ISOLATION OF SALICYLATE AND THREE OTHER NEW COMPOUNDS FROM UNRIPE FRUITS OF AEGLE MARMELOS CORR.

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ABSTRACT

Four novel compounds one salicylate, two esters and one acid isolated from Aegle marmelos Corr. (bael) unripe fruits. On the basis of spectral data, their structures were characterized and identified as Isophytyl salicylate, n-Decanyl godoleate (2), n-Docosanyl oleate (3) and n-Dotriacontanoic acid.

Keywords: Aegle marmelos, Isophytyl salicylate, n-Decanyl godoleate (2), n-Docosanyl oleate (3) and n-Dotriacontanoic acid.

Graphical Abstract

Isophytyl salicylate, n-Decanyl godoleate (2), n-Docosanyl oleate (3) and n-Dotriacontanoic acid isolated for the first time from Aegle marmelos Corr. (bael) Unripe fruits.

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INTRODUCTION

*Aegle marmelos* (L.) (Rutaceae) commonly known as bael or koovalam (Malyalam, India) growing wildly throughout deciduous forest of India, ascending to an altitude of 1,200 m in western Himalayas and also occurring in Andaman Islands. The fruits and leaves are valued in indigenous medicine [1]. The plant has been employed for long time in folk therapy [2]. Fruits, leaves, stem and roots of this tree at all stages of maturity are used as ethnomedicines against various ailments [3].

Extensive chemical investigations on various parts of the tree have been carried out and more than 100 compounds have been isolated [4, 5]. Many of these compounds including skimmianine, aegelin, lupeol, cineole, citral, citronellal, cuminumaldehyde, eugenol, marmesinin, marmelosin, luvangetin, aurapten, psoralen, marmelide, fagarine, marmin and tannins have been proved to be biologically active against various major and minor diseases including cancer, malaria, ulcers, diabetes, and gastroduodenal disorders [6-9]. It is therefore of interest to investigate the presence of new phytoconstituents in *Aegle marmelos* fruits. Therefore, the objective of our study describes the isolation and characterization of four new compounds, one salicylate, two esters and an acid.

EXPERIMENTAL

**Plant materials:** The fruits were collected from the local market of Delhi. The drug was authentified as *Aegle marmelos* (L.) Correa ex Roxb. by Dr. H.B. Singh (Taxonomist), National Institute of Science Communication and Information Resources, NISCAIR, New Delhi. The reference no is NISCAIR/RHMD/Consult/-2010-11/1509/107. A voucher specimen is preserved in the NISCAIR department of New Delhi.

**Chemicals:** All the solvents (petroleum ether 60-80, ethyl acetate and methanol) used in extraction and isolation were of analytical grade. The silica gel used in column for isolation was of 60-120 mesh (Lobochemie) and the silica gel G used used in Thin Layer Chromatography (TLC) was purchased from CDH Laboratory Reagents.
**Extraction:** Air dried fruits were size reduced and 1.5kg of powdered drug was subjected to hot continuous extraction in a soxhlet apparatus with methanol for 8 hrs. The extract was concentrated to half of its volume with a rotary evaporator under a reduced pressure at 30°C. The concentrated methanolic extract was then treated with petroleum ether using separating funnel.

The methanolic extract was poured in the separating funnel and then sufficient amount of petroleum ether was added to it and the mixture was shaken vigorously in a circular motion for a minute so as to separate the petroleum ether soluble components. The procedure was repeated until full methanolic concentrated extract was free of petroleum ether soluble components. The petroleum ether soluble extract was then concentrated to dryness for its final use in separation through column chromatography.

**Preparation of Petroleum Ether Extract for Column:** The petroleum ether extract (15g) was taken in a china dish and minimum quantity of activated (heated for 30 min at 110°C in hot air oven) silica gel (60-120#) was added to it to get uniform consistency. It was air dried and larger lumps were broken to get uniform particle size.

**Fractionation and isolation:** The petroleum ether extract (15 g) was subjected to column chromatography on a silica gel column (100 × 2 cm, 60-120 meshes) and then was equilibrated with petroleum ether. Elution was performed sequentially with petroleum ether and ethyl acetate in a ratio of 100% petroleum ether, Petroleum ether : Ethyl acetate (99 : 1); Petroleum ether : Ethyl acetate (98 : 2); Petroleum ether : Ethyl acetate (96 : 4). Fractions of 15ml each were collected and subjected to TLC analysis. The TLC analysis was performed on precoated silica gel G plates (2mm thickness, self made) with petroleum ether : ethyl acetate (4 : 6) and spots were detected by ultraviolet illumination (254 and 365mm) and iodine stream. Then the effluents of similar composition were combined into fractions A, B, C and D. All the fractions were evaporated under reduced pressure at a room temperature to remove the solvent to give a percentage yield of 20%, 1.66%, 39.33% and 1.2% respectively.

**Molecular weight estimation:** A MS system (micrOTOF-Q II 10262), equipped with a Hewlett-Packard 9000 computer system, was used to determine the molecular weight of each compound. Mass spectra were recorded at a heat capillary voltage of 4500V and a temperature of 180°C. The scan range of m/z was 60-3000.
**Determination of melting point:** Melting points were recorded with a digital electrothermal melting point apparatus (Electrothermal 9100, Electrothermal Engineering Ltd.).

**NMR Identification:** $^1$H and $^{13}$C NMR spectra were recorded with a Bruker AVANCE spectrometer 300Mz. Coupling constants are expressed in hertz, and chemical shifts are given on a δ (ppm) scale with DMSO (dimethylsulphoxide) or solvent signal as an internal standard.

**Determination of absorption maximum:** The absorption maxima i.e. $\lambda_{\text{max}}$ of the compounds were taken with Ultra Violet Spectrophotometer (Shimadzu), equipped with computer aided software UV Probe 1700. The wavelength range was set between 250 nm to 400 nm. The measuring mode was the absorbance and the slit width was 1.0 nm.

**RESULTS**

**Compound A (Isophytyl salicylate)**

Compound A was obtained as a translucent semisolid material. Its molecular formula of C_{27}H_{44}O_{2} was established on the basis of the mass spectrum ([M$^+$ m/z 400]). The compound had R_f value of 0.81 and a melting point of 30°C. The $\lambda_{\text{max}}$ was 285 nm.

**IR $\nu_{\text{max}}$(KBr):** 3127, 2925, 2854, 1746, 1654, 1462, 1400, 1377, 1163, 1118, 722 cm$^{-1}$.

$^1$H NMR (CDCl$_3$): δ 7.51 (1H, m, H-3’), 7.29 (1H, m, H-6’), 7.18 (1H, m, H-4’), 6.96 (1H, m, H-3’), 5.26 (1H, m, H-15), 4.93 (1H, brs, H$_2$-16a), 4.80 (1H, brs, H$_2$-16b), 2.26 (1H, m, H-14), 1.93 (2H, m, CH$_2$), 1.86 (2H, m, CH$_2$), 1.84 (1H, m, H-6), 1.82 (1H, m, H-10), 1.71 (2H, m, CH$_2$), 1.61 (6H, m, 3 × CH$_2$), 1.71 (4H, m, 2 × CH$_2$), 1.39 (6H, brs, Me-1, Me-17), 1.24 (3H, m, Me-18), 1.16 (2H, m, CH$_2$), 0.95 (3H, d, J=6.6 Hz, Me-19), 0.85 (3H, d, J=6.9 Hz, Me-20).

$^{13}$C NMR (CDCl$_3$) δc: δ 19.85 (C-1), 85.68 (C-2), 31.40 (C-3), 30.94 (C-4), 29.25 (C-5), 33.07 (C-6), 29.06 (C-7), 28.59 (C-8), 26.56 (C-9), 3.91 (C-10), 26.32 (C-11), 24.25 (C-12), 24.76 (C-13), 34.17 (C-14), 143.96 (C-15), 105.46 (C-16), 20.58 (C-17), 13.93 (C-18), 13.37 (C-19), 11.22 (C-20), 152.36 (C-1’), 163.39 (C-2’), 128.90 (C-3’), 114.06 (C-4’), 124.25 (C-5’), 128.33 (C-6’), 171.15 (C-7’).

**Description**

$^{13}$C NMR spectra shows 1 carbonyl carbon signal at δ 171.15 (C-7’) and 1 hydroxyl carbon
signal δ 163.39 (C-2’).

\(^1\)H NMR spectra showed 1 proton multiplet at δ 7.51’ assigned to H-3’ proton. A proton multiplet signal at δ 7.29 was attributed to H-6’ proton adjacent to ester group. 6 proton signal at δ 1.39 was attributed to methyl H-1 and H-17 protons nearly (-COO) group. 6 protons doublet signal at δ 1.39 was attributed to methyl H-19 and 20 protons. 1 proton multiplet signal at δ 5.26 was assigned to H-15 proton and 2 proton signals at δ 4.93 and 4.8 was assigned to H- 16 protons.

**Compound B (n- Decanyl godoate (2))**

The compound B was obtained as a oily, lemon yellow colored material, having Rf at 0.42.

**IR \( \nu_{\text{max}} \) (KBr):** 2926, 2854, 1740, 1645, 1463, 1400, 1245, 1170, 722 cm\(^{-1}\)

\(^1\)H NMR (CDCl\(_3\)): δ 5.29 (1H, m, H-9), 5.27 (1H, m, H-10), 4.52 (2H, m, H\(_2\)-1’), 2.72 (2H, m, H\(_2\)-2), 2.23 (2H, m, H\(_2\)-8), 1.98 (2H, m, H\(_2\)-11), 1.53 (4H, m, 2 \times \text{CH}_3), 1.23 (10H, brs, 5 \times \text{CH}_2), 1.18 (28H, brs, 14 \times \text{CH}_2), 0.83 (3H, t, J= 6.2 Hz, Me-20), 0.78 (3H, t, J=7.2 Hz, Me-10’)

**Mass Data:** TOF MS m/z (relative intensity): 450 [M]\(^+\) (C\(_{30}\)H\(_{58}\)O\(_2\)) (43.1)

**Description**

\(^1\)H NMR spectra of compound B showed 2 proton multiplet at δ 4.52 which was assigned to H-1’ proton near ester group. 2 proton multiplets at δ 5.29 and 5.27 were assigned H-9 and H-10 protons of ethylene group. 2 proton multiplet signals at 2.72 were assigned to H-2 protons near C-O group. 4 proton multiplet signals at δ 2.23 and 1.98 were attributed to H-8 and H-11 protons near the double bond. 6 proton triplet signal at δ 0.83 (J= 6.2 Hz) and 0.78 (J = 7.2 Hz) was assigned to methyl protons at C-20 and C-10’.

**Compound C (n- Docosanyl oleate (3))**

The compound C was obtained as a oily, lemon yellow colored material, having Rf at 0.71.

**UV \( \lambda_{\text{max}} \) (MeOH):** 551nm

**IR \( \nu_{\text{max}} \) (KBr):** 2925, 2854, 1746, 1654, 1464, 1378, 1237, 1163, 722 cm\(^{-1}\)

\(^1\)H NMR (CDCl\(_3\)): δ 5.34 (1H, m, H-9), 5.28 (1H, m, H-10), 4.28 (1H, m, H\(_2\)-1’a), 4.15 (1H, m, H\(_2\)-1’b), 2.79 (2H, m, H\(_2\)-2), 2.31 (2H, m, H\(_2\)-8), 2.03 (2H, m, H\(_2\)-11), 1.60 (2H, m, CH\(_2\)), 1.30 (10H, brs, 5 \times \text{CH}_2), 1.25 (50H, brs, 25 \times \text{CH}_2), 0.88 (3H, t, J=6.2 Hz, Me-18), 0.85 (3H, t, J=6.0 Hz, Me-22’).
$^{13}$C NMR (CDCl$_3$) δc: δ 171.15 (C-2), 128.90 (C-9), 128.33 (C-10), 63.01 (C-1’), 34.20 (CH$_2$), 33.94 (CH$_2$), 30.96 (CH$_2$), 26.35 (34 × CH$_2$), 29.06 (CH$_2$), 24.79 (CH$_2$), 23.98 (CH$_2$), 22.07 (CH$_2$), 13.97 (C-18), 12.96 (C-22’)

**Mass Data:** + ve TOF MS m/z (relative intensity): 590 [M]$^+$ (C$_{40}$H$_{78}$O$_2$) (3.8)

**Description:** 1H NMR showed 1 proton multiplet at δ 5.34 which was assigned to H-9. Again 1 proton multiplets were observed at δ values 5.28, 4.28, 4.15 for H-10, H-1’a and 1’b respectively. 8 proton multiplet signals were seen at δ values 2.79, 2.31, 2.03 and 1.60 for H$_2$-2, H$_2$-8, H$_2$-11 and CH$_2$ respectively. δ value 1.30 was observed for 10 protons of 5 methyl groups. 6 proton triplets signal were observed at δ values 0.88 and 0.85 for Me groups at 18 and 22’ having J values 6.2 Hz and 6 Hz respectively.

**13C NMR:** δ values 128.90 and 128.33 were observed at C-9 and C-10 which are bonded by double bonds. δ value 171.15 was assigned to C-1 which was found be an ester. δ value 63.01 was assigned to the C-1’ which lies adjacent to an ester group.

**Compound D (n-Dotriacontanoic acid)**
The compound D was obtained as off-white colored crystals, having R$_f$ at 0.34.

**IR$_{\nu \text{max}}$ (KBr):** 3235, 2925, 2845, 1701, 1460, 1265, 830, 720 cm$^{-1}$

$^1$H NMR (CDCl$_3$): δ 2.27 (2H, m, H$_2$-2), 1.98 (2H, m, CH$_2$), 1.53 (4H, m, 2 × CH$_2$), 1.18 (52H, brs, 26 × CH$_2$), 0.81 (3H, t, J=6.1 Hz, Me-32)

$^{13}$C NMR (CDCl$_3$) δc: δ 179.73 (C-1), 33.99 (CH$_2$), 31.94 (CH$_2$), 29.71 (CH$_2$), 29.60 (20 × CH$_2$), 29.44 (CH$_2$), 29.38 (CH$_2$), 29.25 (CH$_2$), 29.07 (CH$_2$), 27.21 (CH$_2$), 24.68 (CH$_2$), 22.71 (CH$_2$)

**Mass Data:** TOF MS m/z (relative intensity): 480 [M]$^+$ (C$_{32}$H$_{64}$O$_2$) (15.3)

**Description:**
1H NMR 4 proton multiplet signals were observed at H$_2$-2 and CH$_2$ carbons having δ values 2.27 and 1.98. 1 triplet peak was observed at δ value 0.81 for methyl group at 32 having J value 6.1 Hz. 52 proton peaks were observed having δ value 1.18 for 26 methyl groups.

13 C NMR δ value 179.73 was observed for C-1 which was found to be an acid.
DISCUSSION
The compound A could be used as medicinal supplements along with the analgesic drugs or in combination with the decongestant ointments. Compound B and Compound C could be used as adjuvants in pharmaceutical preparations in near future as they are long chain of hydrocarbon and Compound D could also be used as medicinal supplements with antimicrobial and antioxidant drugs.

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REFERENCES


