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# 3-DIMETHYLALLYLINDOLE: AN ANTIBACTERIAL AND ANTIFUNGAL METABOLITE FROM *MONODORA TENUIFOLIA*

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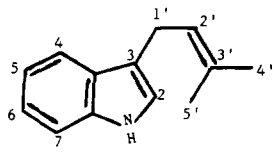
*Monodora tenuifolia* (Benth.) W. Ash (Annonaceae) is a tree found in evergreen and transition forests along the west coast of Africa. The morphological parts of the plant are used in traditional medicine for the treatment of diseases such as dysentery (leaves), dermatitis (root), headache (stem bark), toothache, and as a vermifuge. The aromatic seeds are used as a condiment in soup or as seasoning (1). Despite the numerous uses of this plant, only one investigation has been carried out on the chemical constituents (2).

In a continuation of the investigation of Nigerian medicinal plants, a prenylated indole has been isolated from the stem bark of *M. tenuifolia*. This paper reports on the isolation, identification, and antimicrobial activity of the prenylated indole.

## RESULTS AND DISCUSSION

Extraction of the stem bark with EtOAc and evaporation of the solvent gave a red, oily residue which was chromatographed consecutively over silica gel 60 and neutral alumina to give a light yellow oil that crystallized with difficulty from *n*-hexane to give compound **1** (mp 45-47°). Compound **1** was pure as evidenced by tlc and gc. The uv spectrum showed absorption bands at λ max 291, 282, and 225 nm characteristic of an indole chromophore, while the ir spectrum showed a characteristic band for an amino group (3400 $cm^{-1}$). The $^1$H-nmr spectrum, in addition to showing aromatic protons characteristic of an indole (δ 7.70, 1H; 7.27, 3H; 6.87, 1H), revealed an olefinic proton resonating as a triplet (δ 5.50, $J$=6Hz), a 2H doublet (δ 3.48, $J$=6Hz), and a 6H singlet [δ 1.77, $=C(CH_3)_2$]. The mass spectrum showed the parent ion peak at *m/z* 185 (85%) and significant fragments at *m/z* 155 (32%) and 117 (73%) corresponding to $M^+$-$2CH_3$ and $M^+$-$C_5H_8$, respectively. These data suggested an indole substituted with a γ,γ-dimethylallylic group. The $^{13}$C-nmr data further confirmed that **1** is a prenylated indole. The placement of the substituent at C-3 follows from a comparison of its $^{13}$C-nmr data with those reported for indole and 2-methyl and 3-methyl indoles (Table 1). It has been observed that alkylation of indoles at position 2 or 3 causes a characteristic downfield shift of 10-13 ppm in the $^{13}$C resonance of the substituted carbon atom (3). In **1**, C-3 resonates at 115.8 ppm, which is shifted 13 ppm downfield relative to the corresponding carbon in indole.




**1**

3-γ,γ-Dimethylallylindole (**1**) has been reported previously from studies on the insertion of isoprene units into indole systems (4,5). A sample of **1** was prepared (5), and it was identical in all respects to that obtained naturally.

An investigation of the antimicrobial activity of **1** using procedures previously reported (6,7) showed antibacterial and antifungal activity (see Table 2). Based on the qualitative antimicrobial

TABLE 1. $^{13}C$-nmr Assignments for **1**, Indole, 2-Methylindole, and 3-Methylindole[a]

| Carbon No. | **1** | Indole | 2-methylindole | 3-methylindole |
|---|---|---|---|---|
| C-2 | 122.0d[b] | 125.2d | 135.7d | 122.7d |
| C-3 | 116.2s | 102.6s | 100.4s | 111.4s |
| C-3a | 127.5s | 128.8s | 129.9s | 129.2s |
| C-4 | 119.1d[c] | 121.3d | 120.3d | 119.4d |
| C-5 | 121.2d[b] | 122.3d | 121.1d | 122.3d |
| C-6 | 119.0d[c] | 120.3d | 119.9d | 119.6d |
| C-7 | 111.0d | 111.8d | 110.9d | 111.7d |
| C-7a | 136.6s | 136.1s | 137.1s | 137.3s |
| C-1′ | 24.1t | | 13.4q | 9.9q |
| C-2′ | 123.2d | | | |
| C-3′ | 131.9s | | | |
| C-4′ | 25.7q | | | |
| C-5′ | 17.7q | | | |

[a]Chemical shifts for **1** are expressed in ppm from internal TMS and were recorded in $CDCl_3$. The data for indole, 2-methylindole, and 3-methylindole were taken from Parker and Roberts (3) and are listed here for comparison. The multiplicities were determined by SFORD. A $^1H$-$^{13}C$ heteronuclear correlation experiment was performed on **1** using an IBM AF-300 spectrometer which further confirmed the assignments as listed.

[b,c]Assignments with the same letter designation may be interchanged.

evaluation, 3-dimethylallylindole exhibited antifungal activity against both yeasts (*Candida albicans*, *Saccharomyces cerevisae*, and *Cryptococcus neoformans*) and filamentous fungi (*Trichophyton mentagrophytes*, *Pycnoporus sanguineus*, and *Helminthosporum* sp.). In addition, **1** showed good antibacterial activity against the acid-fast bacterium *Mycobacterium smegmatis* and the gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. Based on these data the minimum inhibitory concentration (MIC) values of **1** were determined against susceptible bacteria and fungi and are summarized in Table 2. 3-Dimethylallylindole is more effective as an antifungal agent than as an antibacterial agent. The dermatophyte *T. mentagrophytes* and the phytopathogen *Helminthosporium* sp. appear to be nearly equally susceptible, with MIC values of 1.56 and 0.78 μg/ml, respectively. Other fungi showed susceptibility at the significantly higher concentrations of 12.5 and 25 μg/ml.

Indole has previously been reported to

TABLE 2. Minimum Inhibitory Concentrations of 3-Dimethylallylindole

| Organism | MIC (μg/ml)[a] |
|---|---|
| *Bacillus subtilis* ATCC 6633 | 12.5 (6.25) |
| *Mycobacterium smegmatis* ATCC 607 | 3.12 (6.25) |
| *Staphylococcus aureus* ATCC 6538 | 12.5 (6.25) |
| *Candida albicans* ATCC 10231 | 25.0 |
| *Candida albicans* NIH B311 | 25.0 |
| *Candida albicans* WH | 25.0 |
| *Cryptococcus neoformans* ATCC 32264 | 12.5 (12.5) |
| *Saccharomyces cerevisiae* ATCC 9763 | 12.5 (12.5) |
| *Helminthosporium* sp. NRRL 4671 | 0.78 (3.12) |
| *Pycnoporus sanguineus* ATCC 14622 | 12.5 (6.25) |
| *Trichophyton mentagrophytes* ATCC 9172 | 1.56 (1.56) |
| *Escherichia coli* ATCC 10536 | inactive |
| *Pseudomonas aeruginosa* ATCC 15442 | inactive |
| *Aspergillus niger* ATCC 16888 | inactive |

[a]Values in parentheses represent duplicate determinations.

exhibit marginal antibacterial activity and slightly better antifungal activity against a wide range of organisms (8). In a later comprehensive study of the effects of lipophilic substituents on the biological activities of substituted indoles, it was found that while 54% of the 400 substituted indoles showed some degree of antimicrobial activity against at least one species of bacteria or fungi, only 36% inhibited two or more species, and only 8% inhibited at least 15 species (9). 3-Dimethylallylindole (**1**) was not included in the study, and this represents the first report of the antimicrobial activity of **1**. In addition to the reports on the antimicrobial activities of substituted indoles, a comprehensive review covering all aspects of the biological activities of indole derivatives is available (10).

Two other prenylated indoles have been reported from plants in the family Annonaceae. 3,6-Bis(γ,γ-dimethylallyl)-indole has been reported from *Uvaria elliotiana* (11) and 7-γ,γ-dimethylallylindole from *Annonidium mannii* (12), but neither has any antimicrobial data reported.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ir spectra were obtained on a PYE Unicam SP 3-300 recording spectrophotometer. $^1$H-nmr spectra were recorded on a Varian EM-390 nuclear magnetic spectrometer at 90 MHz using TMS as internal standard. Uv spectra were taken on a PYE Unicam SP8-100 recording spectrophotometer. The $^{13}$C-nmr spectra were recorded on a JEOL-FX60 Fourier Transform nmr spectrometer at 15.03 MHz. Mass spectra were recorded on Finnigan 3200 and Kratos MS 25 mass spectrometers. Spot detection on tlc plates was achieved by spraying with 10% $H_2SO_4$ and heating for 5 min at 100°.

PLANT MATERIAL.—The stem bark and fruit of *M. tenuifolia* was collected in January 1983, on the University of Ife campus, Nigeria. The plant material was identified by Dr. Z.O. Gbile of the Forestry Research Institute of Nigeria. Herbarium specimens representing this collection are deposited in the Herbaria of the Forestry Research Institute of Nigeria, Ibadan and Botany Department, University of Ife, Nigeria.

EXTRACTION AND CHROMATOGRAPHIC SEPARATION.—The fresh stem bark of *M. tenuifolia* (270 g) was cut into small pieces and extracted exhaustively in a Soxhlet extractor with EtOAc. The EtOAc extract was concentrated in vacuo to give a red, oily residue (8.0 g). The dried marc was extracted exhaustively with MeOH. The MeOH extract was concentrated in vacuo at 40° to give a gummy residue (10 g).

The EtOAc extract (8.0 g) was adsorbed onto silica gel 60 (70-230 mesh) and chromatographed on a column of silica gel 60 slurry-packed in petroleum ether (40°-60°). The column was eluted stepwise with petroleum ether, increasing percentages (1,2,4,10, 50%) of EtOAc in petroleum ether, EtOAc, and, finally, MeOH (500 ml each).

3-DIMETHYLALLYLINDOLE.—Elution with 10% EtOAc in petroleum ether gave an oily fraction which contained a major component by tlc. This fraction (800 mg) was purified by column chromatography over neutral alumina (activity grade I, 40 g) slurry-packed with petroleum ether (60°-80°). Elution with 10% $Et_2O$ in petroleum ether gave a light yellow oil (500 mg) that was crystallized from *n*-hexane mp 45-47° [*lit.* mp 43-45° (4)] and shown to be pure by tlc and gc. 3-Dimethylallylindole was also obtained from the volatile oil of the pericarp of the fresh fruit (26 kg) by hydrodistillation, and crystallization of the oil from *n*-hexane, (524 mg), mp 45-46°. The uv, $^1$H-nmr and ms data were essentially the same as reported previously (4,5). For $^{13}$C-nmr (see Table 1).

To a stirred solution of 468 mg (0.004 mole) of indole in 30 ml of acetic buffer solution (HOAc-$H_2O$-NaOAc, 100 ml:20 ml:8 g) was added 596 mg (0.004 mole) of 3-methyl-but-2-enyl bromide under $N_2$ at room temperature. The solution was diluted with distilled $H_2O$ (100 ml) after stirring for 5 h, neutralized with saturated $NaHCO_3$ solution, and extracted with $Et_2O$ (100 ml×3). The combined $Et_2O$ extract was concentrated in vacuo to give 986 mg of a red oil which was chromatographed over silica gel (270-430 mesh, E. Merck, 40 gm) slurry packed with *n*-hexane. Elution with 10% EtOAc/*n*-hexane gave 85 mg of **1**, mp 44-46° [lit (5) mp 43-45°].

ANTIMICROBIAL ASSAY.—The MICs of 3-dimethylallylindole (**1**) against susceptible bacteria and fungi (based on qualitative assessment) were determined by two fold serial dilution as previously described (6,7).

## ACKNOWLEDGMENTS

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