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PHYTOCHEMICAL STUDIES ON *CAPSICUM FRUTESCENS*

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Ortho-hydroxy-N-benzyl-16-Methyl-11, 14-diene-octadecamide and 9, 12-diene-octadecanoic acid

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ABSTRACT

Two chemical compounds have been isolated and their structures were elucidated by spectroscopic studies including VLC, TLC and NMR. The isolated compounds are Ortho- hydroxy- N- benzyl- 16- Methyl- 11, 14- diene-octadecamide (1) and 9, 12-diene-octadecanoic acid (2).

INTRODUCTION: *Capsicum frutescens* (Family: Solanaceae) is an annual or short-lived perennial herb. It grows well all over in Bangladesh. It's local name is morich or lanka. Probably native of the tropics, but the original habitat is obscure. The stem of *Capsicum frutescens* almost striate, glabrous, highiet between 1-4 feet depending on climate and growing conditions. The leaves are elliptical, slightly leathery, dark green and smooth, and measure 2½ inches long and 1 inches wide.

The flowers are typically conical or funnel form with five petals, usually fused and color is white. The fruits are erect, ellipsoid-conical to lanceoloid, 10-20 mm long, 3-7 mm in diameter. These fruits have range in color from green when immature to purple, red, orange or yellow when ripe [1, 3]. A few compounds had been isolated from *C. frutescens* like ester, terpenoids, noncarotenoids, lipoxygenase derivatives, carbonyls, alcohols, hydrocarbons 8, capsaicin, dihydrocapsaicin [4], capsiconinoid [11], capsinoid [9]. Medicinally, *C. frutescens* has been used to treat diabetes mellitus by traditional healers in Jamaica [12], decrease fat intake [5], decrease plasma glucose level [2], as antifungal agents [10], as cosmetics & food supplement, effective treatment for psoriasis [7].

METHODS AND MATERIALS: Collection of plant materials: Two kilogram morich bought from Anondabazar, near Dhaka university campus in March, 2006. The fruit of *Capsicum frutescens* was identified by Professor Dr. Abul Hasan of the Department of Botany, University of Dhaka. The seeds of *C. frutescens* were separated manually from skin and dried in air. The weight of the seeds was 700 gram & seeds were ground by using a cyclotech grinding machine. A voucher specimen for this collection has been maintained in Bangladesh National Herbarium (DACB accession number 35444) Dhaka, Bangladesh.

Extraction of the powdered seeds: The powdered seeds extracted in a soxlet apparatus with petroleum ether (40°C- 60°C) solvent. At first the dried powder of the seeds taken in a number of precleaned cloth bags.

Each bag (150 g) placed in the soxhlet apparatus and then solvent (petroleum ether) added. This was subjected to extraction by refluxing for several hours until the material extract appeared light yellowish color. The extract was filtered and concentrated under reduced pressure using a rotary vacuum evaporator.

Analysis of the Concentrated Extract: The petroleum ether extract (40^oC- 60^oC) was examined by TLC. Its TLC examination showed (solvent system: 86% petroleum ether and 14% ethyl acetate) four distinct spots along with light yellow tailing. The mixture of the compound was separated by VLC using suitable solvent**.**

Fractionating the Extract: Fractions obtained by VLC technique: The effluent collected in 191 (each 25ml) test tubes. The effluent of each test tube was monitored by TLC over silica gel (G-60, PL254). The effluent of similar behavior were poured into a small conical flask and thus designated as PEE1, PEE2, PEE3, PEE4, PEE5, PEE6, and PEE7.

Analysis of the Designated Fractions: On concentration and keeping undisturbed at room temperature the fractions PEE$_{2,}$ PEE$_{3,}$ PEE$_{4,}$ PEE$_{5,}$ gave a light yellow gummy material (63mg) & then this gummy material washed with petroleum-ether solvent. It was dissolved in chloroform and spotted on the TLC plate. The plate developed in 86% petroleum-ether and 14% ethyl acetate. When the solvent front reached just one cm below the upper edge of the plate taken out from the chamber and dried at room temperature in regular air flow until solvent was evaporated from the plate. Then, the vanillin- sulphuric acid reagent was sprayed on the plate and the plate was heated at 106^oC for 10 minutes. One spot was observed and then fractions were concentrated by vacuum rotavapour to yield gummy mass (15 mg).

The fractions PEE$_{6,}$ PEE$_7$ gave white residue. It was collected and washed with water, petroleum-ether, and *n*-hexane respectively. This material also dissolved in chloroform and spotted on TLC plate (over silica gel). The plate was developed in 86% petroleum-ether and 14% ethyl acetate. When the solvent front reached just one cm below the upper edge of the plate, the plate was taken out from the chamber and dried at room temperature in regular air flow until solvent was evaporated from the plate then the vanillin - sulphuric reagent was sprayed on the plate and then plate was heated at 106^o for 5 min. One pink spot was observed at the middle on the plate. This fraction was concentrated by hot air blow to yield oily mass (amount: 7mg).

Properties of the Isolated Compounds:

Properties of Compound -1:

Physical state: Gummy; Solubility: Sparingly soluble in petroleum-ether but highly soluble in chloroform and ethyl acetate; R$_f$ value: 0.81 (solvent mixture: Petroleum-ether and ethylacetate).

Spectral Characteristics:

IR Spectral data (cm^{-1})**:** 3400 (N—H**),** 3000**,** (C-H attached to >C=C<), 1750 (-CONH$_2$), 1640 (>C=O), 1450-720(-CH$_2$-), 1100-1240 (C—O), 1360 [(CH$_3$)$_2$CH-]**.**

^{1}H NMR Spectrum (400MHz, CDCl3, TMS)**:** δ_H value (ppm) vs indication; (7.1-6.8 , substituted aromatic ring protons**),** 5.3(d, J=6Hz, olefenic protons), 4.3(dd, J=10Hz,-CH$_2$=CH$_2$-); 4.14(J=6Hz,-CH$_2$=CH$_{2-}$), 2.8 (t, -CH$_2$- next to >C=C<), 2.08(d, J=7Hz, -CH$_3$), 1.6 (s,-CH$_3$)$_,$ 0.092[br, (CH$_3$)$_2$CH-].

^{13}C spectrum: δ_c value (ppm) vs indication; 173(>C=O), 172 (olefenic carbon or aromatic carbon or both)**,** 86.45(quarternary carbon), 68.8-62(oxygenated carbon), 13.9-34.07 (>CH$_3$ or -CH$_2$-).

Properties of Compound -2:

Physical state: Oily, Solubility: Sparingly soluble in petroleum-ether but highly soluble in chloroform and ethyl acetate, R$_f$ value: 0.83 (solvent mixture: Petroleum-ether and ethyl acetate).

Spectral Characteristics:

IR Spectral data (cm^{-1})**:** 2900 ***(***N—H***)*,** 1700 (>C=O in -COOH)**,** 1650(>C=C<), 1450 ***(***-CH$_2$- or -CH$_3$).

^{1}H NMR Spectrum (400MHz, CDCl$_3$, TMS): δ_H value (ppm) vs. indication; 5.3(dd, J=6Hz, olefenic protons), 2.6(dd, J=10Hz, -CH$_2$-CH$_2$-next to >C=C<), 2.3(d, J=7Hz,-CH$_2$ attatched to -COOH), 1.2 -1.6 (s, CH$_2$-), 0.87(t**,**-CH$_3$ next to -CH$_{2-}$).

^{13}C spectrum: δ_c value (ppm) vs. indication; 127-130 (olefenic carbon), 25.5(-CH_2- joined two >C=C<), 27(-CH_2- adjacent to >C=C<), 33.8 (-CH_2-adjacent to >C=O), 14(-CH_3), 179 (C-O), 27-31 (-CH_2-).

RESULTS AND DISCUSSION:

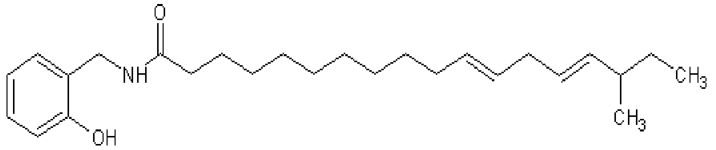
Spectral Characteristics of Compound -1:

IR spectrum: Its IR spectrum showed a sharp peak at 3400 cm^{-1} indicative the presence of -NH group. Another peak at 1750 cm^{-1} along with a band at 1640 cm^{-1} was indicative of the presence of -$CONH_2$ and >CO groups(R-CONH-).The band at 1450 cm^{-1} together with the band at 720 cm^{-1} was suggestive of the presence of -CH_2- group. The bands at 1100 cm$^{-1\ to}$ 1240 cm^{-1} were exhibitive of C-O stretching. The band at 1360 cm^{-1} along with a shoulder band 1340 cm^{-1} was indicative of the presence of isopropyl [-CH-$(CH_3)_2$] group. A sharp peak just above 3000 cm^{-1} was demonstrative of H-C=C- group i.e., C-H stretching attached to a >C=C< bond.

^{1}H NMR spectrum: Its ^{1}H NMR spectrum showed multiplexes within δ_H value**;** 7.13-6.8 indicative of the presence of substituted aromatic ring protons**,** 4.1-5.3 were indicative of the presence of vinyl hydrogen**,** 1.6 to 2.6 were indicative of the presence of allylic hydrogen i,e hydrogen attached to a carbon adjacent to double bond [13]. A broad singlet at δ_H value 1.2 was indicative of the methyl group**,** doublet at 2.07 was the indicative of -CH-CO-NH-. A 6H intensity broad singlet at 0.92 was indicative of [$(CH_3)_2$-CH-] group.

^{13}C spectrum**:** The ^{13}C spectrum and DEPT ^{13}C spectrum showed two >C=O carbons at 173 and 172. The δ_c at 128.1, 128.7, 127.8, 125 were indicative of aromatic carbons. A tiny signal at 86.45 showed the presence of quaternary carbon. The signals at 68.8 and 62 were indicative of oxygenated carbon. Moreover, the ^{13}C spectrum showed 18 signals within δ_c value 34.07, 13.97 were indicative of the presence of either -CH_3 or -CH_2- carbon or both types of carbons.

Inference: Based on the IR, **^{1}**H NMR and **^{13}**C NMR spectral data, the compound-1 has been proposed to have the following structure**.**



Compound-1: Ortho hydroxyl-N benzyl-16- methyl-11, 14- diene-octadecamide

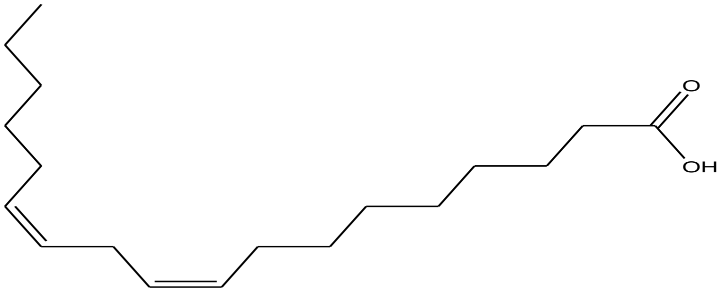
Spectral Characteristics of Compound -2:

IR spectrum: Its IR spectrum showed a sharp peak at 2900 cm^{-1} was indicative of the presence of C-H stretching. A peak at 1700 cm^{-1} was suggestive of >C=O stretching in -COOH group. Another peak at 1650 cm^{-1} was indicative of the presence of >C=C< group. A sharp peak at 1450 cm^{-1} was indicative of the presence of -CH_2- or -CH_3 group.

^{1}H NMR spectrum: Its ^{1}H NMR spectrum showed doublet at 5.3 indicative of the presence of olefenic protons. Another doublet of doublet at 2.6 was indicative of the presence of -CH_2- protons which joined two >C=C< moieties. A broad peak at 2.0 was suggestive of -CH_2- protons next to a >C=C< moiety. A triplet at 2.3 was indicative of the presence of -CH_2- protons attached to the -COOH group. Another triplet at 0.87 was suggestive of -CH_3 group next to a -CH_2- group. The peaks from 1.2-1.6 were indicative of the presence of -CH_2- protons.

^{13}C NMR spectrum: The ^{13} C spectrums and DEPT spectrum showed four olefenic carbons at 127.8-130.1. At 25.5 was indicative of the presence of -CH_2- carbon which joined two >C=C< moieties. A signal at 27 was suggestive of -CH_2- carbon adjacent to >C=C< group. Another signal at 14 was suggestive of terminal -CH_3 carbon. A tiny signal at 33.8 was indicative of the presence of -CH_2- carbon adjacent to >C=O group. Another tiny signal at 179 was suggestive of oxygenated carbon. Moreover, this ^{13}C spectrum showed -CH_2- carbon at 27-31.

Inference: Based on the IR, **^{1}**H NMR and **^{13}**C NMR spectral data, the compound-2 has been proposed to have the following structure.



Compound-2: 9, 12-diene-octadecanoic acid [6]

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CONCLUSION: The chemical study of the stem bark of *Capsicum frutescens* afforded two purified compounds Ortho hydroxy- N benzyl- 16- methyl- 11, 14- diene-octadecamide (1) and 9, 12-diene-octadecanoic acid (2) whose structures were established by extensive spectroscopic studies as well as comparison with published results.

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