J. Antibiot. 60(4): 285–288, 2007

NOTE

THE JOURNAL OF ANTIBIOTICS

Serialynic Acid, a New Phenol with an Isopentenyne Side Chain from *Antrodia serialis*

Tetsuo Kokubun, Dianne Irwin, Mike Legg, Nigel C. Veitch, Monique S. J. Simmonds

Received: January 11, 2007 / Accepted: March 12, 2007

Abstract A new phenolic compound serialynic acid was isolated from an agar culture of the basidiomycete *Antrodia serialis*, through bioactivity-guided fractionations. It showed weak growth inhibitory activity towards phytopathogenic fungi and a dose-independent anti-*Pythium graminicola* activity.

Keywords *Antrodia serialis*, serialynic acid, isopentenynylphenol, antifungal activity, Basidiomycete

Introduction

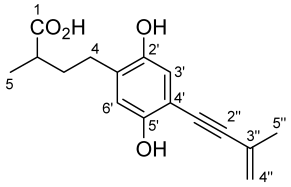
During a systematic screening for antimicrobial and/or novel compounds from a collection of British fungi, a strain of *Antrodia serialis* (Fr.:Fr.) Donk (Coriolaceae), when cultured, was found to produce an antifungal compound in large amounts. Subsequent culturing of this strain and bioactivity-guided fractionations led to the isolation of serialynic acid (**1**).

A. serialis is a brown rot fungus mainly growing on coniferous wood and constitutes one of the major timber- and woodwork-degrading fungi. A large number of triterpenoids of the lanostane and ergostane type, and simple phenolic metabolites have been isolated from the fruiting bodies of *A. cinnamomea*, which is used in Chinese folk medicines [1～6], and cytotoxic pyrrole derivatives have been found in the cultured mycelia of *A. camphorata* [7]. We report here the isolation and characterisation of **1** from *A. serialis*.

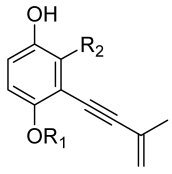
Experimental

General

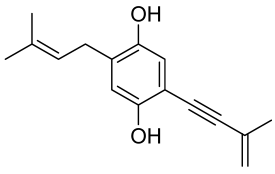
UV spectra were obtained with a Shimadzu UV-1601 spectrophotometer. NMR spectra were recorded on a Bruker Avance 400 spectrometer in CD_3OD at 30°C, with TMS as the internal standard (400 MHz for proton, and 100 MHz for carbon). Mass spectra were recorded using a



Serialynic acid (**1**)



Siccayne (**2**) R_1=H, R_2=H
Eulatinol (**3**) R_1=CH_3, R_2=H
Frustulosinol (**5**) R_1=H, R_2=CH_2OH



Culpin (**4**)

M. S. J. Simmonds (Corresponding author), **T. Kokubun, N. C. Veitch:** Royal Botanic Gardens, Kew, Richmond TW9 3AB, UK, E-mail: m.simmonds@kew.org

D. Irwin, M. Legg: Syngenta, Jealott's Hill, International Research Centre, Bracknell, Berks, RG42 6EY, UK

Thermo Finnigan LCQ Classic in both positive and negative atmospheric pressure chemical ionisation (APCI) modes. TLC was run on silica gel F_{254} precoated plates (Merck 5554) with $CHCl_3$ - MeOH - H_2O, 73 : 24 : 3 (in volume), and spots were detected by UV light and bioautography [8] (see below). Final purification of **1** was carried out using a Waters HPLC system consisting of a 600 pump, a 717 plus autosampler and a 996 photodiode array detector. The column used was Genesis C_{18} (Jones Chromatography, 10 mm i.d.×250 mm, *dp* 4 μm) at 30°C, eluting with a linear gradient of 40 to 100% MeOH in H_2O over 20 minutes at a flow rate of 4 ml/minute.

Fungal Strain, Fermentation and Isolation

The fruiting body was found growing on a windowsill near Fishguard, Wales, UK, in November, 1995 and identified by Dr. Peter Roberts of the Mycology Section, Jodrell Laboratory, Royal Botanic Gardens, Kew. The dried specimen was deposited in the mycological herbarium of the Gardens (K(M)33565). The mycelial culture (strain KC920) raised from uncontaminated tissue of the same specimen was maintained on a malt extract - agar medium under oil at 16°C.

The seed culture was grown on a medium containing malt extract (Oxoid L39, 2%) and agar (1.5%) at 25°C. The well grown culture at approx. 4 weeks old was cut into small pieces and transferred (3-point inoculation) to forty 9 cm-diameter Petri dishes containing the same medium, and grown at 25°C for 24 days with artificial illumination (white light, 4×40 W).

The whole culture was extracted twice with $CHCl_3$ - MeOH (2 : 1, v/v). The combined organic phase was concentrated *in vacuo* at 40°C, and the residue (2.81 g) was chromatographed over silica using mixtures of $CHCl_3$ - MeOH - H_2O (100 : 0 : 0, 87 : 12 : 1, 73 : 24 : 3, 16 : 9 : 1 and 5 : 5 : 1, in volume). The fractions with antifungal activity against the test strain *Cladosporium herbarum* IMI300461 were combined (82.3 mg) and further purified by reverse phase HPLC. Serialynic acid eluted at 14.3 minutes, yielding 8.3 mg of pure compound (**1**).

The chemical structure of **1** was determined mainly by one-dimensional proton and carbon NMR at 400 and 100 MHz, respectively, and ^{1}H-^{1}H COSY, HSQC and HMBC experiments.

Physico-chemical Properties

Serialynic acid (4-[2,5-dihydroxy-4-(3-methylbut-3-en-1-ynyl)phenyl]-2-methylbutanoic acid, **1**) was obtained as a pale yellow needles from MeOH - H_2O. Mp (uncorr.) 102～104°C. APCI-MS (pos. mode) *m/z* 275 $[M+H]^+$, 257, 187, 159; (neg. mode) *m/z* 547 $[2M-H]^-$, 273 $[M-H]^-$, 255, 229, 174. UV λ_{max} (MeOH) nm (log ε) 209

Table 1 ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data, and HMBC correlations of serialynic acid (**1**) in CD_3OD (δ in ppm, *J* in Hz)

Position	δ (^{1}H)	δ (^{13}C)	^{1}H-^{1}H COSY	HMBC
1		181.0		
2	2.42 (1H, m)	40.6	H-3, H-5	C-1, 3, 4, 5
3	1.91 (1H, m)	35.0	H-3, H-4	C-1, 2, 4, 5, 1′
	1.66 (1H, m)		H-3, H-4	C-1, 2, 4, 5, 1′
4	2.56 (2H, t, 7.7)	29.2	H-3	C-2, 3, 1′, 2′
5	1.18 (3H, d, 7.0)	17.8	H-2	C-1, 2, 3
1′		132.5		
2′		149.2		
3′	6.67 (1H, s)	119.3		C-4, 1′, 2′, 4′, 5′, 6′, 1″
4′		109.6		
5′		152.2		
6′	6.59 (1H, br s)	118.0	H-4	C-3, 4, 1′, 2′, 3′, 4′, 5′, 1″, 2″
1″		86.1		
2″		94.6		
3″		128.9		
4″	5.32 (1H, m)	121.4	H-4″	C-2″, 3″, 5″
	5.25 (1H, m)		H-4″, H-5″	C-2″, 5″
5″	1.96 (3H, dd, 1.6, 1.0)	23.8	H-4″	C-2″, 3″, 4″

Chemical shifts are shown with reference to TMS as the internal standard.

Table 2 Antifungal activity of serialynic acid (**1**)[a]

Microorganisms	Concentration in μg/ml and in (μM)		
	70 (255)	140 (511)	280 (1022)
Botrytis cinerea	−[b]	−	−
Fusarium graminearum	−	−	−
Pyrenophora teres	−	−	++[b]
Rhizoctonia solani	−	−	−
Septoria tritici	−	−	−
Stagonospora nodorum	−	−	+[b]
Pythium graminicola	+	+	+

[a] Test compounds were dissolved in DMSO/isopropyl alcohol and Tween 20, and dispersed into minimal medium containing a calibrated number of spores. After incubation period (4~6 days depending on the test organisms) the growth of the test strains were visually assessed and given scores.
[b] Score −: no inhibition compared to the control; +: arbitrary level of inhibition compared to the control; ++: complete inhibition.

(4.4), 265 (4.22), 279 (4.24), 328 (4.1). ^{1}H (400 MHz, CD_3OD) and ^{13}C (100 MHz, CD_3OD) NMR, see Table 1.

Biological Assays

The fungus *C. herbarum* IMI300461 was used as the test strain to trace the activity on the TLC bioautography [8]. The growth inhibitory activity of the compound was tested against a panel of phytopathogenic organisms (listed in Table 2), using 96-well microplates in a series of concentrations (70, 140 and 280 μg/ml).

Results and Discussion

The UV spectrum of **1** was virtually identical with that of siccayne (**2**) and eulatinol (**3**) [9], indicating the presence of a chromophore similar to 3-methyl-3-penten-1-ynyl substituted hydroquinone. The positive APCI-mass spectrum showed a protonated ion peak at 275 $[M+H]^+$, while 273 $[M-H]^-$ and 547 $[2M-H]^-$ were major ions in the negative mode, indicating the relative molecular mass to be 274. The high-resolution ESI MS of **1** gave a pseudo-molecular ion peak at *m/z* 292.1541 ($[M+NH_4]^+$, calcd. for $C_{16}H_{22}NO_4$, 292.1543, Δ=0.8 ppm), corresponding to a molecular formula of $C_{16}H_{18}O_4$.

The ^{13}C NMR spectrum revealed the presence of nine sp^2 carbons, including one carboxylic acid at δ 181.0 and two oxygenated ones (δ 152.2 and 149.2). Two *sp* carbons at δ 94.6 and 86.1 ppm were also detected. From the ^{1}H-^{1}H COSY, HSQC and HMBC spectra, and by comparison with literature data [9, 10], a partial structure corresponding to siccayne (**2**) was deduced. Despite the difference in the solvents used, the ^{13}C chemical shift values of the isopentenynyl moiety and the aromatic carbons 2′ through to 5′ were practically identical to the literature values measured in $CDCl_3$ [9, 10]. Serialynic acid was hardly soluble in $CHCl_3$ but more soluble in MeOH or Me_2CO. The two sp^2 carbons directly adjacent to the acetylenic bonds *i.e.* C-4′ (δ 109.6) and C-3″ (δ 128.9) showed correlations in the HMBC spectrum with H-6′ and H-4″ and -5″, respectively; therefore the chemical shift assignments given for the corresponding carbons in support of the structure of foeniculoxin [11] should be interchanged.

The remaining fragment consisting of $C_5H_9O_2$ was readily identified as 2-methylbutanoic acid through interpretation of the ^{1}H-^{1}H COSY, HSQC and HMBC data. This substituent was placed at the *para*-position to the isopentenyne group on the basis of HMBC correlations between H-3′ (δ 6.67) and C-4 (δ 29.2), and that no coupling was observed between the two aromatic protons (δ 6.59 and 6.67). This conclusion was further corroborated by the weak five-bond ($^5J_{HC}$) HMBC correlation observed from H-6′ to C-2″. Similar long range couplings *via* conjugated triple and double bonds have been recorded in the literature [12]. The structure of **1** was therefore determined to be 4-[2,5-dihydroxy-4-(3-methylbut-3-en-1-ynyl)phenyl]-2-methylbutanoic acid. The absolute configuration of the single stereogenic centre has not been determined.

Serialynic acid belongs to a small group of simple phenolic compounds bearing isopentenyne substructures, all of which have been isolated from fungi of both ascomycetes [9, 13, 14] and basidiomycetes [10, 15~17],

in addition to a hyphomycete [18]. They have variably been shown to have weak to no antimicrobial and phytotoxic activities [9, 10, 13, 14, 16∼19]. Of these **1** possesses the closest structural resemblance to culpin isolated from *Preussia* sp. which showed a weak antimicrobial activity [14].

Serialynic acid was tested against several standard in-house test organisms at the Jealott's Hill International Research Centre, and shown to have weak to no activity against the representative strains of phytopathogenic fungi (Table 2). Interestingly, the straminipile *Pythium graminicola* was found most susceptible to the compound, and its growth was retarded at the lowest concentration tested (70 μg/ml). Frustulosinol **5** [15], isolated from a culture of *Gloeocystidiellum porosum* (Berk. & M.A. Curtis) Donk (Stereaceae) strain KC1004 (unpublished result), was devoid of activity in the assays performed against the same test organisms concurrently.

Acknowledgments We are grateful to CABI Bioscience (Egham, Surrey, UK) for provision of a test organism, Dr. Geoffrey Kite for the acquisition of LC-MS data, and the EPSRC National Mass Spectrometry Service Centre, University of Wales, Swansea, for high resolution mass spectral data. Thanks are also due to Ms. Gill Butterfill and Dr. Eleanor Saunders for isolation and maintenance of the living collections at Kew.

References

1. Cherng IH, Chiang HC, Cheng MC, Wang Y. Three new triterpenoids from *Antrodia cinnamomea*. J Nat Prod 58: 365–371 (1995)
2. Chen CH, Yang SW, Shen YC. New steroid acids from *Antrodia cinnamomea*, a fungal parasite of *Cinnamomum micranthum*. J Nat Prod 58: 1655–1661 (1995)
3. Chiang HC, Wu DP, Cherng IW, Ueng CH. A sesquiterpene lactone, phenyl and biphenyl compounds from *Antrodia cinnamomea*. Phytochemistry 39: 613–616 (1995)
4. Cherng IH, Wu DP, Chiang HC. Triterpenoids from *Antrodia cinnamomea*. Phytochemistry 41: 263–267 (1996)
5. Yang SW, Shen YC, Chen CH. Steroids and triterpenoids of *Antrodia cinnamomea*—a fungus parasitic on *Cinnamomum micranthum*. Phytochemistry 41: 1389–1392 (1996)
6. Shen YC, Yang SW, Lin CS, Chen CH, Kuo YH, Chen CF. Zhankuic acid F: a new metabolite from a Formosan fungus *Antrodia cinnamomea*. Planta Med 63: 86–88 (1997)
7. Nakamura N, Hirakawa A, Gao JJ, Kakuda H, Shiro M, Komatsu Y, Sheu CC, Hattori M. Five new maleic and succinic acid derivatives from the mycelium of *Antrodia camphorata* and their cytotoxic effects on LLC tumor cell line. J Nat Prod 67: 46–48 (2004)
8. Homans AL, Fuchs A. Direct bioautography on thin-layer chromatograms as a method for detecting fungitoxic substances. J Chromatogr 51: 327–329 (1970)
9. Molyneux RJ, Mahoney N, Bayman P, Wong RY, Meyer K, Irelan N. *Eutypa* dieback in grapevines: differential production of acetylenic phenol metabolites by strains of *Eutypa lata*. J Agric Food Chem 50: 1393–1399 (2002)
10. Kupka J, Anke T, Steglich W, Zechlin L. Antibiotics from Basidiomycetes. XI. The biological activity of siccayne, isolated from the marine fungus *Halocyphina villosa* J. & E. Kohlmeyer. J Antibiot 34: 298–304 (1981)
11. Evidente A, Lanzetta R, Abouzeid MA, Corsaro MM, Mugnai L, Surico G. Foeniculoxin, a new phytotoxic geranylhydroquinone from *Phomopsis foeniculi*. Tetrahedron 50: 10371–10378 (1994)
12. Nishimura S, Matsunaga S, Shibazaki M, Suzuki K, Harada N, Naoki H, Fusetani N. Corticatic acids D and E, polyacetylenic geranylgeranyltransferase type I inhibitors, from the marine sponge *Petrosia corticata*. J Nat Prod 65: 1353–1356 (2002)
13. Renaud JM, Tsoupras G, Tabacchi R. Biologically active natural acetylenic compounds from *Eutypa lata* (Pers:F.) Tul. Helv Chim Acta 72: 929–932 (1989)
14. Johnson JH, Meyers E, O'Sullivan J, Phillipson DW, Robinson G, Trejo WH, Wells JS. Culpin, a novel hydroquinone antibiotic of fungal origin. J Antibiot 42: 1515–1517 (1989)
15. Nair MSR, Anchel M. Frustulosinol, an antibiotic metabolite of *Stereum frustulosum*: revised structure of frustulosin. Phytochemistry 16: 390–392 (1977)
16. Hautzel R, Anke H, Sheldrick WS. Mycenon, a new metabolite from a *Mycena* species TA 87202 (Basidiomycetes) as an inhibitor of isocitrate lyase. J Antibiot 43: 1240–1244 (1990)
17. Dubin GM, Fkyerat A, Tabacchi R. Acetylenic aromatic compounds from *Stereum hirsutum*. Phytochemistry 53: 571–574 (2000)
18. Ishibashi K, Nose K, Shindo T, Arai M, Mishima H. Siccayne: a novel acetylenic metabolite of *Helminthosporium siccans*. Ann Sankyo Res Lab 20: 76–79 (1968)
19. Smith LR, Mahoney N, Molyneux RJ. Synthesis and structure-phytotoxicity relationships of acetylenic phenols and chromene metabolites, and their analogues, from the grapevine pathogen *Eutypa lata*. J Nat Prod 66: 169–176 (2003)