JOURNAL OF NATURAL PRODUCTS

Subscriber access provided by ISTANBUL TEKNIK UNIV

Antifungal Activity of Meridine, a Natural Product from the Marine Sponge Corticium sp.

Peter J. McCarthy, Tara P. Pitts, Geewananda P. Gunawardana, Michelle Kelly-Borges, and Shirley A. Pomponi

J. Nat. Prod., **1992**, 55 (11), 1664-1668• DOI: 10.1021/np50089a016 • Publication Date (Web): 01 July 2004

Downloaded from http://pubs.acs.org on April 4, 2009

More About This Article

The permalink http://dx.doi.org/10.1021/np50089a016 provides access to:

- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

ACS Publications
High quality. High impact.

Journal of Natural Products is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Journal of Natural Products
Vol. 55, No. 11, pp. 1664-1668, November 1992

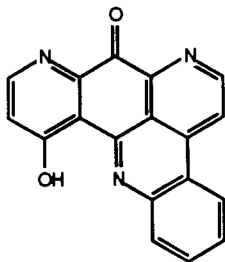
ANTIFUNGAL ACTIVITY OF MERIDINE, A NATURAL PRODUCT FROM THE MARINE SPONGE *CORTICIUM* SP.[1]

PETER J. MCCARTHY,* TARA P. PITTS, GEEWANANDA P. GUNAWARDANA,[2] MICHELLE KELLY-BORGES and SHIRLEY A. POMPONI

Division of Biomedical Marine Research, Harbor Branch Oceanographic Insitutiton, 5600 Old Dixie Highway, Fort Pierce, Florida 34946

ABSTRACT.—Meridine [**1**], a polycyclic alkaloid derived from the marine sponge *Corticium* sp., was found to inhibit the growth of *Candida albicans* and *Cryptococcus neoformans*. Activity was also observed against *Trichophyton mentagrophytes* and *Epidermophyton floccosum*. Studies of the mechanism of action of this agent have shown an inhibition of nucleic acid biosynthesis.

In our search for antifungal agents from marine sources, an extract of the sponge *Corticium* sp. (Class Demospongiae, Order Homosclerophorida, Family Plakinidae) collected at a depth of 450 feet at Great Inagua Island in the Bahamas showed excellent activity against *Candida albicans*. Bioassay-guided purification of the active constituent led to the isolation of meridine [**1**], a compound previously described from the South Australian marine ascidian *Amphicarpa meridiana* (1). The structure of the compound was confirmed as meridine by ^{13}C and ^{1}H nmr. The report of Schmitz *et al.* (1) describes the biological activity for a series of polycyclic alkaloids: no biological activity is reported for meridine itself although the related compounds shermilamine and ascididemin are shown to have activity against various tumor cell lines. In this paper we describe the antifungal activity of meridine and discuss evidence for a probable mechanism of action.



1

The present study showed that meridine was highly active against the pathogenic yeasts *Can. albicans* and *Cryptococcus neoformans* (Table 1). The activity of meridine against *Can. albicans* was found to be fungicidal with the minimum fungicidal concentration (MFC) occurring at the same concentration as the minimum inhibitory concentration (MIC). Interestingly, there was a far wider difference between the MIC and the MFC for *Cr. neoformans* (MIC=0.8 μg/ml; MFC=6.2 μg/ml), indicating fungistatic activity at the lower concentrations. Meridine was also tested against a panel of filamentous fungi with significant activity being observed against *Trichophyton mentagrophytes* and *Epidermophyton floccosum*. No activity was seen against the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. Meridine inhibited the growth of the Gram-positive bacterium *Bacillus subtilis*, giving an MIC of 3.1 μg/ml.

The activity of meridine against *Can. albicans* was growth-medium dependent with greater than tenfold higher activity being observed on RPMI-1640 compared with the conventional test media Sabouraud dextrose broth (SDB) and Yeast Nitrogen Base+glucose (YNBG). Our studies have shown similar results for dercitin, a related marine alkaloid (2) which was inactive on YNBG but showed

[1]This paper is Harbor Branch Oceanographic Institution Contribution #929.

[2]Current address: Abbott Laboratories, D47P AP9A, Abbott Park, IL 60064.

TABLE 1. Antifungal Activity of Meridine [1].

Organism	Growth medium	MIC [a]	MFC [b]
Candida albicans	Sabouraud dextrose broth	3.1	3.1
Candida albicans	YNBG	2.5	NT [c]
Candida albicans	RPMI-1640	0.2	0.2
Cryptococcus neoformans	Emmon's SDB	0.8	6.2
Trichophyton mentagrophytes	Sabouraud dextrose agar	6.2	NT
Epidermophyton floccosum	Sabouraud dextrose agar	1.6	NT
Microsporum gypseum	Sabouraud dextrose agar	25	NT
Trichosporon beigellii	Sabouraud dextrose agar	>25	NT
Sporothrix schenkii	Sabouraud dextrose agar	>25	NT
Scopulariopsis brevicaulis	Sabouraud dextrose agar	>25	NT

[a]Minimum inhibitory concentration (μg/ml).
[b]Minimum fungicidal concentration (μg/ml).
[c]NT=Not tested.

an MIC against *Can. albicans* of 1.6 μg/ml when tested on RPMI-1640. *Can. albicans* is a dimorphic fungus: it can grow in either a yeast or mycelial form. When grown in RPMI-1640 under the conditions described in this paper, *Can. albicans* was observed to grow in the mycelial form rather than as a yeast as seen on SDB or YNBG. This leads to increased sensitivity towards imidazole antifungal agents such as miconazole and ketoconazole (3). In the present study a similar increase in sensitivity towards imidazoles was observed: the activities of miconazole and ketoconazole were 25 and 0.062 μg/ml, respectively, when tested on SDB, while these MIC values were reduced to 0.03 and 0.008 μg/ml when tested on RPMI-1640. One of the principal differences between the growth media RPMI-1 640 and YNBG was pH: under the conditions used in our laboratory, the pH of RPMI-1640 was 7.2 while that of YNBG was 5.4. A simple explanation for the difference in the activity of meridine when tested on the two growth media would therefore be pH sensitivity of the antifungal activity. The activity of meridine against *Can. albicans* was not significantly affected by pH (Table 2). Therefore the effect of growth medium on activity could not be explained by the difference in the pH of the media. Since the yeast form of *Can. albicans* grown on either SDB or YNBG showed less sensitivity to meridine than the mycelial form grown on RPMI-1640, these data suggest that this compound showed preferential activity towards the mycelial form of *Can. albicans*. A similar technique has

TABLE 2. Antifungal Activity of Meridine [1] Against *Candida albicans* at Various Initial pH Values of the Culture Medium.

pH	MIC [a] RPMI-1640	MIC [a] YNBG
4	0.62	2.5
5	0.62	2.5
6	0.31	1.2
7	0.31	1.2
8	0.31	1.2
No buffer [b]	0.31	2.5

[a]Minimum inhibitory concentration (μg/ml).
[b]Test media prepared by manufacturer's directions: RPMI-1640 pH 7.2; YNBG pH 5.4.

TABLE 3. Concentrations (μg/ml) of Inhibitors Producing 50% Inhibition of Radiolabel Incorporation Compared with Control.

Compound	IC_{50} Incorporation into TCA precipitable material			
	U-^{14}C adenine	U-^{14}C leucine	U-^{14}C glucose	U-^{14}C acetate
Meridine	1	50	>100	>100
5-Fluorocytosine	6	>100	>100	>100
Blasticidin S	>100	6	>100	>100

been used to find agents with preferential activity against the mycelial form of *Can. albicans* and has led to the discovery of Sch 40873 (4). The mycelial form of *Candida* has been implicated in the pathogenesis of this organism, and it is therefore possible that agents which are active against this form will have useful activity in treating infections caused by this organism.

MECHANISM OF ACTION.—To define the mechanism of action of meridine, we studied the incorporation of radiolabelled precursors into macromolecules. Incorporation of all labels was found to be linear over a 60-min period. The effects of meridine and two control compounds, 5-fluorocytosine (5-FC) and blasticidin S, are shown in Table 3. It can be seen that 5-FC inhibited the incorporation of adenine into nucleic acid, as would be expected from its mechanism of action, disruption of nucleic acid biosynthesis (5). Blasticidin S is an inhibitor of protein biosynthesis in fungi (6): the reduction in the incorporation of radiolabelled leucine can be seen in the results. Meridine had little effect on the incorporation of ^{14}C-glucose or ^{14}C-acetate, demonstrating that there was no general inhibition of metabolism or membrane transport. The incorporation of ^{14}C-leucine was affected at high concentrations of meridine suggesting a secondary effect on protein biosynthesis. ^{14}C-adenine incorporation was inhibited by very low concentrations of meridine (IC_{50} = 1.6 μg/ml) which corresponded to the MIC on YNBG. This indicated that one site of action for meridine was nucleic acid biosynthesis. Similar results were observed for RPMI-grown cells: the IC_{50} for adenine incorporation was found to be 1.5 μg/ml. Schmitz *et al.* (1) report that the related compounds shermilamine B and ascididemin inhibit the topoisomerase II system of mammalian cells. No data are reported concerning either the cytotoxicity of meridine or its interaction with topoisomerase II. Our data showed an interaction with nucleic acid biosynthesis. Further studies are required to show whether this was due to an interaction with the fungal topoisomerases which are possible targets for antifungal agents (7).

It is interesting to note that meridine was isolated from a deep-water Bahamian sponge in our laboratory, while Schmitz *et al.* (1) isolated meridine from an Australian ascidian. The diverse sources of the same metabolite suggest a common microbial source for this compound.

EXPERIMENTAL

CHEMICALS AND MEDIA.—RPMI-1640 was purchased from Gibco. All other media were from Difco. Radiochemicals were from NEN-Dupont. Blasticidin S (800 U/mg) was a gift from the Kaken Chemical Company, Japan. All other chemicals were from Sigma.

The sponge *Corticium* sp. was collected at a depth of 450 ft at Great Inagua Island, Bahamas, using the Johnson-Sea-Link I manned submersible. It grew as a thin encrustation on a steep rock slope. The sponge is a deep reddish purple, both alive and in EtOH fixative. The sponge is most closely related to *Corticium tetralophum* (8) and *Corticium versatile* (9), both from the West Indies. *Corticium* sp. differs from these species in coloration and in the larger size and extreme density of the spicules throughout the sponge. A taxonomic voucher of the sponge is deposited with the Harbor Branch Oceanographic Museum (catalog number 003:00047, DBMR number 10-X-88-3-15).

ISOLATION OF MERIDINE [**1**].—The sponge (wet wt 21.1 g) was soaked in MeOH for 2 h, put

through a blender, and exhaustively extracted with CH_2Cl_2-MeOH (1:1) (3×100 ml). The crude extract thus obtained (0.33 g) was separated by planetary coil countercurrent chromatography (pccc) using an MeOH-CH_2Cl_2-H_2O (5:5:3) system (upper phase stationary, 20-ml fractions were collected). The antifungal active fractions (2–5) were combined and evaporated to give a yellow pigment (13 mg). This was further purified by passing through a short column of RP NH_2 silica (10 g, Lichrosorb, EM Science) using CH_2Cl_2-MeOH (9:1) saturated with NH_3, to yield **1** as a yellow amorphous solid (8 mg): mp>250°; λ max (MeOH) 203 nm (ε 25000), 227 (38000), 285 (17000), 370 (11000); ν max (KBr) 3070, 1674 cm^{-1}; hrfabms $[M+H]^+$ 300.0768 ($C_{18}H_{10}N_3O_2$ requires 300.0785) (Δ 1.7 mmu). Originally this compound was referred to as corticimine in our laboratory, and its structure was established as **1** through the extensive use of long-range proton carbon correlation information obtained by HMBC experiments (10). Subsequently, we came to know of the isolation of meridine by Dr. F. Schmitz through personal communications. Spectral comparison, mixed tlc and mixed mp with authentic material showed corticimine and meridine to be the same compound.

MICROORGANISMS.—Strains were obtained from the American Type Culture Collection unless otherwise indicated. *Can. albicans* 44506, *Cr. neoformans* 32045, *Esch. coli* HBOI strain 001 and *Ps. aeruginosa* strain 27853 were stored at −80° in the presence of 10% (v/v) glycerol. *Trichophy. mentagrophytes* 9533, *Epi. floccosum* 52066, *Microsporum gypseum* 42960, *Scopulariopsis brevicaulis* 36840. *Trichosporon beigellii* 28592, and *Sporothrix schenkii* 14284 were maintained on Sabouraud dextrose agar (SDA) and subcultured monthly. *B. subtilis* 6633 was purchased as a spore suspension from Difco. Cell densities were determined microscopically using a Reichert hemocytometer.

All biological data were determined in triplicate and are expressed as the average value.

BROTH DILUTION MIC.—Twofold dilutions of the test compound were prepared in the appropriate culture medium, giving a total volume of 40 μl in the wells of a 96-well plate. Wells were inoculated with the microorganism, bringing the total volume to 50 μl and the cell density to 10^3 ml^{-1}. Plates were incubated at 37° for 18–24 h (48 h for *Cr. neoformans*). RPMI plates were incubated in an atmosphere of 5% CO_2. The MIC was determined visually as the lowest concentration of the test compound that produced complete inhibition of growth. Bacterial MICs were determined using Mueller-Hinton broth supplemented with Mg^{2+} and Ca^{2+} (11). When required, the pH of the growth medium was adjusted by the addition of either Tris-citrate or Tris-phospate buffer. The final concentration of Tris in the test system was adjusted to 100 mM; buffered RPMI-1640 plates were incubated in the absence of added CO_2.

MINIMUM FUNGICIDAL CONCENTRATION.—The MFC was determined from the MIC plates described above. After overnight incubation the plate was mixed to resuspend the cell pellet. Part of the suspension (1 μl) was removed and streaked on to the surface of an agar plate (SDA for *Can. albicans* or Emmon's SDA for *Cr. neoformans*). After 24–48 h incubation at 37°, plates were scored for growth. The MFC was defined as the lowest concentration of the test compound resulting in 99% killing of the inoculum.

AGAR DILUTION MIC.—Dilutions of the test compound were prepared in SDA held at 45°, and 2 ml of each dilution was added to the wells of a Quad-Petri dish. Suspensions of the filamentous fungi were prepared in SDB and inoculated on to the surface of the agar using a Steer's replicator. This delivered an inoculum of 1 μl containing 10^5 $cfu \cdot ml^{-1}$. Plates were incubated for 7 days at 25°, at which point the MIC was determined as being the lowest concentration which completely inhibited growth of the fungus.

MACROMOLECULAR SYNTHESIS.—Macromolecular synthesis in *Can. albicans* was studied by standard methods as described by Hamamoto *et al.* (12). Briefly, *Can. albicans* was grown overnight in YNBG to mid-logarithmic phase. The cells were then harvested, washed with saline, and resuspended in YNB lacking the precursor being studied. The test compound was added and, following a 30 min incubation at 37°, the precursor (^{14}C-labelled adenine, leucine, acetate, or glucose) was added. Incorporation was found to be linear over a 60-min time period. Therefore after this time, ice-cold tricholoroacetic acid (TCA) was added to a final concentration of 5% (v/v). Counts incorporated into TCA precipitable material were determined.

ACKNOWLEDGMENTS

We thank Professor Francis Schmitz for the meridine reference sample and Dr. Amy Wright for her help and advice in the preparation of this manuscript.

LITERATURE CITED

1. F. Schmitz, F.S. DeGuzman, M.B. Hossain, and D. van der Helm, *J. Org. Chem.*. **56**, 804 (1991).
2. G.P. Gunawardana, S. Komoto, S.P. Gunasekera, O.J. McConnell, and F.E. Koehn, *J. Am. Chem. Soc.*. **110**, 4856 (1988).
3. J.R. Ryley and W.G. Rathmell, in: "Mode of Action of Antifungal Agents." Ed. by A.P.J. Trinci and J.R. Ryley, Cambridge University Press, Cambridge, 1984, Chapter 4, pp. 63–87.

4. V. Gullo, I. Gunnarson, V. Hegde, A. Horan, D. Loebenberg, J. Marquez, M. Patel, M. Puar, and J. Schwartz, *J. Ind. Microbiol.*, **8**, 65 (1991).
5. A. Polak and H.J. Scholer, *Chemotherapy*, **21**, 113 (1975).
6. T.K. Huang, T. Misato, and E. Asuyama, *J. Antibiot.*, **17A**, 65 (1964).
7. D.P. Figgitt, S.P. Denyer, P.M. Dewick, D.E. Jackson, and P. Williams. *Biochem. Biophys. Res. Commun.* **160**, 257 (1989).
8. G.J. Hechtel, "A Systematic Study of the Demospongiae of Port Royal, Jamaica," Yale University Peabody Museum of Natural History Bulletin No. 20, 1965, p. 77.
9. O. Schmidt, "Die Spongien des Meerbusen von Mexico (und des Caraibischen Meeres)," G. Fischer, Jena, 1880, pp. 1–90.
10. A. Bax and M.F. Summers, *J. Am. Chem. Soc.*, **108**, 2093 (1986).
11. L.D. Thrupp, in: "Antibiotics in Laboratory Medicine." Ed. by V. Lorian, Williams and Wilkins, Baltimore, 1986, Chapter 4, pp. 93–150.
12. T. Hamamoto, T. Uozumi, and T. Beppu, *J. Antibiot.*, **38**, 1573 (1986).

Received 20 March 1992