds chloroatranorin, physodic acid, atranorin, and olivetoric acid from the chemical races of *P. furfuracea* and determined their MIC values against the same microorganisms. The Kirby and Bauer method was employed for determination of MIC values of the extracts and the compounds.

Antimicrobial activity of the extracts of P. furfuracea var. furfuracea and var. ceratea

All of the extracts from both chemical races inhibited the growth of eight bacteria and two yeasts

Table II. MIC values of the extracts of *P. furfuracea* var. *furfuracea* and var. *ceratea* for bacteria and yeasts.

Microorganism	MIC of the extract (against 10^7 cells)							
	var. *furfuracea*[b]				var. *ceratea*[c]			
	E[d] [μg]	C[d] [μg]	DEE[d] [μg]	A[d] [μg]	E[d] [μg]	C[d] [μg]	DEE[d] [μg]	A[d] [μg]
A. hydrophila	800	907	267	–[a]	46	98	277	19
B. cereus	100	453	8.3	40	46	98	69	37
B. subtilis	100	113	8.3	40	46	49	69	75
E. coli	–	–	–	–	–	–	–	–
K. pneumoniae	–	–	–	–	–	–	–	–
L. monocytogenes	6400	227	133	–	46	25	69	37
P. aeruginosa	–	–	–	–	–	–	–	–
P. syringae	–	–	–	–	–	–	–	–
P. vulgaris	800	113	133	160	46	394	69	19
S. typhimurium	–	–	–	–	–	–	–	–
S. aureus	50	907	17	160	46	98	69	37
S. faecalis	800	453	267	20	46	394	277	19
Y. enterocolitica	100	57	8.3	20	46	49	69	37
C. albicans	25	14	8.3	20	11	25	17	9.3
C. glabrata	25	14	17	20	11	25	17	9.3

[a] –, Inactive.
[b] The concentrations of the extracts of var. *furfuracea* were: ethanol, 15.0 mg/ml; chloroform, 8.5 mg/ml; diethyl ether, 5.0 mg/ml; acetone, 6.0 mg/ml.
[c] The concentrations of the extracts of var. *ceratea* were: ethanol, 6.8 mg/ml; choloroform, 7.4 mg/ml; diethyl ether, 5.2 mg/ml; acetone, 5.6 mg/ml.
[d] E, ethanol extract; C, chloroform extract; DEE, diethyl ether extract; A, acetone extract.

with only one exception; the growth of *A. hydrophila* was not inhibited by the acetone extract of var. *furfuracea* (Table II). The inhibited bacteria and yeasts were *A. hydrophila*, *B. cereus*, *B. subtilis*, *L. monocytogenes*, *P. vulgaris*, *S. aureus*, *S. faecalis*, *Y. enterocolitica*, *C. albicans*, and *C. glabrata*. However neither of the extracts either from var. *furfuracea* or from var. *ceratea* showed antimicrobial activity against the bacteria *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. syringae*, and *S. typhimurium*. Similar activities or inactiveness of the extracts toward the same microorganism may be attributed to the similar contents of the extracts (Table I). In general the extracts inhibited the growth of Gram-positive bacteria. The MIC values obtained for the acetone and diethyl ether extracts of both chemical races were generally lower than those obtained with the ethanol and chloroform extracts with some exceptions and most of the MIC values of the extracts against the bacteria and yeasts were lower than 100 μg. Cosar *et al.* (1988) observed antimicrobial activity of the ethanol extract of *P. furfuracea* only against two strains of *S. aureus* in concentrations of 100 μg/ml and 1000 μg/ml. Among their test microorganisms, *E. coli*, *K. pneumoniae*, *S. typhimurium*, and *C. albicans* were not inhibited by their extract whereas we observed the growth inhibition of *C. albicans* by our *P. furfuracea* extracts.

If the antimicrobial activities and MIC values of our extracts against the bacteria and yeasts are compared to the ones reported previously by us for the various extracts of *C. foliacea*, *R. farinacea*, and *H. tubulosa* (Yılmaz *et al.*, 2004, 2005; Tay *et al.*, 2004), generally speaking, the extracts of the chemical races of *P. furfuracea* showed antimicrobial activity against about the same bacteria and yeasts but the MIC values of the extracts of the chemical races of *P. furfuracea* are comparable higher than the ones previously reported.

The most remarkable result of this study is the observation of antifungal activity of the ethanol, chloroform, and acetone extracts of both chemical races against some filamentous fungi (Table III). Aside from few exceptions, these extracts showed antifungal activity against eight of the tested eleven filamentous fungi; *A. alternata*, *A. rabiei*, *A. niger*, *F. culmorum*, *F. moniliforme*, *F. oxysporum*, *F. solani*, and *P. notatum*. However there was no antifungal activity of these extracts against the

Table III. MIC values of the extracts of *P. furfuracea* var. *furfuracea* and var. *ceratea* for filamentous fungi.

Microorganism	MIC of the extract (against 10^7 spores)					
	var. *furfuracea*[b]			var. *ceratea*[c]		
	E[d] [μg]	C[d] [μg]	A[d] [μg]	E[d] [μg]	C[d] [μg]	A[d] [μg]
A. alternata	800	–[a]	1280	730	787	299
A. citri	–	–	–	–	–	–
A. rabiei	800	1813	1280	365	787	597
A. tenuissima	–	–	–	–	–	–
A. niger	800	1813	–	2920	3148	2390
F. culmorum	800	1813	320	1460	787	597
F. moniliforme	400	1813	640	1460	787	597
F. oxysporum	800	1813	640	1460	1574	597
F. solani	800	907	640	1460	1574	597
G. graminis	–	–	–	–	–	–
P. notatum	400	3627	640	1460	787	1195

[a] –, Inactive.
[b] The concentrations of the extracts of var. *furfuracea* were: ethanol, 15.0 mg/ml; chloroform, 8.5 mg/ml; diethyl ether, 5.0 mg/ml; acetone, 6.0 mg/ml.
[c] The concentrations of the extracts of var. *ceratea* were: ethanol, 6.8 mg/ml; choloroform, 7.4 mg/ml; diethyl ether, 5.2 mg/ml; acetone, 5.6 mg/ml.
[d] E, ethanol extract; C, chloroform extract; A, acetone extract.

fungi *A. citri*, *A. tenuissima*, and *G. graminis*. In most cases the obtained MIC values of the extracts against the fungi were below 1.0 mg. On the other hand, the diethyl ether extracts of both chemical races were totally inactive against all of the tested filamentous fungi. In the aforementioned studies of us, we have never observed any antifungal activity of the lichen extracts of *C. foliacea*, *R. farinacea*, and *H. tubulosa* against the filamentous fungi, most of which were tested in this study.

Physodic acid, chloroatranorin, atranorin, and olivetoric acid constituents of the lichen P. furfuracea

After finding antimicrobial activities and determining MIC values of the extracts of both chemical races of *P. furfuracea* against some microorganisms, we isolated the major constituents in the chemical races and then tested the antimicrobial activities of these constituents. Olivetoric acid, physodic acid, chloroatranorin, and atranorin are widely reported compounds in the chemical races of *P. furfuracea*. Olivetoric acid and physodic acid are a depside-depsidone pair with exactly corresponding structure (Fig. 1). An oxidative cyclization reaction between the hydroxy group at the 2-position of ring A and the 5′-position of ring B in a depside converts it to a depsidone (Culberson, 1979). Although olivetoric acid and physodic acid can be found together in individual specimens, in this study, we have not detected physodic acid in var. *ceratea* which contains olivetoric acid. Al-

Table IV. MIC values of chloroatranorin, physodic acid, and olivetoric acid for bacteria and yeasts.

Microorganism	MIC of the substance (against 10^7 cells)					
	Chloroatranorin		Physodic acid		Olivetoric acid	
	[μg/25 μl]	[mM]	[μg/25 μl]	[mM]	[μg/41.7 μl]	[mM]
A. hydrophila	78	7.7	–[a]		104	5.3
B. cereus	156	15.3	78	6.6	26	1.3
B. subtilis	156	15.3	156	13.3	26	1.3
E. coli	–		–		833	42.3
K. pneumoniae	–		–		–	
L. monocytogenes	78	7.7	78	6.6	104	5.3
P. aeruginosa	–		–		–	
P. syringae	–		–		–	
P. vulgaris	156	15.3	625	53.1	104	5.3
S. typhimurium	–		–		833	42.3
S. aureus	156	15.3	625	53.1	26	1.3
S. faecalis	–		625	53.1	417	21.2
Y. enterocolitica	156	15.3	78	6.6	26	1.3
C. albicans	313	30.6	78	6.6	52	2.6
C. glabrata	313	30.6	78	6.6	52	2.6

[a] –, Inactive.

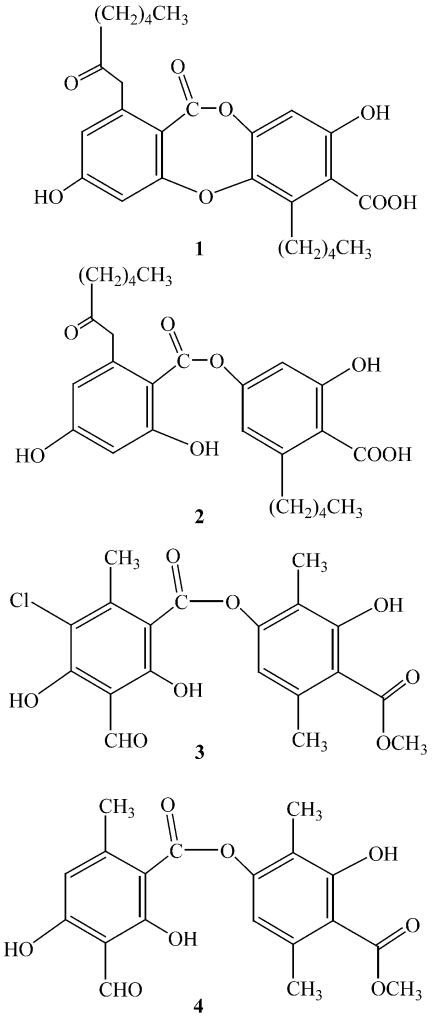

Fig. 1. Chemical structures of physodic acid (**1**), olivetoric acid (**2**), chloroatranorin (**3**), and atranorin (**4**).

though we have observed the presence of chloroatranorin and atranorin in both chemical races, we isolated them from the var. *furfuracea*. Chloroatranorin and atranorin are β-orcinol *para*-depsides and frequently occur together (Culberson, 1979).

Antimicrobial activity of physodic acid, chloroatranorin, olivetoric acid, and atranorin

We have already reported the antimicrobial activity of atranorin isolated from the lichen *C. foliacea* against some bacteria and yeasts previously (Yılmaz *et al.*, 2004). It showed antimicrobial activity against seven bacteria and two yeasts with MIC values varying from 31.2 μg to 500 μg. Physodic acid, chloroatranorin, and olivetoric acid showed antimicrobial activity against the same bacteria and yeasts as the extracts of both chemical races (Table IV). Furthermore, olivetoric acid was active against *E. coli* and *S. typhimurium* although the obtained MIC values for them were quite high. Probably these high MIC values are the reason for the inactiveness of the extracts of var. *ceratea* against these bacteria in which the amount of olivetoric acid may be lower than its MIC values for them. If we compare the MIC values of these three compounds against some of the bacteria and yeasts to those values obtained in our previous studies for (–)-usnic acid, (+)-usnic acid, fumarprotocetraric acid, norstictic acid, atranorin and 3-hydroxyphysodic acid (Yılmaz *et al.*, 2004, 2005; Tay *et al.*, 2004), the MIC values reported here are quite higher than the ones previously reported. Nevertheless this is yet the first report on the antimicrobial activity of chloroatranorin and olivetoric acid against bacteria and yeasts. For physodic acid, Gollapudi *et al.* (1994) reported its antibacterial activity against *S. aureus* with a 25 μg/ml MIC value and *M. smegmetis* with a 100 μg/ml MIC value and they did not observe any antimicrobial activity of it against *E. coli*, *S. gallinarum*, *K. pneumoniae*, *C. albicans*, and *P. aeruginosa*.

We also believe that it will be interesting to compare the antimicrobial activity of physodic acid to that of 3-hydroxyphysodic acid reported recently (Yılmaz *et al.*, 2005). The only difference in both acids is the substitution of a hydrogen atom with a hydroxy group on the third carbon atom of ring A. More or less physodic acid showed antimicrobial activity against the same microorganisms as 3-hydroxyphysodic acid but the MIC values of physodic acid were significantly higher than those of 3-hydroxyphysodic acid. For example the lowest MIC value of physodic acid was 78 μg for five microoganisms whereas the MIC values of 3-hydroxyphysodic acid were 1.95 μg for *C. albicans*, *S. typhimurium*, *S. faecalis*, and *L. monocytogenes*.

As in the assay of the antifungal activity of the extracts of the *P. furfuracea* chemical races, chloroatranorin and olivetoric acid showed remarkable antifungal activities (Table V). Chloroatranorin inhibited the growth of the tested filamentous fungi except for *A. niger* and *G. graminis* whereas olivetoric acid inhibited the growth of seven filamentous fungi. Olivetoric acid was inactive against also *A. niger* and *G. graminis* as well as *A. citri* and *A.*

Table V. MIC values of chloroatranorin and olivetoric acid for filamentous fungi.

Microorganism	MIC of the substance (against 10^7 spores)			
	Chloroatranorin		Olivetoric acid	
	[μg/40 μl]	[mM]	[μg/50 μl]	[mM]
A. alternata	300	18.4	1000	42.3
A. citri	300	18.4	–[a]	
A. rabiei	300	18.4	500	21.2
A. tenuissima	300	18.4	–	
A. niger	–		–	
F. culmorum	300	18.4	500	21.2
F. moniliforme	300	18.4	500	21.2
F. oxysporum	300	18.4	500	21.2
F. solani	300	18.4	500	21.2
G. graminis	–		–	
P. notatum	300	18.4	1000	42.3

[a] –, Inactive.

tenuissima. The fungi inhibited by chloroatranorin with a 300 μg/40 μl MIC value were *A. alternata*, *A. citri*, *A. rabiei*, *A. tenuissima*, *F. culmorum*, *F. moniliforme*, *F. oxysporum*, *F. solani*, and *P. notatum*.

Physodic acid showed no antifungal activity against all of the tested eleven filamentous fungi. This is an intriguing result because olivetoric acid and physodic acid have closely related structures as mentioned above and only one of them showed antifungal activity against some of the tested filamentous fungi. One may speculate that the antifungal activity of olivetoric acid is due to its flexible structure. Because the lack of the linkage between the carbon atom at 2-position of ring A and the carbon atom at 5′-position of ring B allows both rings in olivetoric acid to rotate freely and independently around the COO ester linkage (Fig. 1). This feature may be allowing this molecule to reach and/or fit the inhibition site in a fungus. On the other hand the presence of the linkage between 2- and 5′-positions of the rings in physodic acid makes this molecule rigid and locks it in a fairly planar geometry. Therefore, this molecule may not be able to reach the inhibition site in a fungus because of its rigidity and planarity.

We also found that atranorin at even high contents (as high as 0.6 mg/disk) was inactive against all tested filamentous fungi. The antifungal activity of chloroatranorin against some of the filamentous fungi tested and its absence in atranorin against all of them is also interesting. The only difference between chloroatranorin and atranorin is the presence of a chlorine atom at 5-position of ring A in chloroatranorin instead of a hydrogen atom in atranorin (Fig. 1). Thus the antifungal activity of chloroatranorin may be only attributed to the presence of the chlorine atom in the structure. It is well known that many chlorine containing compounds are used as a pesticide or herbicide today.

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