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SHORT COMMUNICATION

ISOLATION AND CHARACTERIZATION OF 3-CARBOMETHOXYPYRIDINE FROM THE LEAVES OF PYRENACANTHA STAUDTII HUTCH AND DALZ (ICACINACEAE)

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Abstract: *Pyrenacantha staudtii* is a medicinal plant used widely in the West African sub-region particularly in Nigeria for the treatment of threatened abortion and dysmenorrhea. This study reports for the first time the chemical isolation and characterization of 3-carbomethoxypyridine from one of the active fractions of the methanolic extract of *Pyrenacantha staudtii* leaves.
The crude methanolic extract was subjected to accelerated gradient chromatography (AGC) to give various fractions. One of the active fractions was further subjected to a reversed phase lobar column chromatography to give the alkaloidal compound using methanol : water as solvent system. The structure was unequivocally determined by physical, chemical and spectral techniques.

Keywords: 3-carbomethoxypyridine, *Pyrenacantha staudtii*, alkaloid, isolation, characterization

Pyrenacantha staudtii is an annual herb found in the light tropical forest and farmland bushes. It is a woody climber. The older leaves bear greenish almost inconspicuous flowers (1,2). The leaves are intensively bitter and the aqueous extract is used for the treatment of dysmenorrhea, intestinal colic and threatened abortion (3-5). Recent reports showed that the leaves have anti-malarial activity (6) and that the aqueous extract of the leaves had a significant anti-ulcer effect in experimental animals (7, 8). Further research in our laboratory also revealed that the crude methanolic extract and some fractions inhibited the contraction of the isolated rat uterus (9).

This paper deals with the isolation and identification of one of the main alkaloidal bitter chemical constituent, 3-carbomethoxypyridine, from one of the pharmacologically active fractions. 3-Carbomethoxypyridine is also distributed in terrestrial plants as well as in marine invertebrates, and was found in algal source (10), and *Platymonas subcordifo* (11).

EXPERIMENTAL

Melting point was measured with a Koffler melting point apparatus and is uncorrected. The IR spectra were recorded on an ATI Mattson Genesis series FTIR spectrometer using KBr discs. The NMR spectra were recorded on a Varian Germini 200 spectrophotometer in DMSO. Chemical shift values are shown in ppm (δ) with tetramethylsilane (TMS) as internal standard. Mass spectra were acquired on a Varian MAT 44S mass spectrometer operating at 70 eV.

Extraction and isolation

Air dried leaves (300 g) were ground to a fine powder and extracted exhaustively by maceration at room temperature with MeOH for 48 h. Removal of the solvent under reduced pressure at 25°C yielded a greenish residue (35 g). The methanolic extract (27 g) was applied on a column of silica gel 230 - 400 mesh and subjected to accelerated column chromatography (AGC). Elution was done with 100% n-hexane followed by a mixture of $CHCl_3$/MeOH of increasing polarity (1:9, 2:8, 3:7, 4:6, 5;5, 6:4, 7:3, 8:2 and 9:1 v/v). Fractions were monitored by TLC and similar fractions were combined to afford fractions M_1 (0.956 g), M_2 (1.945 g), M_3 (0.256 g), and M_4 (2.174 g). Part of the fraction M_4 (1.074 g) was chromatographed on a column of reversed-phase C_{18} silica gel eluted with MeOH/H_2O (30 : 70, 40 : 60 and 50 : 50 v/v, each 200 mL) to yield compound 3-carbomethoxypyridine (690 mg). The plates (normal and reversed-phase) were visualized with ninhydrin and Dragendorff spray reagents, and UV lamp at 254 nm and 366 nm.The needle like crystals of the alkaloidal compound were subjected to various spectral techniques such as IR, ^{1}H-NMR, ^{13}C- NMR,

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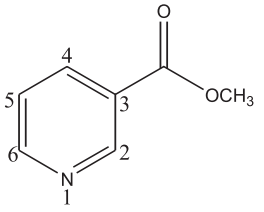
mass spectrometry and elemental analysis.

RESULTS AND DISCUSSION



3-carbomethoxypyridine

Compound: 3-carbomethoxypyridine (methyl nicotinate) (R_f 0.33, pale yellow to brown crystals) m.p. = 40 - 42°C. (Lit. mp. 42-43°C) (12,13).
IR (KBr cm-[1]: 1721 CO), 3551 (CH), **^{1}H-NMR** (DMSO-d_6) δ_H (ppm): 9.20 (s,H-2), 8.88 (1H, d, H-6),8.70 (1H, d, H-4), 8.00 (1H, t, H-5, *J* 6.2,7.9), 4.30 (s, OCH_3). **^{13}C-NMR** (DMSO-d_6) δ (ppm): 162.03 (COO), 145.85 (C_6), 144.47 (C_2),143.86 (C_4), 140.24 (C_3), 126.57 (C_5), 47.46 (CH_3). **MS** (m/z): 138 (M^+ +1), 137 (M^+) $C_7H_7NO_2$: 137.138.
Elemental analysis: Calcd.: C, 61.30%, H, 5.15%, N, 10.21%; found: C, 61.20% H, 5.04%, N, 10.34%.

The compound gave positive reactions with Lassaigne, ninhydrin and Dragendorff reagents indicating its nitrogenous nature. This was confirmed by the IR absorption bands for carbonyl (1721 cm^{-1}). The ^{13}C spectrum showed seven carbon atoms with the carbonyl group appearing at 162.03. The ^{1}HNMR spectrum of AD1 determined in deuterated DMSO, revealed the presence of an OCH_3 group as a singlet at δ 4.20. The assignment of this compound is obscured because it has two isolated carbons. After straightforward assignment of H2 and H4-6, we found very small H, H-couplings from H2 and H6 to the methyl group in the COSY spectrum. The position of carboxyl group was identified by long-range C, H correlations to H2 and H4. Homonuclear experiments are especially useful for sorting out proton spectra. One form includes the COSY (Correlation Spectroscopy), which is used to establish which nuclei (usually protons) are coupled to which. From these data, the structure of 3-carbomethoxypyridine was assigned to the compound. The structure elucidation of 3-carbomethoxypyridine was achieved by a combined interpretation of ^{1}H-NMR, ^{13}C-NMR and H, H-COSY measurements. The relative configuration of the C-atoms bearing the methyl group was determined by long range couplings from the H^+, H-COSY spectrum.

From the chemotaxonomic point of view this is the first report of the occurrence of 3-carbomethoxypyridine in a natural source from *Pyrenacantha staudtii* leaves.

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