Novel Lipid Constituents from the Leaves of *Psidium guajava* L. (leaves)

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ABSTRACT

Psidium guajava belongs to family myrtaceae. In Vedic literature its name has been given as "amrude" (amar and udar), which means a kind of *amrit* in all stomach trouble. A lot of scientific literature is also available in which extract of its leaves was used as astringent in diarrhoea. The powdered leaves are applied in rheumatism and extract is used in epilepsy. Here, we report three new aliphatic compounds from bezene and hexane fraction of ethanol extract. Hexaeicosan-16-ol, Pentatetracosan-10, 25-diol and Untricontan-11, 19-ol were the identified by spectral (IR, 1H NMR spectra, mass spectrum) and chemical analysis. These are novel compounds and being reported first time by us.

INTRODUCTION

Psidium guajava L. of family myrtaceae, commonly known as guava, is a native plant of tropical America[1]. Different parts of the same plant possess different biological activities and thus used in the indigenous system of medicine for the treatment of various human ailments such as wounds, Ulcers, cholera, etc.[2]. Pharmacological investigations indicated that its bark, fruits and leaves possess hypoglycemic, anti-inflammatory, analgesic, antipyretic, spasmolytic, and CNS depressant activities[3]. The fruit contains a high percentage of vitamin C, carotene, vitamin B_1, B_2, B_6, free sugars (glucose, fructose and sucrose), pectin and water soluble arabanose[1]. Root bark is an astringent and given to children in diarrhoea and root preparation with fruit is considered to be useful in jaundice[4]. Guava consumption has been reported to significant, reduce serum total cholesterol, triglycerides and blood pressure and help in the increase high-density lipoprotein (HDL) or good cholesterol[5,6]. Furthermore high concentration of pectin in guava fruit may play a significant role in reduction of cholesterol and thereby decreases the risk of cardiovascular diseases[7]. In traditional practice, guavas

leaves are still employed as a natural medicine. Locally decoction of the leaves is an efficacious gargle for the swollen gums, ulceration of the mouth and in scurvy. In cholera, for arresting vomiting and diarrheoic symptoms the decoction of the leaves is used. Also applied commonly in rheumatism, epilepsy, cerebral affection and nephritis. Ground leaves are used as poultice. The plant is used for prevention and treatment of scurvy in Asia and Africa. Guava juice has been reported to possess hypoglycemic activity in both mice and humans (Cheng and Yang, 1983). In addition, guava leaves have been used to treat many ailments including cough and pulmonary diseases in Bolivia and Egypt. Young guava leaves are used in India as a remedy against cough. People in China use guava leaves as an anti-inflammatory and haemostatic agent. A decoction of the leaves is used for the treatment of cholera[8].

2. EXPERIMENTAL

2.1. General procedures

Melting points (mp) are uncorrected. 1H NMR was recorded on 300 MHz Varian XL spectrometer, ^{13}C NMR spectra were recorded on Varian XL 75 MHz spectrometer, IR spectra were recorded in KBr disk on Perkin Elmer-377 spectrometer, EIMS on Jeol-JMS D 300 mass spectrometer. All chemical shifts (d) are given in ppm and Me4Si was used as internal standard. The carbon type (CH_3, CH_2, and CH) was determined by DEPT experiments. Chemicals are of analytical-reagent grade and column chromatography was carried out on alumina grade III and TLC on silica gel G (CDH/Glaxo laboratories). Spots were visualized by exposure to iodine vapor or by spraying with H_2SO_4-vanillin solution followed by heating at 105 °C for 5 min.

2.2. Plant material

The leaves (8 kg) of Psidium guajava Linn were collected from the gardens of Ujjain city and University campus and were identified by the authorities of the Institute of Environment Management and Plant Science, Vikram University, Ujjain. A voucher specimen was deposited in the herbarium of the School of Studies in Botany, Vikram University, Ujjain, India.

2.3. Extraction and isolation

The leaves (6 kg) were shade dried, cleaned, coarsely powdered and extracted with n-hexane, benzene, benzene: acetone and ethanol in soxhlet-extractor for 72 h. Removal of solvent under reduced pressure afforded solid extracts. The yield of ethanol extract was quite good it was taken for the isolation of active aliphatic compounds by repeated column chromatography. The three aliphatic compounds are isolated from hexane and benzene fraction of ethanolic extract by repeated column chromatography on silica gel. The column was eluted by gradient elution in increasing order of polarity like hexane, benzene was eluted by gradient elution in increasing order of polarity like hexane, benzene, EtOAc and methanol. Benzene fraction was rechromatographed on silica on the basis of increasing order of polarity

of eluents. The column was successively eluted with the hexane, benzene, EtOAc and methanol and their mixtures of increasing polarity. Fractions (a and b) (hexane: benzene v/v 6:4 and hexane: benzene v/v 2:3) were purified and identified as Hexaeicosan-16-ol and Pentatetracosan-10, 25-diol. The compound was analyzed by IR, ^{1}H NMR and mass spectrometry with the literature data. Hexane fraction was rechromatographed on silica on the basis of increasing order of polarity of eluents. The column was successively eluted with the hexane, benzene, EtOAc and methanol and their mixtures of increasing polarity. Fractions (c) (hexane: benzene v/v 9:1) were purified and identified as Untricontan-11, 19-ol. The compound was analyzed by IR, ^{1}H NMR and mass spectrometry. Fraction (c) yielded alcoholic compound 3 which was in crystal form.
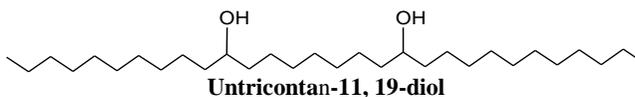
1.

OH

Hexaeicosan-11-ol

2.

OH

OH

Pentatetracosan-10, 25-diol

3.

OH   OH

Untricontan**-11, 19-diol**
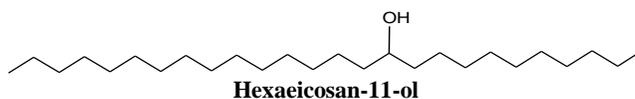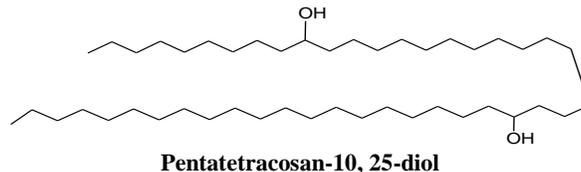
2.4. Compound 1: Hexaeicosan-16-ol (1) EIMS m/z (% intensity) 382 [M+] 382(13.0), 381(52.0), 365(34.5), 347(35.0), 333(22.0), 319(20.1), 301(42.0), 289(54.0), 275(63.0), 261(20.0), 245(24.0), 242(43.2), 231(83.1), 217(65.0), 203(26.5). $C_{26}H_{54}O$ (30 mg, CDCl$_3$) m.p. 167 °C, isolated from hexane: benzene (7:3, v/v) fraction, TLC hexane:benzene (8:2 v/v) as solvent system, it showed single clear spot. IR (KBr) tmax: 3454, 2917, 2849, 1463, 1120, 1020 and 730-719cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$, TMS): 0.88 (t, 6H, -2CH$_3$, J=7.5 Hz), 3.61 (m, 1H, -CH, J=6.9Hz), 1.56 (s, 1H, -OH), 1.25 (s, 46H, -23CH$_2$).

2.5. Compound 2: Pentatetracosan-10, 25-diol (2) EIMS m/z (% intensity) 664 [M+] 664(8.2), 529(5.2), 485(9.0), 437(19.0), 413(44.2), 385(18.3), 343(26.0), 311(12.0), 301(20.0), 289(13.0), 247(24.5), 219(59.0), 202(100.0), 161(12.3), 159(70.1), 149(30.0), 132(20.0). $C_{45}H_{92}O_2$ (25 mg, CDCl3) m.p. 194 °C, isolated from hexane: benzene (7:3, v/v) fraction, TLC hexane: benzene (2:8 v/v) as solvent system, it showed single clear spot. IR (KBr) tmax: 3454, 2916, 2849, 1455, 1378, 1122, 1018 and 729- 719 cm^{-1}. ^{1}HNMR (300 MHz, CDCl$_3$, TMS) : 0.88 (t, 6H, -2CH$_3$, J=7.5 Hz), 1.56 (s, 1H, OH), 3.66 (m, 1H, -CH J=6.5HZ), 1.25 (s, 82H, - 41CH$_2$).

2.6. Compound 3: Untricontan-11, 19-ol (3) EIMS m/z (% intensity) 454 [M+] 454(8.2), 487(7.8), 415(12.0), 391(11.9), 375(21.0), 353(9.0), 331(13.2), 317(16.0), 316(31.0),

311(19.0), 301(36.0), 289(60.0), 279(30.2), 261(10.0), 247(18.7), 231(25.0), 221(75.0), 202()72.0, 187(38.0), 175(55.0), 172(76.2), 160(42.0), 148(77.0), 134(52.3), 126(63.0), 118(100.0), 116(87.2), 113(19.6.). $C_{30}H_{62}O_2$ (30 mg, $CDCl_3$), m.p. 210°C isolated from hexane: benzene (9:1, v/v) fraction, TLC Benznene:ether (9.5:0.5, v/v) as solvent system, it showed single clear spot. IR (KBr) tmax: 3433, 2917, 2849, 1473, 1463, 1362, and 730-719cm^{-1}. ^{1}HNMR (300 MHz, $CDCl_3$, TMS) : 0.88 (t, 6H, J = 7.5 Hz, 2CH$_3$), 3.69 (s, 1H, -CH, J = 6.9 Hz), 1.54 (s, 1H, -OH), 1.25 (s, 54H, -26CH$_2$).

3. RESULT AND DISCUSSION

The novel aliphatic compounds were identified mainly by their IR, ^{1}H NMR, and Mass spectrometry analysis including a comparison with the literature data. The mass spectrum of Hexaeicosan-11-ol 1 indicated the molecular ion peak at m/z 382 suggesting its molecular formula $C_{26}H_{54}O$. IR spectrum showed a band at 3454 cm^{-1} for the presence of hydroxyl group in the molecule. Bands at 2917, 2849 and 1463 cm^{-1} were due to –CH stretching and bending vibrations, where as bands at 1020 and 730-719 cm^{-1} revealed the long chain aliphatic nature of the molecule. Thus the IR spectrum indicated the compound may be a long chain aliphatic alcohol[9-12]. ^{1}HNMR spectrum showed triplet (J=7.5Hz) at δ 0.88 for the six protons of terminal methyl groups. The hydroxyl proton resonated at δ 1.56 as a broad singlet. A multiplet at δ 3.61 was due to carbinolic proton. The rest of the methylene protons merged into a singlet at δ 1.25[13,14]. Thus on the basis of the above evidences the compound is identified as Hexaeicosan-11-ol, it is a novel compound and being reported first time by us.

The mass spectral analysis of diol compound gave the molecular formula as $C_{45}H_{92}O_2$. IR spectrum showed bands at 3454 cm^{-1} for the presence of hydroxyl group. Bands at 2916, 2849 and 1455 cm^{-1} was due to –CH stretching and bending vibrations, where as bands at 1018 and 729-719 cm^{-1} revealed the long chain aliphatic nature of the molecule. Thus the IR spectrum indicated the compound may be a long chain aliphatic alcohol[9-12]. ^{1}HNMR spectrum showed triplet (J=7.5Hz) at δ 0.88 for the six protons of terminal methyl groups. The hydroxyl protons resonated at δ 1.56 as a broad singlet. A multiplet at δ 3.66 was due to carbinolic protons. The rest of the methylene protons merged into a singlet at δ 1.25 [13,14]. The mass spectrum showed the molecular ion peak at m/z 664 suggesting its molecular formula as $C_{45}H_{92}O_2$. A gradual decreases in the intensity of the peaks with the increasing molecular mass is the characteristic pattern of long chain hydrocarbon. The formation of abundant fragments at m/z 157 and formed by α cleavage indicated the position of hydroxyl groups at C-10 and C-25. The other abundant fragments at m/z 619, 557, 481, 439, 413, 385, 311, 202, 159 and 113 were characteristic nature of the long chain aliphatic alcohol[15,16]. Thus on the basis of the above evidences the compound is identified as Pentatetracosan-10, 25-diol., it is a novel compound and being reported first time by us.

The molecular formula of naturally isolated Untricontan-11, 19-ol 5 was $C_{30}H_{62}O_2$. IR absorption bands showed band at 3433 cm^{-1} for the presence of hydroxyl group. Bands at 2917, 2849 and 1463 cm^{-1} was due to –CH stretching and bending vibrations, where as bands at

1020, and 730-719 cm^{-1} revealed the long chain aliphatic nature of the molecule[16]. Thus the IR spectrum indicated the compound may be a long chain aliphatic alcohol[18]. ^{1}HNMR spectrum showed triplet (J=7.5Hz) at δ 0.88 for the six protons of terminal methyl groups. The hydroxyl protons resonated at δ 1.56 as a broad singlet. A multiplet at δ 3.64 was due to carbinolic protons. The rest of the methylene protons merged into a singlet at δ 1.25 [19,20]. The mass spectrum showed the molecular ion peak at m/z 454 suggesting its molecular formula as $C_{30}H_{62}O_2$. A gradual decrease in the intensity of the peaks with the increasing molecular mass is the characteristic pattern of long chain hydrocarbon. The formation of abundant fragments at m/z 172 by α cleavage indicated the position of hydroxyl group. The other abundant fragments at m/z 439, 289, 231, 202, 187, 172 and 148 were characteristic nature of the long chain aliphatic alcohol. Based on the above spectral evidences the compound 3was characterized as Untricontan-11, 19-ol and being reported for the first time by us.

4. CONCLUSION

From the survey of the literature to the best of our knowledge all the three compounds were novel and being reported first time by us from A. odoratissimus (leaves) and further examination of the constituents of this plant is currently in progress.

5. ACKNOWLEDGEMENTS

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