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Chemical investigation of aliphatic compounds of Piper betle (leaf stalk)

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ABSTRACT

The hexane extract was separated by column chromatography using silica gel as adsorbent. The column was eluted by solvents in their increasing order of polarity. Compounds were isolated and purified by repeated silica gel, reverse phase silica gel, column chromatography and thin layer chromatography. Hexane fraction of Piper betle(leaf stalk) yields four aliphatic compounds in pure form i.e. Pentadecyl 6-hydroxytridecanoate(1), Pentatriacontanol, (2), Methyl hexacos-7-enoate (3) and 6, 9-heptacosa diene (4). Their structures were determined by spectroscopic and chemical methods.

Keywords: *Piper betle* (leaf stalk), IR, ^{1}HNMR, ^{13}CNMR, and Mass spectroscopy structure determination

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INTRODUCTION

Piper betle Linn. (local name 'Pan') Piperaceae, a dioecious, perennial creeper, climbing by many short adventitious rootless, widely cultivated in hotter and damper parts of the country is widespread in damp forests and is cultivated in India and other countries in South-East Asia, such as Vietnam and China. A concoction of indigenous Indian drugs containing *P.betel* dry extract was found to be an effective long-lasting oral contraceptive [1]. The flowers of this area used as ingredient for the chewing food know as betel quid in South-East Asia [2]. Mouthwashes and tablets containing pulverized betel nut were used for the treatment of dental and periodontal diseases [3]. Betel leaves were reported to have high antioxidant effects [4,5] antidiabetic [6] Radio protective [7] Antibacterial Effect [8], Pro-apoptotic effect [9].The leaves possess antibacterial properties and are beneficial in the treatment of purulent parodontosis in the form of a collutory made of the juice or extract. A poultice of the leaves and a wash with the decoction are used in treating wounds, burns, impetigo, furunculosis, eczema and lymphangitis. The leaves if topically applied to the chest cure cough and asthma and if applied to the breast arrest lactation. Friction of the spinal column with the leaves is recommended for treating colds. The roots (8 to 12 g) are used in treating rheumatism [10]. The essential oil of *P.betel* showed hypertensive, cardiac and respiratory depressant effects [11]. Hydroxychavicol[12] showed antidiabetic effect, Allylpyrocatechol showed gastric ulcer-healing action, Chavibetol showed

Anti-inflammatory effect, Piperbetol showed Photoprotective/radioprotective, Methylpiperbetol, Piperol A, Piperol B showed Platelet hyperactivity/cardiovascular effects. Eugenol was identified as antifungal principle in the oil [13].

Methyl undecanote, Dodecanoic acid, methyl ester (methyl laurate), Tridecanoic acid,methyl ester, Hexadecane (also called cetane), 1-Dodecanol (lauryl alcohol) [14] were isolated by the same plants. Two new ceramides, (2*S*,3*S*,4*R*)-2-*N*-[(2′*R*)-2′-hydroxypentacosanoylamino]-nonacosane-1,3,4-triol and (2*S*,3*S*,4*R*,8*E*)-2-*N*-[(2′*R*)-2′-hydroxytetracosanoylamino]-8-eicosylene-1,3,4-triol [15] were also reported.

MATERIALS AND METHODS

Plant material

The *P. betle* plant material was collected from Kolkata (West Bengal). The leaf stalk studied was collected from plants grown in Kolkata, West Bengal. A voucher specimen has been deposited at the Herbarium of Vikram University. The taxonomic identification of the plant material was obtained from the authorities of the Institute of Environment Management and Plant Sciences, Vikram University, Ujjain (M.P.) India.

T.L.C. of fingerprinting of plant material

T.L.C. of the alcoholic extract of *P. betle* plant material on Silica gel 'G' plate using Toluene: Ethylacetate (9 : 1, v/v) as mobile phase shows five spots at Rf. 0.11 (green), 0.18 (light green), 0.23 (yellow), 0.34 (grey) and 0.61 (greyish green).

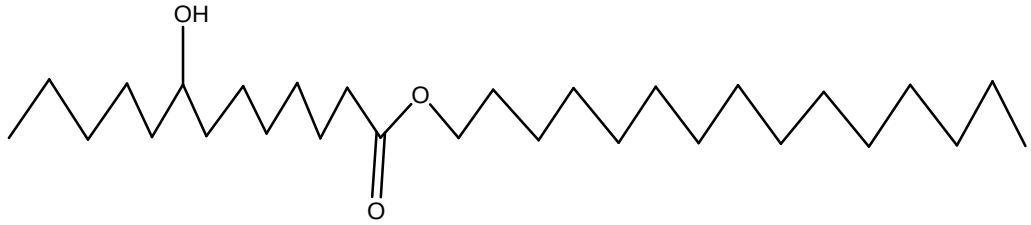
A. Under U.V. light (366 nm) seven fluorescent zones are visible at Rf. 0.11, 0.16 (both pink), 0.23 (brown), 0.34 (pink), 0.43 (pink), 0.61 (pink) and 0.76 (grey).
B. On exposure to Iodine vapour seven yellow spots appear at Rf.0.10, 0.12, 0.18, 0.34, 0.61, 0.76 and 0.88.
C. On spraying with Vanillin-Sulphuric acid reagent and heating the plate for ten minutes at 110°C seven spots appear at Rf. 0.08, 0.11, 0.18 (greenish grey), 0.34 (grey), 0.43 (violet), 0.61 and 0.76 (light green)[16]

Extraction

The air dried *P. betel* (leaf stalk) was Soxhlet extracted with n-hexane and fractionated on silica gel column. The column was eluted with different solvents in their increasing order of polarity.

Identification of the compounds

The hexane extract was analyzed by TLC, which revealed the presence of eight spots. To separate it the extract was subjected to column chromatography on silica gel as adsorbent. The column was eluted with different solvents in their increasing order of polarity. The column afforded five compounds in pure form designated as pentadecyl 6-hydroxytridecanoate, *pentatriacontanol*, methyl hexacos-7-enoate and 6, 9-heptacosa diene.



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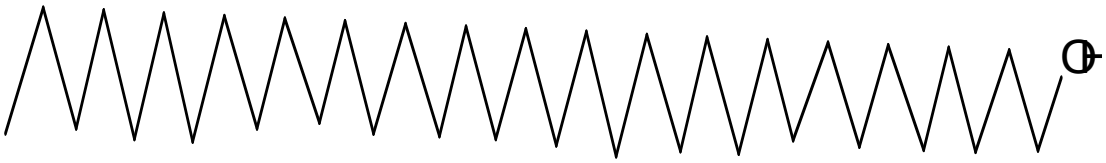

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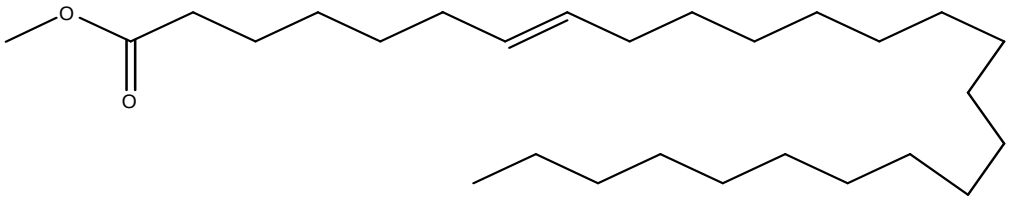

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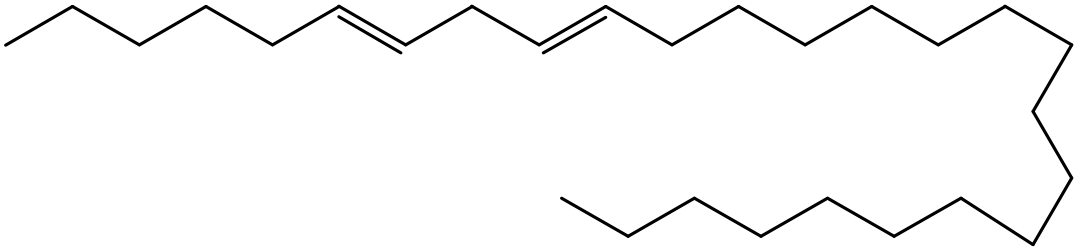

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Compound 1:

White crystal M^+ 440, mp $C_{28}H_{56}O_3$ IR spectrum showed absorption bands at 3480 cm $^{-1}$ and 1752 cm $^{-1}$showed the presence of hydroxyl group and ester group respectively. Bands at 2926, 1450 cm^{-1} were due to -CH stretching and bending vibrations [17,18], while bands at 1073, 1050 and 779-719 cm^{-1}diagnostic of (CH_2) n revealed the aliphatic long chain nature of the molecule. ^{1}H NMR showed a triplet at δ_H 0.88 for terminal methyl groups. While carbinolic proton resonated at δ_H 1.55 as multiplet. The rest of the methylene groups were merged into a single peak at δ_H 1.28 as singlet [19,20]. The carbonyl carbon of the ester resonated at δ_C 178.2 ppm. A bunch of peaks at δ_C 29.7 corresponds to the remaining methylene carbons. The peak at δ_C18.6 corresponds to the end methyl carbons rest of methylene groups resonated at δ_C 29.7 and 27.2 were assigned to the α and β of the methylene groups attached to the methyl group [18, 19, 21]

The separation of most of the peaks by 14 and 28 mass units and appearance of $C_nH_{2n+1}^+$ and $C_nH_{2n-1}^+$ ion series also confirmed its long chain aliphatic nature. The peaks at m/z 57 and 201 resulted from α cleavage and ester group respectively confirming the position of hydroxyl and ester group. Based on the above spectral evidences the compound 1 was characterized as **Pentadecyl 6-hydroxytridecanoate.**

Compound 2:

M^+ 508. M.F. $C_{35}H_{72}O$ IR spectrum, band at 3402 cm^{-1} indicating showed the presence of hydroxyl group in the molecule. Bands at 2926, 1450 cm^{-1} were due to -CH stretching and bending vibration, while bands at 1073, 1050 and 779-719 cm^{-1} revealed the aliphatic long chain nature of the molecule [18, 22].^{1}H NMR spectrum showed triplet at δ_H 0.82 for the three end protons while the methylene group α to the hydroxyl group resonated at δ_H 3.50 as triplet. A triplet at δ_H 3.71 was due to carbinolic proton [23]. The rest of the methylene protons merged in to a singlet at δ_H 1.26. The methylene protons β-to carbinolic group resonated at δ_H 1.56 with OH

proton. ^{13}C NMR spectrum a sharp peak at 63.5 ppm corresponds to the carbon attached to the OH group (CH_2OH). The peaks at 32.83 ppm and 31.89 ppm correspond to the methylene carbons attached at α and β positions of the primary alcoholic group. A bunch of peaks at δ_C 29.7ppm corresponds to the remaining methylene carbons. The peak at 18.6ppm corresponds to the end methyl carbon. The peaks at δ_C 22.63 and 25.73 ppm were assigned to α and β of the methylene groups attached to the methyl group [19, 20].

The EIMS showed the molecular ion peak at m/z 508. The mass fragmentation was characteristic of long chain hydrocarbon [22].

Based on the above spectral evidences the compound 2 was characterized as *pentatriacontanol.*

Acetylation:

Compound (10 mg), Ac_2O and C_5H_5N (2.0 ml each) was allowed to stand over night at room temperature. On usual work-up, the mixture afforded white crystals of acetylated $AOHC_6$-4 (7mg), m.p. 200^0C. IR: 1742, 1240 etc.

Compound 3:

M^+ 408, M.F. $C_{27}H_{52}O_2$.IR spectrum showed an intense band at 1737 cm^{-1} confirmed the presence of ester group. An olefinic bond showed weak absorption band at 1635 cm^{-1} indicating the unsaturation in the compound.

^1H NMR spectrum showed triplet at δ_H 0.88 for six protons of terminal methyl groups. Methoxy proton resonated at δ_H 3.65 as a singlet. The olefinic protons resonated at δ_H 5.02 as triplet. The methylene protons β to ester group and α to double bond resonated at δ 1.58 as weak multiplet. The rest of the methylene protons were displayed at δ_H 1.25 as intense sharp signal [17, 19]. ^{13}C NMR spectrum a sharp peak at δ_C 174.6 for ester carbon, the peak at δ_C 65.2 for the methoxyl carbon and unsaturated carbon resonated at δ_C112.0. Peak at δ_C 38.5 correspond to the methylene carbons attached at the α position of the ester group. A bunch of peaks at δ_C 29.3 corresponds to the remaining methylene carbons. The peak at 18.6 corresponds to the end methyl carbon [17, 19].

The EIMS showed the mass fragmentation was characteristic of long chain unsaturated ester. The peaks at m/z 74 resulted from Mc Lafferty rearrangement respectively confirming the position of ester group. An abundant peak at m/z 278 formed by α- cleavage indicated the position of double bond. The separation of most of the peaks by 14 and 28 mass units confirmed its long chain aliphatic nature.

Based on the above spectral evidences the compound 3 was characterized as **methyl hexacos-7-enoate**.

Compound 4:

M^+ 376, M.F. $C_{27}H_{52}$.IR spectrum showed absorption bands at 1637, 1617 cm^{-1} indicating the unsaturation in the compound. Band at 730-720 cm^{-1} was due to long chain aliphatic nature of the molecule. ^1H NMR spectrum showed six proton triplet at δ_H 0.88 for terminal methyl groups. The olefinic protons resonated at δ_H 4.8 as triplet. The methylene groups between two double bonds were displayed at δ_H 2.16, while methylene groups adjacent to olefinic protons resonated at δ_H 1.62 [17, 19].

^{13}C NMR showed peak at δ_C 120.1 was due to double bonds. A bunch of peaks at δ_C 29.3 corresponds to the remaining methylene carbons. The peak at δ_C 18.6 corresponds to the end methyl carbons [17,19,21]. The peak at δ_C 39.47 was due to methylene carbon between two double bonds.The FAB Mass Spectrum showed the molecular ion peak at m/z 399 suggesting its molecular formula as $C_{27}H_{52}Na$. The separation of most of the peaks by 14 and or 28 mass units confirmed its long chain aliphatic nature.

Based on the above spectral evidences the compound 4 was characterized as **6, 9-heptacosa diene**.

Experimental

M.P.s are uncorrected. The I.R. spectra were recorded on Perkin-Elmer grating 377 I.R. spectrometer in KBr phase (range 4000-400 cm^{-1}). 1HNMR spectra were recorded on varian DRX 200 MHz and 400 MHz spectrometer, using TMS as an internal standard and $CDCl_3$ as a solvent. In column chromatography alumina Brochmann Gr.III was used. It was made grade III by the addition 7% of distilled water and mixing thoroughly. Silica gel (Qualigens (Glaxo) and ACME 60-120 mesh) was used. The 2D NMR spectra were recorded on varian DRX 400 MHz spectrometer.

RESULTS AND DISCUSSION

The 3kg leaf stalks were grinded in mechanical stirrer and squeezed to remove water. The squeezed leaf stalk were dried and extracted with n- hexane in hot condition. The extract was dried in vacuum and subjected to TLC analysis. TLC showed the presence of four compounds and hence it was put on silica gel column. The hexane eluate yielded four compounds in pure form after rechromatography from different fractions.

M+ 440, $C_{28}H_{56}O_3$. (Hexane: Benzene 4:6, 15 mg) m.p.123-127 º C,
Isolated from Hexane fraction of Hexane: Benzene eluate of the column.
TLC solvent system Hexane: Benzene: Ether (7.5:2:0.5, v/v/v, Rf 0.84)
I.R. (KBr):3480(OH), 2926, 1752, 1450, 1073, 1050 and 779-719 cm^{-1}
^{1}HNMR(400MHz, $CDCl_3$,TMS) 0.88 (t, 6H,2X -CH_3), 1.55 (m, 1H-CH), 4.09 (t, 2H-CH_2OCO), 2.30 (t, 2 CH_2, CH_2COO), 1.6 (br, 8H, 4 CH_2) and 1.28 (s,36H $(CH_2)_{18}$)

EIMS (m/z, rel, int) m/z 440(6.0), 425(9.0), 411(12.0), 409(15.5), 397(14.8), 383(16.5), 366(21.0), 355(25.0), 341(15.0), 327(19.5), 313(22.5), 299(30.5), 285(24.85), 271(15.2), 267(18.0), 257(22.7), 243(23.2), 229(26.8), 225(29.3), 215(28.5), 201(25.1), 197(34.0), 187(23.0), 183(33.0), 169(22.0), 155(23.0), 141(18.0), 129(48.1), 115(47.0), 113(30.0), 99(34.5), 85(27.7), 71(30.1), 59(41.0), 57(100), 45(39.0), 43(13.0), 31(69.9), 39(40.5) and 15(34.0).

^{13}C NMR 75 MHz, $CDCl_3$, TMS) 63.5, 38.5, 32.2, 29.7, 18.6, 29.7, 27.2 and178.2 ppm
M+ 408, $C_{27}H_{52}O$
Benzene: EtOAc (8:2, 17 mg) M.P., 234º C,
Isolated from hexane fraction of Benzene: EtOAc eluate
TLC solvent system,Hexane: Benzene: Acetic acid (6.5:3.5:0.5, v/v)State White powder
I.R. (KBr) 3402, 2926, 2849, 1450, 1073, 1050, 780-719 cm^{-1}.

^{1}H NMR (300MHz,$CDCl_3$,TMS) 0.88(t, 3H, -CH_3, *J*= 7.5 Hz), 3.65 (t, 3H-OCH_3), 5.02 (t,2H, -CH=CH-), 1.58 (m, 6H, 3 CH_2) 1.25 (s, 3H, 19 CH_2).

EIMS (m/z, rel, int) m/z; 410(3.0), 4.09(4.0), 408(6.0), 379(9.5), 369(12.5), 355(9.5),349(18.0),321(12.5),307(22.0),293(25.5), 278(19.5),253(24.0),239(29.5),225(34.0),211(29.0),197(24.0), 83(36.5) 174(24.0), 169(34.0), 155(43.5), 141(21.0), 115 (39.0), 101(24.5), 94(44.0), 77(6.0), 57(45.0), 45 (10.0), 35(15.29), 31(29.3).

^{13}C NMR (75MHz, $CDCl_3$, TMS) 63.2, 38.9, 29.7, 22.63 and 25.73 ppm
M+408, $C_{35}H_{72}O$, Benzene: EtOAc (8:2, 21mg) eluate m.p. 267º C isolated from hexane fraction of benzene: EtOAc eluate of the column.

TLC solvent system Hexane: Benzene (4: 3, v/v)
I.R. (KBr) 3402, 2926, 1450, 1073, 1050 and 779-719 cm-1
1H NMR (400 MHz, DCl3,TMS) 0.82(t, 3H, -CH3, J=6.03 Hz), 3.71(t, 2H- CH_2OH) J=8 Hz), 1.56 (m,2H, -CH_2-CH_2OH) 1.26(s, n CH2).

EIMS (m/z, rel, int)m/z: 508(2.0), 507(3.5), 475(11.0), 463(10.0), 449(8.0), 439(6.5), 435(8.0), 415(7.0), 407(16.5), 393(19.0), 379(18.0), 351(27.5), 327(29.0), 309(21.0), 295(24.5), 281(24.5), 253(26.0), 239(27.0), 225(29.0), 197(23.5), 183(26.5), 169(37.0), 155(36.0), 145(40.0), 141(37.5), 123(27.0), 99(34.0), 73(3.0), 57(100.0), 31(57.0), 29(69.5) and 15(69.5).

13C NMR 63.5, 32.83, 31.89, 29.7, 22.63 and 25.73 ppm
M+ 376, $C27H_{52}$ (Hexane: Benzene 6:4, 19 mg)
M.P., 187º C, isolated from hexane fraction of hexane: benzene: eluate of the column.
TLC solvent system, Hexane: Benzene: Acetic Acid (4:6:0.4, Rf 0.41)
I.R. (KBr): 2920, 2850.1637, 1439, 1384, 1055, 759 and 730-720cm^{-1}
1HNMR (300MHz, CDCl3, TMS) 0.88 (t, 6H, 2-CH3, J=6.3 Hz), 4.8 (t, 4H, 2 CH=CH-), 2.16 (d, 2 H, CH_2-CH=CH-), 1.62 (d, 4H, 2 CH_2) 1.30 (s 36 H, 18 CH_2)
FABMS (m/z, rel, int)
M^+399 (M+Na) (11.11), 334(11.84), 316(11.38), 304(16.45), 290(11.98), 276(13.36), 262(14.13), 248(15.06), 159(15.10) 150(27.37), 136(31.05), 119(5.01) 122(50.06), 110(58.77), 96(80.55), 86 (97.83), 72(100.00) 58(97.22).
13C NMR (75 MHz, $CDCl_3$, TMS) 120.01, 18.6, 39.47,34.4,29.3.

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