Secondary Mold Metabolites: Part 46. Hericenes A-C and Erinapyrone C, New Metabolites Produced by the Fungus Hericium erinaceus

Alberto Arnone, Rosanna Cardillo, Gianluca Nasini, and Orso Vajna de Pava

J. Nat. Prod., **1994**, 57 (5), 602-606• DOI:
10.1021/np50107a006 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 5, pp. 602-606, May 1994

SECONDARY MOLD METABOLITES: PART 46.[1] HERICENES A–C AND ERINAPYRONE C, NEW METABOLITES PRODUCED BY THE FUNGUS *HERICIUM ERINACEUS*

ALBERTO ARNONE, ROSANNA CARDILLO, GIANLUCA NASINI,* and ORSO VAJNA DE PAVA

CNR Centro di Studio per le Sostanze Organiche Naturali, Dipartimento di Chimica del Politecnico di Milano, Via Mancinelli 7, I 20123, Milano, Italy

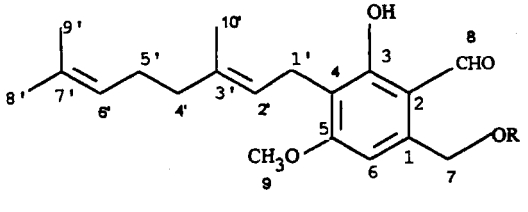
ABSTRACT.—Investigation of culture extracts of *Hericium erinaceus* led to the isolation of three new phenols, hericenes A–C [**1–3**], and a new pyrone, erinapyrone C [**5**]. Their structures were elucidated on the basis of ^{1}H- and ^{13}C-nmr evidence and by gc-ms analysis.

In the course of our continuing search for new active compounds from Basidiomycetes (fungi) grown on artificial media, we have studied *Hericium erinaceus* (Bull.) Pers., an edible mushroom cultured in Japan which possesses interesting biological properties. Previous investigations on the fruiting bodies of the fungus resulted in the isolation of small amounts of biologically active compounds having cytotoxic effects on HeLa cells, hericenones A and B (2) and erinapyrones A and B (3), and compounds that stimulated the synthesis of nerve growth factor (NGF), hericenones C-H (4,5).

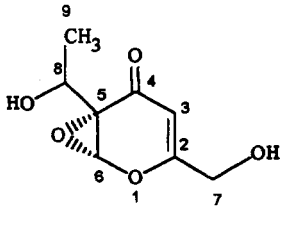
In the present study, five strains of *Hericium erinaceus* have been analyzed in order to optimize their growth and to orient their metabolite production. Strain CBS 233.87 afforded the best results with hard corn bran or MPGA cultures. Three phenolic derivatives, named hericenes A–C [**1–3**], have been isolated from the less polar fractions of the culture extracts. These compounds, among which hericene A [**1**] is prevalent, are characterized by having three different fatty acids bound to the same benzyl alcohol moiety and differ from hericenones C–E in the absence of the carbonylic group at position 5′. Hericene B [**2**] also has an oleic acid residue. From the more polar chromatographic fractions, a new γ-dihydropyrone, named erinapyrone C [**5**], with moderate activity against Gram-positive bacteria, has been isolated. Strain CBS 233.87 consistently produces these metabolites in high yield. Work is in progress to evaluate the activity of the isolated metabolites in promoting the synthesis of NGF.

RESULTS AND DISCUSSION

The strain of *Hericium erinaceus* was grown on MPGA (malt extract-peptone-glucose-agar) or hard corn bran for three weeks. Ethyl acetate extracts of the mycelia were



1 R=-CO(CH$_2$)$_{14}$CH$_3$
2 R=-CO(CH$_2$)$_7$CH=CH(CH$_2$)$_7$CH$_3$
3 R=-CO(CH$_2$)$_{16}$CH$_3$
4 R=H



5

[1]For Part 45, see Arnone *et al.* (1).

evaporated to dryness, combined, and the residues subjected to repeated Si gel cc, prep. tlc, and hplc to give hericenes A–C [**1–3**] in a 5:3:2 ratio, and erinapyrone C [**5**].

Mass spectrometry established the molecular formulae of the hericenes A–C [**1–3**] as $C_{35}H_{56}O_5$, $C_{37}H_{58}O_5$, and $C_{37}H_{60}O_5$, respectively, while the ir spectra showed absorptions at 3450, 1730, and 1630 cm^{-1} indicating the presence of hydroxy and carbonyl groups. Methanolysis of the mixture of **1–3** afforded methyl esters of palmitic, oleic, and stearic acid in a 5:3:2 ratio from the acid portion and compound **4** as the only product derived from the alcoholic portion. The esters were identified by gc-ms and comparison with authentic samples, while the structure of **4** followed from nmr evidence. A comparison of the ^{1}H- and ^{13}C-nmr spectra of hericenes [**1–3**] (Table 1) with those of hericenones C–E (4) revealed that the alcoholic portion of these compounds share the same basic structure, the only difference being the presence in **1–3** of a -CH_2-CH_2- grouping in place of a -CH_2C=O fragment. In fact, the ^{1}H-nmr spectra of **1–3** showed two multiplets at δ_H 1.97 and 2.03 (H_2-4′ and H_2-5′), correlated by a HETCOR experiment with the methylene carbons at δ_C 39.77 and 26.67, which exhibited vicinal couplings of 6.8 Hz with H-6′ and allylic couplings of 1.5 Hz with H-2′, respectively. Moreover, the nOe enhancements shown in Figure 1 confirmed the relative disposition of the aromatic substituents and the stereochemistry of the -C(2′)H=C(3′) double bond.

TABLE 1. ^{1}H- and ^{13}C-Nmr Data for Compounds **1–4**[a,b] in $CDCl_3$.

H	Compound		C	Compound
	1	**4**		**1**
	δ_H	δ_H		δ_C
6	6.52 s	6.52 s	1	138.41
7	5.32 s	4.94 s	2	112.87
8	10.10 s	10.20 s	3	162.88
9	3.91 s	3.92 s	4	118.06
3-OH	12.37 s	12.37 s	5	163.47
1′	3.34 br d (J=7.0 Hz)	3.33 br d (J=7.0 Hz)	6	105.60
2′	5.17 br t (J=7.0 Hz)	5.16 br t (J=7.0 Hz)	7	62.96
4′	1.97 m	1.97 m	8	193.09
5′	2.03 m	2.03 m	9	55.90
6′	5.05 br t (J=6.8 Hz)	5.06 br t (J=6.9 Hz)	1′	21.35
8′	1.63 br s	1.63 br s	2′	121.19
9′	1.60 br s	1.59 br s	3′	131.23[c]
10′	1.77 br s	1.77 br s	4′	39.77
2″	2.33 t (J=7.5 Hz)		5′	26.67
3″	1.60 m		6′	124.36
16″	0.88 t (J=6.5 Hz)		7′	135.75[c]
			8′	25.67
			9′	17.65
			10′	16.10
			1″	173.21
			2″	34.23
			16″	14.14

[a]The ^{1}H-nmr data of the alcoholic portion of **2** and **3** paralleled those of **1**; the vinylic protons of the oleic portion of **2** resonate at 5.34 ppm while the remaining protons of **1–3** resonate between 2.4 and 0.8 ppm.

[b]The ^{13}C-nmr chemical shifts of the alcoholic portion of **2** and **3** paralleled those of **1**; the vinylic carbons of the oleic portion of **2** resonate at 130.02 and 129.71 ppm while the remaining carbons of **1–3** resonate between 32.0 and 14.0 ppm.

[c]Assignments may be interchanged.

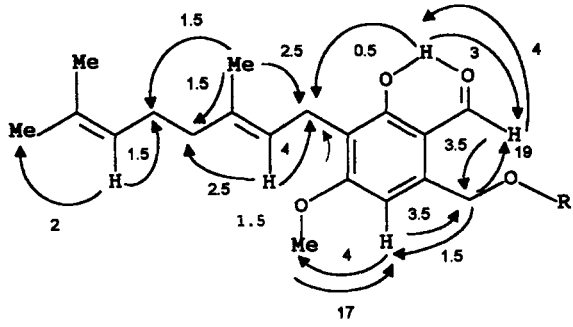


FIGURE 1. The nOe connectivity pattern observed for the alcoholic portion of hericenes A–C [**1–3**]. Numbers depicted near the arrows are percent enhancements upon irradiation of the appropriate protons.

As a consequence, hericenes A–C [**1–3**] are, respectively, the palmitic, oleic, and stearic esters of 4-(3′,7′-dimethyl-2′,6′-octadienyl)-2-formyl-3-hydroxy-5-methoxybenzyl alcohol [**4**]. It may be pointed out that compound **4** has a structure related to mycophenolic acid, an antibiotic produced by *Penicillium* spp. (6).

Erinapyrone C [**5**] was isolated as a solid, mp 72°, $[\alpha]^{25}D$ +141° (c=1, MeOH). The ir spectrum exhibited absorption bands at 3400 and 1650 cm^{-1} due to hydroxy and conjugated carbonyl groups while the molecular formula was determined as $C_8H_{10}O_5$ by cims and elemental analysis.

The ^{1}H-nmr spectrum of **5** (Table 2) showed the presence of two exchangeable resonances which were assigned to one primary and one secondary hydroxy group since their protons were vicinally coupled to H-7a and H-7b and H-8, respectively. In addition, the spectrum contained a methyl doublet (CH_3-9) exhibiting vicinal coupling with H-8 and two methine signals which were assigned to H-3 and H-6. As expected, acetylation of **5** afforded the diacetate **6** in which H-7a, H-7b, and H-8 were shifted downfield (see Experimental). The ^{13}C-nmr spectrum of **5** exhibited eight signals which were attributed by DEPT experiments to one methyl, one methylene, three methine, and three quaternary carbon atoms. The carbons at δ_C 189.47, 170.61, and 101.60 (C-4, C-2, and C-3) were assigned on the basis of chemical-shift criteria as those of an α,β-unsaturated carbonyl system. Furthermore, the CH_2OH group was placed at C-2 since the methylene protons showed two- and three-bond couplings with C-2 and C-3 [^2J(C,H)=11 and 5.5 Hz; ^3J(C,H)=3 and 2.5 Hz] and allylic couplings with H-3 [^4J(H,H)=1.2 Hz]. The remaining C_2HO_2 fragment contains a trisubstituted oxirane moiety in which C-6 [δ_C=80.98; ^1J(C,H)=228 Hz] is linked to an additional oxygen atom in order to explain the large value of the one-bond (C,H) coupling (7). The chemical

TABLE 2. ^{1}H- and ^{13}C-Nmr Data of Compound **5** in $CDCl_3$-DMSO-d_6 (1:1).

H	δ_H	C	δ_C	^1J(CH)
3	5.72 t (J=1.2 Hz)	2	170.61 s	
6	5.74 s	3	101.60 d	169.5
7a	4.16 ddd (J=16.7, 6.1, and 1.2 Hz)	4	189.47 s	
7b	4.07 ddd (J=16.7, 6.1, and 1.2 Hz)	5	64.90 s	
8	4.41 dq (J=6.1 and 6.4 Hz)	6	80.98 d	228
9	1.28 q (J=6.4 Hz)	7	59.98 t	143.5
7-OH	5.40 t (J=6.1 Hz)	8	60.68 d	145
8-OH	4.67 d (J=6.1 Hz)	9	18.17 q	127.5

shift values of C-4 and C-8 (δ_C=189.47 and 60.68) imply that these carbons are part of α,β-unsaturated carbonyl and alcoholic moieties but not of lactone or acetalic functions, with this fact indicating that they must all be linked to C-5. It is therefore apparent that O-1 is linked to C-2 in the structure of erinapyrone C [**5**] and that H-6 and the CHOHMe grouping are through necessity cis-disposed.

From a biogenetic point of view erinapyrone C may be derived from glucose with a following addition of an acetate unit at position 5, as is kojic acid (6). Determination of the relative stereochemistry was unsuccessful because of the facile opening of the oxirane ring.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Kofler apparatus and are uncorrected. Ir and uv spectra were recorded with a Perkin-Elmer 177 Instrument and a Jasco Uvidec-510 spectrophotometer, respectively; optical rotations on a Jasco DIP-181 polarimeter, and mass spectra on a Finnigan-MAT TSQ70 spectrometer. Nmr spectra were acquired on a Bruker AC 250L spectrometer operating at 250.1 MHz for ^{1}H and 62.9 MHz for ^{13}C; chemical shifts are in ppm (δ) from TMS as internal standard. Flash cc was performed with Merck Si gel (0.04–0.06 mm) and tlc with Merck HF_{254} or RP-18 F_{254} Si gel.

CULTIVATION OF FUNGUS AND ISOLATION OF HERICENES A–C [**1–3**] AND ERINAPYRONE C [**5**].—Strain (CBS 233.87) of *Hericium erinaceus* was inoculated in 10 Roux flasks containing 100 ml of hard corn bran (or MPGA malt extract-peptone-glucose-agar, 20:5:20:15 g/liter) with a mycelium suspension at 24°. After three weeks, flasks were extracted with EtOAc containing MeOH (1%) and the extracts were evaporated to give a mixture of crude metabolites (3 g) (2.7 g from 40 flasks containing MPGA). The mixture was chromatographed on a Si gel column using hexane-EtOAc (4:1) to yield hericenes A–C [**1–3**] (400 mg), fatty acids (palmitic, linoleic, and oleic acids in a ratio 1:3:1, 1.2 g) and glyceryl trioleate (300 mg); the residual material was then eluted with EtOAc containing MeOH (1%) to give erinapyrone C [**5**] (150 mg) (250 mg from 40 MPGA flasks). Further purification of the above mixture of compounds [**1–3**] by prep. tlc (RP-18 plates Me_2CO-H_2O, 8:1) and hplc with a SI-60 column [250×50 mm, 6 ml min^{-1}, hexane-EtOAc (98:2), detection at 298 nm] gave hericenes A–C [**1–3**] in a 5:3:2 ratio.

Hericene A [**1**].—Oil (125 mg); eims *m/z* 556, 300, 257; cims *m/z* 557; ir (liquid film) ν max 3450, 1730, 1630, 1570 cm^{-1}.

Hericene B [**2**].—Oil (75 mg); eims *m/z* 582, 300, 283.

Hericene C [**3**].—Oil (50 mg); eims *m/z* 584, 300 285.

Methanolysis of hericenes A–C [**1–3**].—A mixture of compounds [**1–3**] (20 mg) was dissolved in 0.1 M methanolic KOH (5.0 ml) and left at 4° for 24 h. Water was added to the reaction mixture, and the solution was neutralized with 0.1 M HCl and extracted with EtOAc. Evaporation of solvent and prep. tlc in hexane-EtOAc (9:1) gave the methyl esters of the palmitic, oleic, and stearic acids in a 5:3:2 ratio, which were identified by gc-ms analysis and comparison with authentic samples and the benzyl alcohol [**4**] as an oil; eims *m/z* 318, 149; ir (liquid film) ν max 3400, 1650 cm^{-1}; ^{1}H-nmr data are reported in Table 1.

Erinapyrone C [**5**].—Eims *m/z* 186, 168, 155; uv λ max (EtOH) (ε) 280 (9700) nm; ^{1}H- and ^{13}C-nmr data are reported in Table 2; *anal.* found % (calcd for $C_8H_{10}O_5$): C, 51.6 (51.3); H, 5.4 (5.7).

Erinapyrone C diacetate [**6**].—Erinapyrone C [**5**] (60 mg) was dissolved in dry pyridine (0.5 ml), Ac_2O (1.0 ml) was added and the solution was kept for 3 h at room temperature. After the usual workup, a diacetate [**6**] was obtained as an oil (40 mg); eims *m/z* 270, 228, 186; ir ($CHCl_3$) ν max 1740, 1670 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 5.66 (1H, t, *J*=1.2 Hz, H-3), 5.64 (1H, q, *J*=6.4 Hz, H-8), 4.72 (1H, dd, *J*=16.2 and 1.2 Hz, H-7a), 4.62 (1H, dd, *J*=16.2 and 1.2 Hz, H-7b), 2.15 and 2.07 (6H, s, 2×OAc) and 1.37 (3H, d, *J*=6.4 Hz, CH_3-9).

ACKNOWLEDGMENTS

This work was supported by Consiglio Nazionale delle Ricerche (CNR) Roma, Progetto finalizzato "Chimica fine II."

LITERATURE CITED

1. A. Arnone, G. Nasini, and O. Vajna de Pava, *J. Chem. Soc., Perkin Trans. I*, 2723 (1993).
2. H. Kawagishi, M. Ando, and T. Mizuno, *Tetrahedron Lett.*, **31**, 373 (1990).

3. H. Kawagishi, R. Shirai, H. Sakamoto, S. Yoshida, F. Oijma, and Y. Ishiguro, *Chem. Lett.*, 2475 (1992).
4. H. Kawagishi, M. Ando, H. Sakamoto, S. Yoshida, F. Ojima, Y. Ishiguro, N. Ukai, and S. Furukawa, *Tetrahedron Lett.*, **32**, 4561 (1991).
5. H. Kawagishi, M. Ando, K. Shinba, H. Sakamoto, S. Yoshida, F. Ojima, Y. Ishiguro, N. Ukai, and S. Furukawa, *Phytochemistry*, **32**, 175 (1992).
6. W.B. Turner and D.C. Aldridge, "Fungal Metabolites," Academic Press, London, 1983.
7. E. Breitmaier and W. Voelter, "^{13}C-Nmr Spectroscopy," Verlag Chemie, Weinheim, 1974.

Received 9 November 1993