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J. Nat. Prod., **1991**, 54 (3), 856-859• DOI:
10.1021/np50075a017 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 3, pp. 856-859, May-Jun 1991

ISOLATION AND CHARACTERIZATION OF TWO ANTIMICROBIAL AGENTS FROM MACE (*MYRISTICA FRAGRANS*)

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ABSTRACT.—The two antimicrobial resorcinols malabaricone B [**1**] and malabaricone C [**2**] were isolated from mace, the dried seed covers of *Myristica fragrans*. Both compounds exhibited strong antifungal and antibacterial activities. Structure modifications by methylation or reduction resulted in diminished activity.

Mace is the fleshy red, net-like skin that covers the seeds of *Myristica fragrans* Houtt. (nutmeg) (Myristicaceae) (1). It is widely used locally as a flavoring agent, a hair dye, and a folk medicine. In a program of screening traditional medicines for biological activities, mace was found to exhibit strong antifungal and antibacterial activities. This note deals with the isolation and characterization of two antimicrobial agents from this source and defines some of the structural parameters needed for activity.

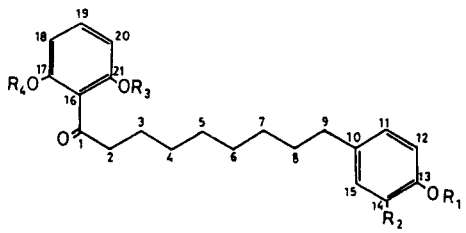
Mace was defatted by extraction with *n*-hexane to give an oily residue that showed only marginal antimicrobial activity. The marc was re-extracted with 95% EtOH in a Soxhlet to yield a reddish residue that exhibited antifungal and antibacterial activities.

Flash chromatography (2) of this residue on Si gel using EtOAc-CH_2Cl_2 (1:39) as eluent provided two main fractions that demonstrated strong antimicrobial activity.

Tlc analysis of the less polar fraction revealed a spot, R_f 0.34, corresponding to malabaricone B [**1**]. Fractional crystallization from C_6H_6 afforded **1** in 1.57% yield. It was obtained as pale yellow crystals, mp 102–103°. Likewise, malabaricone C [**2**], R_f 0.10, crystallized from the more polar fraction in 0.53% yield using $CHCl_3$ as a solvent to give yellow needles, mp 117–118°.

The identity of both compounds was established by comparing their physical and spectral data with those reported for the compounds previously isolated from the fruit rind of *Myristica malabarica* (3) and from the bark and seeds of *Myristica dactyloides* (4,5).

Malabaricone B [**1**] and malabaricone C [**2**] exhibited a good level of antimicrobial activity when tested against a variety of microorganisms, including *Staphylococcus aureus* and *Candida albicans* (Table 1). The availability of **1** in sufficient amounts made it possible to study some of the structural parameters



1 $R_1=R_2=R_3=R_4=H$
2 $R_1=R_3=R_4=H$, $R_2=OH$
3 $R_1=R_2=R_4=H$, $R_3=Me$
4 $R_3=R_4=Me$, $R_1=R_2=H$
5 $R_1=R_3=Me$, $R_2=R_4=H$
6 $R_1=R_3=R_4=Me$, $R_2=H$

TABLE 1. Minimum Inhibitory Concentrations (μg/ml) of Compounds **1–8** after 24 h of Incubation.[a]

Compound	*Staphylococcus aureus*	*Bacillus subtilis*	*Streptococcus durans*	*Candida albicans*		
				strain A	strain B	strain C
1	1(1)	1(1)	1(2)	4(8)	8(8)	16(16)
2	4(8)	2(2)	4(8)	8(8)	8(8)	32(32)
3	4(4)	4(4)	2(4)	<32	<32	<32
4	32(<32)	<32(<32)	<32(<32)	<32(<32)	<32(<32)	<32(<32)
5	<32	<32	<32	<32	<32	<32
6	<32	<32	<32	<32	<32	<32
7	8(16)	4(4)	8(16)	32(<32)	<32(<32)	<32(<32)
8	4(4)	4(4)	4(8)	8(8)	8(8)	16(16)
Chloramphenicol	4(4)	4(4)	1(4)	NT[b]	NT	NT
Nystatin	NT	NT	NT	2(4)	2(4)	2(4)
2′,6′-Dihydroxy-acetophenone	<32	<32	<32	<32	<32	<32

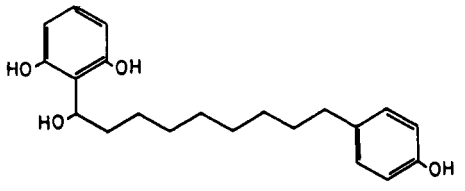
[a]Values in parentheses refer to MICs after 48 h of incubation. For sources, strain numbers and place of deposition of all microorganisms used, see Experimental.

[b]NT: not tested.

needed for antimicrobial activity. Methylation by MeI or Me_2SO_4 afforded the methyl ethers **3–6**, which except for **3**, exhibited neither antibacterial nor antifungal activity. The methyl ether **3**, on the other hand, was devoid of anticandidal activity but retained, to a large extent, its antibacterial activity. It should be noted that the methyl ether **3** was also reported (5) to occur in the stem bark of *M. dactyloides*. These results suggest that the phenolic hydroxyl groups are necessary for antimicrobial action. However, it appears that the activity also depends upon other structural elements in the molecule of **1** as a whole, because 2′,6′-dihydroxyacetophenone was found to be inactive (see Table 1).

$NaBH_4$ reduction of the ketone group of **1** yielded the secondary alcohol **7**, which possessed some antibacterial activity, but lost the anticandidal action. Reductive dehydroxylation of **7** using ammonium formate (6) provided **8** with restored antifungal effect (see Table 1).

Both compounds **1** and **2** are structurally related to gingerenone A [**9**], a constituent of *Zingiber officinale* (ginger), re-



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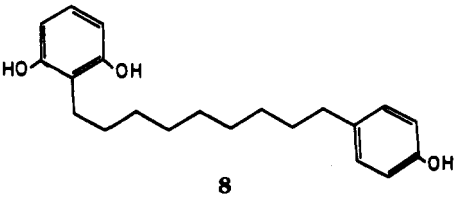

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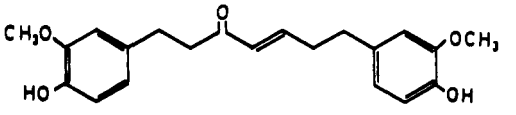

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cently reported (7) to have antifungal activity. Furthermore, all of these compounds are structurally similar to a number of 5-alkylresorcinols that were found to promote DNA strands scission (8). Whether malabaricone B and malabaricone C exert their antimicrobial action through a similar mechanism remains to be seen.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Ir spectra were obtained on a Perkin-Elmer 580 IR spectrometer. Nmr spectra were determined on a Varian XL 200 spectrometer or a Varian VSR-300 spectrometer at 300 and 75 MHz for ^1H nmr and ^{13}C nmr, respectively, and chemical shift values are given in δ (ppm) with TMS as internal standard. Standard Varian pulse sequences were used for DEPTGL, APT, and HETCOR spectra, which aided nmr assignments. Low-resolution ei mass spectra were obtained using an E.I. Finnigan model 3200 (70 eV ionization potential) with INCOS data system or an E.I. Finnigan model 4600 quadrupole system. Tlc analysis was performed on Si gel G plates using 2.5% EtOAc in CH_2Cl_2 as the solvent system, unless otherwise specified, and visualized by exposure to short wavelength uv (λ max = 254) or by spraying with anisaldehyde spray reagent (9). The antimicrobial screening program used is essentially the agar dilution method described by Mitscher *et al.* (10) and designed for the evaluation of antimicrobial activity in extracts of higher plants. In our procedure, however, chloramphenicol and nystatin were used as positive controls for bacteria and fungi, respectively, instead of streptomycin sulfate. Thus, each test substance was initially evaluated at a concentration of 320 μg per 0.1 ml in DMSO by dilution with 10 ml of warm nutrient agar (45–50°) in 90-mm Petri plates and allowed to harden. Test organisms were streaked in a radial pattern, and the plates were incubated for 48 h at 30° for *Candida albicans* and 37° for bacteria. The lowest drug concentration at which no growth was observed was considered to be the MIC. Three strains of *C. albicans*, strains A, B, and C, were used in this study. Strains A and B, No. 3153 and 3110, respectively, were obtained from the Mycological Reference Laboratory, London School of Hygiene and Tropical Medicine, London, England. Strain C, No. 10231, was obtained from the American Type Culture Collection (ATCC) 12301 Parklawn Drive, Rockville, Maryland 20852. The bacteria used were obtained from National Collection of Type Cultures (NCTC), Central Public Health Laboratory, Colindale Avenue, London, England. They included *Staphylococcus aureus* (No. 6571), *Bacillus subtilis* (No. 10400), and *Streptococcus durans* (No. 8307). All microorganism are deposited at the Microbiology Unit, Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia.

The plant material was purchased locally in Riyadh, Saudi Arabia, and its identity was established by Dr. Sultan Ul-Abidin. This was accomplished by examining it morphologically and microscopically and by comparison with an authentic sample. A voucher specimen was deposited at the herbarium of MAPPRC.

ISOLATION OF MALABARICONE B [**1**] AND MALABARICONE C [**2**] FROM MACE.—Mace (375 g) was defatted by extraction with *n*-hexane for 48 h in a Soxhlet apparatus. The marc was re-extracted with 95% EtOH for 48 h, and the deep red extract was evaporated in vacuo at 40° to give 155 g of a semisolid residue.

Flash chromatography of this residue (3.4 gm) on a column of Si gel 14.0 × 3.0 cm, using 2.5% EtOH in CH_2Cl_2 as solvent, provided 129 mg of crude malabaricone B [**1**] that crystallized from C_6H_6 to give pale yellow needles, mp 102–103° [lit. (4) 100–102°].

Malabaricone C [**2**] eluted next in the form of a yellow gum (44 mg) that readily crystallized from $CHCl_3$ to give yellow needles, mp 117–118° [lit. (4) 122–124°]. The ir, ms, ^1H-nmr, and ^{13}C-nmr spectra of both **1** and **2** were indistinguishable from those previously reported (3–5).

METHYLATION OF MALABARICONE B [**1**] TO **6**.—Malabaricone B (20 mg) was stirred for 2 h with Me_2SO_4 (0.4 ml) in 2 ml of 95% EtOH. The medium was kept alkaline by dropwise addition of 4 N NaOH. Acidification with 2 N HCl and Et_2O extraction yielded 16 mg of **6** as colorless crystals, mp 43–44° [lit. (5) unreported], with ir, nmr, and ms data indistinguishable from those reported (5) for the product of methylating **3** with CH_2N_2.

METHYLATION OF MALABARICONE B [**1**] TO **3**, **4**, **5**, AND **6**.—Malabaricone B (200 mg) was refluxed for 2 h in Me_2CO solution (20 ml) with 4.0 ml of MeI in the presence of 2.0 gm of anhydrous K_2CO_3. The products, R_f 0.50, 0.32, 0.78, and 0.70, corresponded to **3**, **4**, **5**, and **6**, respectively. Flash chromatography (2) on Si gel using CH_2Cl_2 as solvent yielded 167 mg of **3**, mp 62–64° [lit. (5) 65–66°]; **5** (31 mg), mp 37–40° [lit. (5) unreported]; and **6** (47 mg), mp 43–44° [lit. (5) unreported]. All three compounds had ir, nmr, and ms data indistinguishable from those reported (4,5).

In addition, the hitherto unreported product **4** (29 mg) was obtained as colorless crystals: mp

96–97°; ir ν max (KBr) (cm^{-1}) 3480 (OH) and 1693 (C=O); ms (rel. int.) *m/z* $[M]^+$ 370 (2); ^1H nmr ($CDCl_3$) δ 7.25 (1H, t, J = 8.4 Hz, H-19), 7.02 (2H, d, J = 8.5 Hz, H-11 and H-15), 6.74 (2H, d, J = 8.5 Hz, H-12 and H-14), 6.55 (2H, d, J = 8.4 Hz, H-18 and H-20), 3.77 (6H, s, 2 × MeO), 2.75 (2H, t, J = 7.3 Hz, H-2), 2.52 (2H, t, J = 7.5 Hz, H-9), and a group of signals between δ 1.8 and 1.2 (12H due to six methylene groups); ^{13}C nmr see Table 2. Calcd for $C_{23}H_{30}O_4$, C 74.56, H 8.10; found C 74.65, H 8.14.

TABLE 2. ^{13}C-nmr Assignments of Compounds **4**, **7**, and **8**.[a]

Carbon	**4**[b]	**7**[c]	**8**[b]
C-1	206.1	70.4	23.1
C-2–C-8 . . .	44.8	37.8	31.6
	31.7	33.0	29.7
	29.3	30.7	29.5
	29.2	30.6	29.4
	29.1	30.5	29.1
	23.5	30.3	29.06
		26.6	
C-9	35.0	36.0	35.0
C-10	134.8	134.9	135.3
C-11, C-15 . .	129.3	130.2	129.4
C-12, C-14 . .	115.1	115.9	115.0
C-13	153.6	156.1	153.3
C-16	120.5	116.9	115.5
C-17, C-21 . .	156.7	157.1	154.6
C-18, C-20 . .	104.0	108.1	107.9
C-19	130.5	128.9	126.7
OMe	55.8	—	—

[a]Multiplicities were determined from the APT and DEPTGL spectra while assignments were confirmed by examining the HETCOR spectra and by corrolation with reported (4,5) data.

[b]Taken in $CDCl_3$.

[c]Taken in CD_3OD.

$NaBH_4$ REDUCTION OF MALABARICONE B [**1**] TO **7**.—Malabaricone B (200 mg) was dissolved in 20 ml of 95% EtOH and stirred for 1 h with $NaBH_4$ (200 mg). After quenching with 10% HOAc and extraction with Et_2O, crude compound **7** (247 mg) was obtained, which was crystallized from CH_2Cl_2/hexane followed by Et_2O/hexane to give 136 mg of colorless crystals, mp 103–110°. This compound was found to decompose quickly unless stored at −20°. Ir ν max (KBr) (cm^{-1}) 3425 (OH) with no carbonyl absorption bands; ms (rel. int.) *m/z* $[M]^+$ 344 (not observed, $[M-H_2O]^+$ 326 (14); ^1H nmr (CD_3OD) δ 6.96 (2H, d, J = 8.5 Hz, H-11 and H-15), 6.86 (1H, t, J = 8.2 Hz, H-19), 6.67 (2H, d, J = 8.6 Hz, H-12 and H-14), 6.25 (2H, d, J = 8.2 Hz, H-19 and H-20), 5.2 (1H, dd, J = 5.3 and 7.4 Hz, H-1), 2.48 (2H, t, J = 7.5 Hz, H-9), and a group of signals due to seven methylene groups between δ 1.2 and 1.8.

REDUCTION OF **7** TO **8**.—The alcohol **7** (215 mg) was stirred under N_2 atmosphere for 1 h at 110° with 5% Pd/C (100 mg) in 2 ml of glacial HOAc containing 250 mg of ammonium formate. The mixture was diluted with 40 ml of $CHCl_3$ and filtered over a bed of celite. The clear filtrate was washed with H_2O, 10% $NaHCO_3$, and dried over anhydrous Na_2SO_4. Evaporation in vacuo left 131 mg of a crystalline residue that was recrystallized from CH_2Cl_2/hexane to give pale yellow needles: mp 76–77°; ir ν max (KBr) (cm^{-1}) 3375 (OH) with no carbonyl absorption bands; ms (rel. int.) *m/z* $[M]^+$ 328 (14); ^1H nmr ($CDCl_3$) δ 7.04 (2H, d, J = 8.6 Hz, H-11 and H-15), 6.91 (1H, t, J = 8.1 Hz, H-19), 6.75 (2H, d, J = 8.6 Hz, H-12 and H-14), 6.40 (2H, d, J = 8.1 Hz, H-18 and H-20), 2.61 (2H, t, J = 7.6 Hz, H-1), 2.52 (2H, t, J = 7.5 Hz, H-9), and a group of seven methylene signals between δ 1.1 and 1.8; ^{13}C nmr see Table 2. Calcd for $C_{21}H_{28}O_3$, C 76.79, H 8.59; found C 76.88, H 8.50.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Charles D. Hufford, School of Pharmacy, University of Mississippi for recording the nmr spectra and Mr. Mustafa O. Khaled for technical assistance with the microbiology work.

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Received 29 May 1990