

# Aliphatic and eudesmalolide esters extracted from the roots of *Inula racemosa* Hook

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## ABSTRACT

**Background:** Phytochemical investigation of hydroalcoholic extract of the root of *Inula racemosa* Hook. **Materials and Methods:** Open silica gel column chromatographic techniques with different solvent systems was used for isolation of aliphatic and eudesmalolide esters from hydroalcoholic extract of the root of *Inula racemosa*. The structure elucidation of the compounds was done on the basis of spectral data analysis, chemical reactions and comparison with literature data. **Results:** Phytochemical investigation of the hydroalcoholic extract of the root of *Inula racemosa* Hook. f. led to the isolation of (5z, 13z)-n-decanyl-n-docos-5, 13-dienoate, a new fatty acid ester, two new sesquiterpenic ester identified as 15-[(13z, 18'z, 20'z)-n-tricos-13, 18, 20-trienyl]-eudesmal-4 (11), 6, 12 (13)-trien-8,14-olide-15-oate and 15-[(16'z), (21'z)-n-tetracos-16', 21'-dienyl]-eudesmal-4 (11) 6, 12 (13)-trien-8, 14-olide-15-oate, two new eudesmanolide ester i.e. 15-[(16z)-n-monadec-16'-enyl]-eudesmal-4 (11) 6, 12 (13)-trien-8,14-olide-15-oate and 15-[(16'z)-n-tetracos-16'-enyl]-eudesmal-4 (11), 6, 12 (13)-trien-8,14-olide-15-oate along with the known compound n-Hexadecanyl n-docosanoate. **Conclusion:** Five new phytoconstituents were identified along with one known compound as aliphatic and eudesmalolide esters from the hydroalcoholic extract of the root of *Inula racemosa*, as mentioned above.

**Key words:** Aliphatic esters, eudesmalolide esters, *Inula racemosa*, phytoconstituents, root extract

## INTRODUCTION

*Inula racemosa* Hook. f. commonly known as 'Pushkarmoola' of family Asteraceae, with its diverse active components, has been utilized as a therapeutic, since time immemorial in both organized (Ayurveda, Unani) and unorganized (folk, tribal, native) form.<sup>[1]</sup> The roots of *I. racemosa* have been used as folk medicine in East Asia and Europe.<sup>[2]</sup> Native Americans used this plant for the treatment of tuberculosis.<sup>[3]</sup> The alantolactone obtained from roots of *I. racemosa* enhances insulin sensitivity, thus proves beneficial in fat reduction.<sup>[4]</sup> Roots of *I. racemosa* are also useful in treating pulmonary and cardiovascular disorders.<sup>[5]</sup> The chemical profiling of the plant roots have shown the presence of alantolactones and isoalantolactones, sitisterol, daucosterol, inunolide, aplotexene, phenylacetone and inosin.<sup>[6]</sup>

The characteristic components of the genus *Inula* are sesquiterpenes, such as eudesmanes, germacrane, guaiane and bis-sesquiterpenes. Recently, much attention has been paid to *Inula* species due to their diverse biological activities.<sup>[7,8]</sup>

During further phytochemical investigation, a new fatty acid ester, two new sesquiterpenic esters and two new eudesmanolide esters were isolated along with known compounds like n-Hexadecanyl n-docosanoate. Herein, we reported the isolation and elucidation of the two new sesquiterpenic esters and two new eudesmanolide esters along with a new fatty acid ester.

## MATERIALS AND METHODS

### General procedure

Melting points were determined on Perfit melting point apparatus and are uncorrected. Fourier transform infrared spectroscopy (FTIR): Jasco FT/IR-5000; UV: Lambda Bio 20 Spectrophotometer, MeOH; <sup>1</sup>H-NMR (400 MHz):

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Advance DRY 400, Bruker spectropin,  $\text{CDCl}_3$ ;  $^{13}\text{C}$  NMR (75 MHz): Advance DRY 100, Bruker spectropin,  $\text{CDCl}_3$  with TMS as an internal standard; MS: FAB ionization on JEOL-JMS-DX 303; CC: silica gel (Qualigens), 60-120 mesh; TLC: Silica gel G (Qualigens). Spots were visualized by exposure to iodine vapours, UV radiation and by the use of spraying reagents.

### Plant material

The root of *I. racemosa* was collected from the local market of Khari Baoli, Delhi one week before commencing the extraction procedure and identified by Dr. M. P. Sharma, taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard (Hamdard University). A voucher specimen No. PRD/JH/09/18 is deposited in the herbarium of the Faculty of Pharmacy, Jamia Hamdard, New Delhi.

### Extraction and isolation

The air-dried root (3 kg) of *I. racemosa* were coarsely powdered, defatted with petroleum ether and then exhaustively extracted in a Soxhlet apparatus with 90% hydro-methanolic mixture for 72h. The extract was concentrated under reduced pressure to obtain dark brown viscous mass, small portion of the extract was analyzed chemically to determine the presence of different chemical constituents. The viscous dark brown mass was adsorbed on silica gel (60-120 mesh) for column after being dissolved in little quantity of methanol for preparation of slurry. The slurry (255 g) was air dried and chromatographed over silica gel column packed in petroleum ether. The column was eluted successively using solvent mixtures e.g., starting with pure petroleum ether, successive mixtures of petroleum ether and chloroform (9:1, 3:1, 1:1 and 1:3), pure chloroform and finally mixture of chloroform and methanol (99:1, 49:1, 24:1, 95:5, 97:3, 9:1) were used to run the column. Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions (having same  $R_f$  values) were combined and crystallized. The isolated compounds were recrystallized to get pure compounds.

## RESULT AND DISCUSSION

### Result

#### IR-1 (*n-Decanyl docosdienoate*)

Elution of the column with petroleum ether gave colorless amorphous powder of IR-1, recrystallized from acetone-methanol (1:1), 78mg (0.035% yield),  $R_f$ : 0.62 (petroleum ether), m.p: 75°C.

UV  $\lambda_{\text{max}}$  (MeOH): 206nm (log $\epsilon$  4.3).

IR  $\gamma_{\text{max}}$  (KBr): 2927, 2856, 1718, 1640, 1460, 1378, 1172, 995, 911 $\text{cm}^{-1}$ .

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  5.79 (1H, m,  $w_{1/2}$  = 14.3 Hz, H-5), 5.04 (1H, m,  $w_{1/2}$  = 17.6 Hz, H-6), 4.96 (1H, m,  $w_{1/2}$  = 13.6 Hz, H-13), 4.91 (1H, m,  $w_{1/2}$  = 14.1 Hz, H-14), 4.52 (1H, ddd,  $J$  = 5.2, 3.6, 10.0Hz,  $\text{H}_2$ -1'a), 4.30 (1H, ddd,  $j$  = 5.2, 5.2, 10.0 Hz,  $\text{H}_2$ -1'b), 2.31 (2H, t,  $J$  = 7.6 Hz,  $\text{H}_2$ -2), 2.05 (2H, m,  $\text{H}_2$ -4), 2.03 (2H, m,  $\text{H}_2$ -7), 1.76 (2H, m,  $\text{H}_2$ -12), 1.63 (2H, m,  $\text{H}_2$ -15), 1.56 (2H, m,  $\text{CH}_2$ ), 1.51 (2H, m,  $\text{CH}_2$ ), 1.36 (8H, brs,  $4 \times \text{CH}_2$ ), 1.28 (6H, brs,  $3 \times \text{CH}_2$ ), 1.24 (20H, brs,  $10 \times \text{CH}_2$ ), 0.94 (3H, t,  $J$  = 7.2 Hz, Me-22), 0.87 (3H, t,  $J$  = 7.2 Hz, Me-22).

$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  171.26 (C-1), 138.75 (C-5), 137.81 (C-6), 119.23 (C-13), 114.47 (C-14), 62.41 (C-1'), 33.78 ( $\text{CH}_2$ ), 33.67 ( $\text{CH}_2$ ), 33.53 ( $\text{CH}_2$ ), 31.93 ( $\text{CH}_2$ ), 29.69 ( $7 \times \text{CH}_2$ ), 29.36 ( $\text{CH}_2$ ), 29.02 ( $\text{CH}_2$ ), 28.95 ( $\text{CH}_2$ ), 28.89 ( $\text{CH}_2$ ), 28.78 ( $\text{CH}_2$ ), 28.72 ( $\text{CH}_2$ ), 28.70 ( $\text{CH}_2$ ), 28.68 ( $\text{CH}_2$ ), 28.62 ( $\text{CH}_2$ ), 28.55 ( $\text{CH}_2$ ), 28.49 ( $\text{CH}_2$ ), 24.55 ( $\text{CH}_2$ ), 22.69 ( $\text{CH}_2$ ), 14.35 (Me-10'), 10.17 (Me-22).

+ive ESI MS  $m/z$  relative intensities: 476  $[\text{M}]^+$  ( $\text{C}_{32}\text{H}_{60}\text{O}_2$ ) (7.8), 363 (22.3), 337 (29.8), 335 (6.8), 319 (8.9), 249 (7.2) [Figure 1].

#### IR-2 (*n-Hexadecanyl behenate*)

Elution of the column with petroleum ether chloroform (3:1) afforded colorless amorphous powder of IR-2, re-crystallized from chloroform-methanol (1:1), 62 mg (0.027% yield),  $R_f$ : 0.5 (petroleum ether-chloroform, 3:1), m.p: 78°C.

UV  $\lambda_{\text{max}}$  (MeOH): 206 nm (log $\epsilon$  3.1).

IR  $\gamma_{\text{max}}$  (KBr): 2997, 2855, 1734, 1457, 1379, 1255, 1173, 723  $\text{cm}^{-1}$ .

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  4.24 (1H, m, H-1'a), 4.09 (1H, ddd,  $J$  = 6.4, 6.0, 10.0 Hz,  $\text{H}_2$ -1'b), 2.29 (2H, t,  $J$  = 7.2 Hz, H-2), 2.02 (2H, m,  $\text{CH}_2$ ), 1.81 (2H, m,  $\text{CH}_2$ ), 1.59 (6H, brs,  $3 \times \text{CH}_2$ ), 1.44 (2H, m,  $\text{CH}_2$ ), 1.27 (8H, brs,  $4 \times \text{CH}_2$ ), 1.23 (46H, brs,  $23 \times \text{CH}_2$ ), 0.86 (3H, t,  $J$  = 5.6 Hz, Me-22), 0.82 (3H, t,  $J$  = 6.5 Hz, Me-1C').

$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  174.39 (C-1), 63.16 (C-1'), 51.42 (C-2), 41.86 ( $\text{CH}_2$ ), 41.11 ( $\text{CH}_2$ ), 36.83 ( $\text{CH}_2$ ), 35.93 ( $\text{CH}_2$ ), 34.12 ( $\text{CH}_2$ ), 31.91 ( $\text{CH}_2$ ), 29.75 ( $10 \times \text{CH}_2$ ), 29.67 ( $7 \times \text{CH}_2$ ), 29.58 ( $\text{CH}_2$ ), 29.43 ( $\text{CH}_2$ ), 29.34 ( $\text{CH}_2$ ), 29.24 ( $\text{CH}_2$ ), 29.14 ( $\text{CH}_2$ ), 29.07 ( $\text{CH}_2$ ), 27.10 ( $\text{CH}_2$ ), 24.71 ( $\text{CH}_2$ ), 22.67 ( $\text{CH}_2$ ), 14.10 (Me-22), 11.97 (Me-16').

+ive ESI MS  $m/z$  relative intensities: 564  $[\text{M}]^+$  ( $\text{C}_{38}\text{H}_{76}\text{O}_2$ ) (19.3), 323 (46.2), 339 (72.6), 241 (34.2), 225 (87.9) [Figure 2].

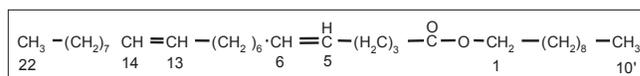


Figure 1: Structure of IR-1 (*n-decanyl docosdienoate*)

**IR-3 (15-tricostrienyl eudesmalolide)**

Elution of the column with the petroleum ether-chloroform (1:1) yielded colorless crystals of IR-3 recrystallized from acetone and methanol (1:1), 70 mg (0.031% yield),  $R_f$ : 0.6 (Chloroform-methanol), mp: 104-105°C.

UV  $\lambda_{max}$  (MeOH): 207, 278, 375nm (log $\epsilon$  4.5, 1.6, 3.2).

IR  $\gamma_{max}$  (KBr): 2993, 2842, 1765, 1742, 1640, 1411, 1235, 1050, 922, 778cm<sup>-1</sup>.

<sup>1</sup>H-NMR (CDCl<sub>3</sub>):  $\delta$  6.20 (1H, d, J = 3.5 Hz, H-6), 5.21 (1H, brs, H<sub>2</sub>-11a), 5.03 (1H, brs, H<sub>2</sub>-11b), 4.86 (1H, brs, H<sub>2</sub>-13b), 4.80 (1H, brs, H<sub>2</sub>-13b), 3.93 (1H, dd, J = 9.5, 9.0 Hz, H-8 $\alpha$ ), 2.49 (1H, d, J = 3.5 Hz, H-5 $\beta$ ), 2.75 (1H, m, H<sub>2</sub>-1a), 1.68 (1H, m, H<sub>2</sub>-1b), 2.19 (2H, m, H<sub>2</sub>-2), 2.02 (1H, dd, J = 6, 7.5 Hz, H<sub>2</sub>-3 $\alpha$ ), 1.93 (1H, dd, J = 4, 10.1 Hz, H<sub>2</sub>-3 $\beta$ ), 2.23 (2H, d, J = 9.5, H<sub>2</sub>-9), 5.36 (2H, m,  $w_{1/2}$  = 5.5 Hz, H-13', H-14'), 5.34 (2H, m,  $w_{1/2}$  = 5.6 Hz, H-18', H-19'), 5.25 (2H, m,  $w_{1/2}$  = 5.5 Hz, H-20', H-21'), 4.16 (2H, t, J = 7.5 Hz, H<sub>2</sub>-1), 2.78 (2H, m, H<sub>2</sub>-15'), 2.47 (2H, m, H<sub>2</sub>-17'), 2.16 (2H, m, H<sub>2</sub>-12), 1.60 (4H, m, 2CH<sub>2</sub>), 1.29 (4H, brs, 2CH<sub>2</sub>), 1.24 (12H, brs, 6CH<sub>2</sub>), 0.83 (3H, t, 7.5 Hz, Me-23').

<sup>13</sup>C-NMR (CDCl<sub>3</sub>):  $\delta$  32.6 (C-1), 31.93 (C-2), 41.01 (C-3), 139.77 (C-4), 52.04 (C-5), 120.20 (C-6), 149.23 (C-7), 85.26 (C-8), 36.28 (C-9), 37.63 (C-10), 109.62 (C-11), 151.24 (C-12), 112.63 (C-13), 170.29 (C-14), 168.46 (C-15), 62.10 (C-1'), 31.53 (C-2'), 30.94 (C-3'), 30.91 (C-4'), 29.70 (C-5'), 29.60 (C-6'), 29.49 (C-7'), 29.27 (C-8'), 29.16 (C-9'), 29.16 (C-10'), 27.20 (C-11'), 47.67 (C-12'), 114.21 (C-13'), 127.12 (C-14'), 45.33 (C-15'), 25.63 (C-16'), 33.77 (C-17'), 127.91 (C-18'), 131.96 (C-19'), 139.11 (C-20'), 130.02 (C-21'), 22.58 (C-22'), 14.09 (C-23').

+ive ESI MS m/z relative intensities: 576 [M]<sup>+</sup>(C<sub>38</sub>H<sub>56</sub>O<sub>4</sub>) (2.9), 333 (23.4), 304 (19.4), 252 (98.4), 243 (4.53), 184 (6.8), 149 (4.1) [Figure 3].

**IR-4 (15-nonadecenyl eudesmalolide)**

Elution of the column with petroleum ether-chloroform (1:1) gave pale yellow crystals of IR-4, recrystallized from acetone-methanol (1:1), 85mg (0.037% yield),  $R_f$ : 0.65 (Chloroform-methanol, 3:2), mp: 120-125°C.

UV  $\lambda_{max}$  (MeOH): 207, 375nm (log $\epsilon$  4.1, 2.2).

IR  $\gamma_{max}$  (KBr): 2928, 2841, 1765, 1735, 1638, 1455, 1383, 1258, 1051, 779 cm<sup>-1</sup>.

<sup>1</sup>H-NMR (CDCl<sub>3</sub>):  $\delta$  6.21 (1H, d, J = 3.6 Hz, H-6), 5.26 (1H, brs, H<sub>2</sub>-13a), 5.06 (1H, brs, H<sub>2</sub>-13b), 4.86 (1H, brs, H<sub>2</sub>-11a), 4.76 (1H, brs, H<sub>2</sub>-11b), 3.95 (1H, t, J = 9.5 Hz,

H-8 $\beta$ ), 2.79 (1H, m, H<sub>2</sub>-1a), 2.75 (1H, m, H<sub>2</sub>-1b), 2.49 (1H, d, J = 3.6 Hz, H-5 $\alpha$ ), 2.26 (2H, d, J = 9.5 Hz, H<sub>2</sub>-9), 2.23 (1H, m, H<sub>2</sub>-2a), 2.16 (1H, m, H<sub>2</sub>-2b), 2.05 (1H, dd, J = 6.5, 14.0 Hz, H<sub>2</sub>-3a), 2.01 (1H, dd, J = 6.5, 5.5 Hz, H<sub>2</sub>-3b), 5.35 (1H, m,  $w_{1/2}$  = 5.5 Hz, H-16'), 5.32 (1H, m,  $w_{1/2}$  = 5.5 Hz, H-17'), 4.14 (2H, t, J = 4.8 Hz, H<sub>2</sub>-1'), 2.86 (2H, m, H<sub>2</sub>-15'), 2.44 (2H, m, H<sub>2</sub>-18'), 1.86 (2H, m, CH<sub>2</sub>), 1.72 (2H, m, CH<sub>2</sub>), 1.62 (2H, m, CH<sub>2</sub>), 1.59 (2H, m, CH<sub>2</sub>), 1.52 (2H, m, CH<sub>2</sub>), 1.29 (6H, brs, 3  $\times$  CH<sub>2</sub>), 1.24 (12H, brs, 6  $\times$  CH<sub>2</sub>), 0.83 (3H, t, J = 6.5 Hz, Me-19').

<sup>13</sup>C-NMR (CDCl<sub>3</sub>): 32.60 (C-1), 31.92 (C-2), 39.82 (C-3), 139.75 (C-4), 52.04 (C-5), 120.19 (C-6), 148.26 (C-7), 85.25 (C-8), 36.26 (C-9), 36.23 (C-10), 109.62 (C-11), 151.93 (C-12), 112.63 (C-13), 171.23 (C-14), 167.85 (C-15), 62.17 (C-1'), 45.13 (C-2'), 30.31 (C-3'), 29.69 (C-4'), 29.34 (C-5'), 31.52 (C-6'), 30.94 (C-7'), 30.31 (C-8'), 29.69 (C-9'), 29.34 (C-10'), 29.11 (C-11'), 28.82 (C-12'), 27.20 (C-13'), 25.53 (C-14'), 47.63 (C-15'), 130.23 (C-16'), 127.91 (C-17'), 22.57 (C-18'), 14.07 (C-19').

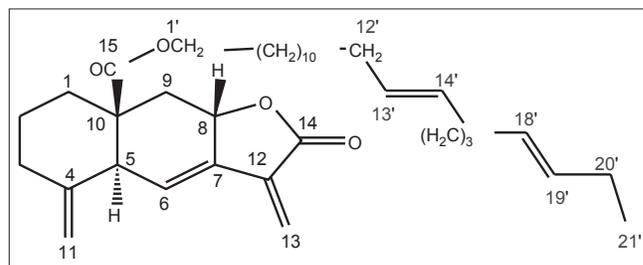
+ive ESI MS m/z relative intensities: 524 [M]<sup>+</sup>(C<sub>34</sub>H<sub>52</sub>O<sub>4</sub>) (4.8), 281 (9.1), 252 (39.8), 243 (3.6) [Figure 4].

**IR-5 (15-Tetracosdienyl eudesmalolide)**

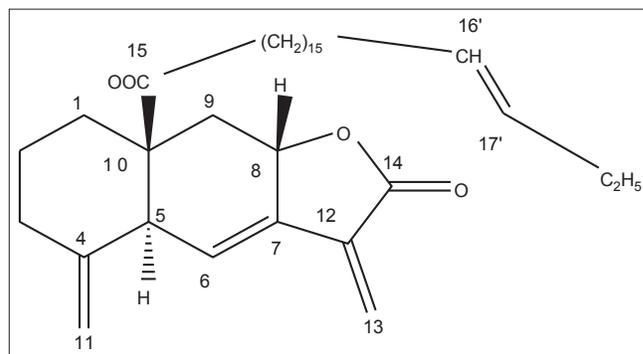
Elution of the column with petroleum ether-chloroform (1:3) afforded light brown crystals of IR-5, recrystallized



**Figure 2:** Structure of IR-2 (n-hexadecanyl behenate)



**Figure 3:** Structure of IR-3 (15-tricostrienyl eudesmalolide)



**Figure 4:** Structure of IR-4 (15-nonadecenyl eudesmalolide)

from acetone-methanol (1:1), 90mg (0.04% yield),  $R_f$ : 0.5 (Petroleum ether-Chloroform, 1:1), mp: 98-100°C.

UV  $\lambda_{max}$  (MeOH): 207, 375 nm (log $\epsilon$  4.1, 1.9).

IR  $\gamma_{max}$  (KBr): 2927, 2855, 1766, 1723, 1640, 1456, 1380, 1258, 1147, 998, 896 $cm^{-1}$ .

$^1H$ -NMR ( $CDCl_3$ ):  $\delta$  6.21 (1H, d, J = 3.6 Hz, H-6), 5.26 (1H, brs, H<sub>2</sub>-13a), 5.06 (1H, brs, H<sub>2</sub>-13b), 4.88 (1H, brs, H<sub>2</sub>-11a), 4.80 (1H, brs, H<sub>2</sub>-11b), 3.95 (1H, t, J = 9.2 Hz, H-8 $\beta$ ), 2.84 (2H, m, H<sub>2</sub>-1), 2.20 (1H, m, H<sub>2</sub>-2a), 2.15 (1H, m, H<sub>2</sub>-2b), 2.04 (1H, dd, J = 6.0, 8.4 Hz, H<sub>2</sub>-3 $\beta$ ), 1.97 (1H, dd, J = 5.2, 8.0 Hz, H<sub>2</sub>-3 $\alpha$ ), 2.50 (1H, d, J = 3.6 Hz, H-5 $\alpha$ ), 2.30 (2H, m, H<sub>2</sub>-9), 3.65 (2H, t, J = 5.6 Hz, H<sub>2</sub>-1'), 5.48 (1H, m,  $w_{1/2}$  = 5.6 Hz, H-21), 5.36 (1H, m,  $w_{1/2}$  = 5.8 Hz, H-16'), 5.31 (1H, m,  $w_{1/2}$  = 5.5 Hz, H-17'), 5.20 (1H, m,  $w_{1/2}$  = 5.6 Hz, H-22'), 2.46 (2H, m, H<sub>2</sub>-15'), 2.26 (2H, m, H<sub>2</sub>-18'), 1.85 (2H, m, H<sub>2</sub>-20'), 1.66 (2H, m, H<sub>2</sub>-23'), 1.62 (2H, m, H<sub>2</sub>-2'), 1.35 (8H, brs, 4  $\times$  CH<sub>2</sub>), 1.29 (18H, brs, 9  $\times$  CH<sub>2</sub>), 0.86 (3H, t, J = 6.8 Hz, Me-24').

$^{13}C$ -NMR ( $CDCl_3$ ):  $\delta$  32.48 (C-1), 31.76 (C-2), 41.86 (C-3), 139.64 (C-4), 52.41 (C-5), 119.89 (C-6), 149.95 (C-7), 83.41 (C-8), 37.62 (C-9), 36.83 (C-10), 109.58 (C-11), 151.68 (C-12), 112.25 (C-13), 173.16 (C-14), 170.24 (C-15), 66.03 (C-1'), 31.62 (C-2'), 130.20 (C-16'), 130.04 (C-17'), 125.05 (C-21'), 128.28 (C-22'), 31.34 (CH<sub>2</sub>), 47.11 (C-15'), 46.92 (C-18'), 44.78 (C-20'), 29.86 (4  $\times$  CH<sub>2</sub>), 29.83 (CH<sub>2</sub>), 29.76 (CH<sub>2</sub>), 29.50 (CH<sub>2</sub>), 28.61 (CH<sub>2</sub>), 28.53 (CH<sub>2</sub>), 27.10 (CH<sub>2</sub>), 25.98 (CH<sub>2</sub>), 25.52 (CH<sub>2</sub>), 24.94 (CH<sub>2</sub>), 22.86 (CH<sub>2</sub>), 14.05 (Me-24).

+ive ESI MS m/z relative intensities: 592 [M]<sup>+</sup>(C<sub>39</sub>H<sub>60</sub>O<sub>4</sub>) (2.1), 350 (41.8), 321 (7.1), 294 (5.2), 252 (62.3), 243 (7.6) [Figure 5].

#### IR-6 (15-tetracosenyl eudesmalolide)

Elution of the column with chloroform gave pale yellow crystals of IR-6, recrystallized from acetone-methanol (1:1), 100 mg (0.04% yield),  $R_f$ : 0.7 (Chloroform-methanol, 97:3), mp: 125°C.

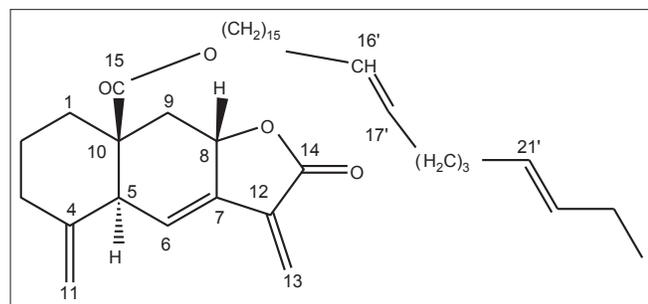


Figure 5: Structure of IR-5 (15-tetracosdienyl eudesmalolide)

UV  $\lambda_{max}$  (MeOH): 208, 375 nm (log $\epsilon$  4.7, 4.2).

IR  $\gamma_{max}$  (KBr): 2928, 2855, 1764, 1720, 1645, 1455, 1381, 1259, 1123, 898  $cm^{-1}$ .

$^1H$ -NMR ( $CDCl_3$ ):  $\delta$  6.20 (1H, d, J = 3.5 Hz, H-6), 5.05 (1H, brs, H<sub>2</sub>-13a), 4.89 (1H, brs, H<sub>2</sub>-13b), 4.69 (1H, brs, H<sub>2</sub>-11b), 3.95 (1H, t, J = 9.5 Hz, H-9 $\alpha$ ), 2.75 (1H, m, H-1a), 1.68 (1H, m, H-1b), 2.18 (1H, m, H<sub>2</sub>-2a), 2.15 (1H, m, H-1b), 2.02 (1H, dd, J = 5.5, 7.7 Hz, H<sub>2</sub>-3 $\alpha$ ), 1.95 (1H, dd, J = 5.5, 5 Hz, H<sub>2</sub>-3 $\beta$ ), 2.29 (2H, d, J = 9.5 Hz, H<sub>2</sub>-9), 2.48 (1H, d, J = 3.5 Hz, H-5 $\beta$ ), 5.33 (1H, m,  $w_{1/2}$  = 6.5 Hz, H-16'), 5.17 (1H, m,  $w_{1/2}$  = 7.2 Hz, H-17'), 4.12 (1H, t, J = 7.6 Hz, H-1'), 2.63 (2H, m, H<sub>2</sub>-15'), 2.45 (2H, m, H<sub>2</sub>-18'), 1.73 (2H, m, CH<sub>2</sub>), 1.68 (2H, m, CH<sub>2</sub>), 1.55 (2H, m, CH<sub>2</sub>), 1.40 (2H, m, CH<sub>2</sub>), 1.78 (5H, brs, 10 CH<sub>2</sub>), 1.24 (18H, brs, 19 CH<sub>2</sub>), 0.85 (3H, t, J = 7 Hz, Me-24').

$^{13}C$ -NMR ( $CDCl_3$ ):  $\delta$  32.60 (C-1), 31.92 (C-2), 44.25 (C-3), 139.75 (C-4), 55.32 (C-5), 120.16 (C-6), 149.22 (C-7), 85.29 (C-8), 36.25 (C-9), 37.48 (C-10), 109.60 (C-11), 151.36 (C-12), 112.63 (C-13), 170.22 (C-14), 167.89 (C-15), 62.15 (C-1'), 45.13 (C-2'), 30.93 (C-3'), 30.30 (C-4'), 30.19 (C-5'), 29.69 (C-6'), 29.35 (C-7'), 29.09 (C-8'), 30.19 (C-9'), 29.69 (C-10'), 29.35 (C-11'), 30.93 (C-12'), 30.30 (C-13'), 30.19 (C-14'), 52.02 (C-15'), 130.19 (C-16'), 113.63 (C-17'), 47.62 (C-18'), 29.69 (C-19'), 29.35 (C-20'), 29.09 (C-21'), 27.20 (C-22'), 22.58 (C-23'), 14.12 (C-24').

+ive ESI MS m/z relative intensities: 594 [M]<sup>+</sup>(C<sub>39</sub>H<sub>62</sub>O<sub>4</sub>) (6.8), 351 (5.2), 252 (57.6), 242 (3.2) [Figure 6].

## DISCUSSION

Compound IR-1, named n-decanyl docosdienoate, was obtained as a colorless crystalline mass from petroleum ether eluent. Its IR spectrum showed distinct absorption bands for ester group (1718  $cm^{-1}$ ), unsaturation (1640 $cm^{-1}$ ) and long aliphatic chain (728 $cm^{-1}$ ). On the basis of mass and  $^{13}C$ -NMR spectra, the molecular ion peak of IR-1 was determined at m/z 476 consistent to the molecular formula

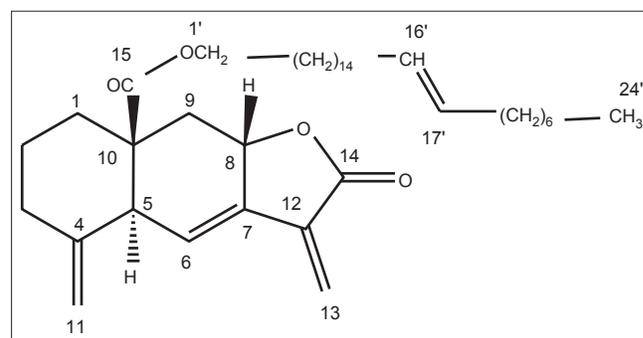


Figure 6: Structure of IR-6 (15-tetracosenyl eudesmalolide)

of a fatty acid ester,  $C_{32}H_{60}O_2$ . The ion peaks arising at  $m/z$  319 [ $M-C_{10}H_{21}$ ] and 319 [ $m-OC_{10}H_{21}$ ]<sup>+</sup> indicated that *n*-docosdienoic acid was esterified with *n*-decanol. The ion peaks generated at  $m/z$  363 [ $C_{14}-C_{15}$  fission]<sup>+</sup>, 337 [ $C_{12}-C_{13}$  fission]<sup>+</sup> and 249 [ $C_4-C_5$  fission,  $CH_3(CH_2)_7CH=CH(CH_2)_6CH=CH$ ]<sup>+</sup> suggested the existence of the vinylic linkages at C-5 (6) and C-13 (14) carbons. The <sup>1</sup>H-NMR spectrum of exhibited four one-proton multiplets at  $\delta$  5.79 ( $w_{1/2} = 14.3$  Hz), 5.04 (1H,  $w_{1/2} = 17.6$  Hz, 4.96 ( $w_{1/2} = 13.6$  Hz) and 4.91 ( $w_{1/2} = 14.1$  Hz) assigned to trans-oriented vinylic H-5, H-6, H-13 and H-14 protons, respectively. Two one-proton triple doublets at  $\delta$  4.52 ( $J = 5.2, 3.6, 10.0$  Hz) and 4.30 ( $J = 5.2, 5.2, 10.0$  Hz) were due to oxygenated methylene  $H_{2-1'}$  protons. A two-proton triplet at  $\delta$  2-31 ( $J = 7.6$  Hz) was ascribed to methylene  $H_2-2$  adjacent to the ester group. The other methylene protons appeared from  $\delta$  2.05 to 1.24. Two three-proton triplets at  $\delta$  0.94 ( $J = 7.2$  Hz) and 0.87 ( $J = 6.0$  Hz) were accounted to the C-10' and C-22 primary methyl protons. The <sup>13</sup>C-NMR spectrum of compound IR-1 displayed signals for ester carbon at  $\delta$  171.26 (C-1), vinylic carbons at  $\delta$  138.75 (C-5), 137.81 (C-6), 119.23 (C-13) and 114.47 (C-14), methylene carbons between  $\delta$  33.78-22.69 and methyl carbons at  $\delta$  14.35 (C-10') and 10.17 (C-22). On the basis of these evidences, the structure of IR-1 has been elucidated as (5*z*, 13*z*)-*n*-decanyl- *n*- docos-5, 13- dienoate. This is a new fatty acid ester. Earlier (5*E*, 13*E*)- docosdienoic acid from *Hybanthus enneaspermus* whole plant was identified, Meadow foam oil and *Eranthis* seed oil.<sup>[9-11]</sup>

Compound IR-2, *n*-hexadecanyl behenate, was obtained as a colorless amorphous powder from petroleum ether chloroform (3:1) eluent. Its IR spectrum showed characteristic absorption bands for ester function (1734  $cm^{-1}$ ) and long aliphatic chain (723  $cm^{-1}$ ). Its mass spectrum displayed a molecular ion peak at  $m/z$  564 corresponding to the molecular formula of a fatty acid ester,  $C_{38}H_{76}O_2$ . The ion peaks arising at  $m/z$  323 [ $CH_3(CH_2)_2CO$ ]<sup>+</sup>, 241 [ $M-323$ ]<sup>+</sup>, 339 [ $CH_3(CH_2)_{20}COO$ ]<sup>+</sup>, and 225 [ $M-339$ ]<sup>+</sup> indicated that behenic acid was esterified with *n*-hexadecanol. The <sup>1</sup>H-NMR spectrum of IR-2 displayed two one proton signals as a multiplet at  $\delta$  4.24 and as a triple doublet at  $\delta$  4.09 ( $J = 6.4, 6.0, 10.0$  Hz) assigned to oxygenated methylene  $H_{2-1'}$  protons. A two proton triplet at  $\delta$  2.29 ( $J = 7.2$  Hz) was ascribed to methylene  $H_2-2$  protons adjacent to the ester carbon. The other methylene protons appeared between  $\delta$  2.02-1.23. Two three proton triplets at  $\delta$  0.86 ( $J = 5.6$  Hz) and 0.82 ( $J = 6.5$  Hz) were accounted to the terminal C-27 and C-16' primary methyl protons respectively. The <sup>13</sup>C-NMR spectrum of compound IR-2 exhibited signals for ester carbon at  $\delta$  174.39 (C-1), oxygenated methylene carbon at  $\delta$  63.16 (C-1'), other methylene carbons from  $\delta$  51.42 to 22.67

and methyl carbons at  $\delta$  14.10 (C-22) and 11.97 (C-16'). The absence of any signal beyond  $\delta$  4.24 in the <sup>1</sup>H-NMR spectrum and between  $\delta$  174.39-63.16 supported saturated nature of the molecule. On the basis of these results, the structure of IR-2 has been characterized as *n*-Hexadecanyl *n*-docosanoate.

Compound IR-3, named 15-tricosterienyl eudesmolide, was obtained from petroleum ether-chloroform (1:1) eluent. Its IR spectrum showed characteristic absorption bands for lactone ring (1765  $cm^{-1}$ ), ester groups (1742  $cm^{-1}$ ), unsaturation (1640  $cm^{-1}$ ) and long aliphatic chain (778  $cm^{-1}$ ). On the basis of mass and <sup>13</sup>C-NMR spectra, the molecular ion peak was determined at  $m/z$  576 corresponding to a sesquiterpenic ester  $C_{38}H_{56}O_4$ . The ion peaks arising at  $m/z$  333 [ $C_{15}-O$  fission,  $O(CH_2)_{12}-CH=CH-(CH_2)_3-(CH=CH)_2C_2H_5$ ]<sup>+</sup> and 243 [ $M-333$ ]<sup>+</sup> suggested that the sesquiterpenic acid was esterified with  $C_{23}$  alcohol. The ion fragments forming at  $m/z$  304 [ $333-C_2H_5$ ]<sup>+</sup>, 252 [ $333-(CH=CH)_2C_2H_5$ ]<sup>+</sup>, 184 [ $C_{12}-C_{13}$  fission,  $O(CH_2)_{12}$ ]<sup>+</sup> and 149 [ $333-184$ ] indicated the location of the arylc linkages at C-13', C-18' and C-20' carbons.

The <sup>1</sup>H-NMR spectrum of IR-3 exhibited a one proton doublet at  $\delta$  4.86 and 4.80 assigned to vinylic H-6 and to exocyclic methylene  $H_2-11$  and  $H_2-13$ , respectively. A one proton double doublet at  $\delta$  3.93 ( $J = 9.5, 9.0$  Hz) and a one proton doublet at  $\delta$  2.49 ( $J = 3.5$  Hz) were attributed to oxygenated methine H-8 $\beta$  and methine H-5 $\alpha$  protons, respectively suggesting olide ring in the molecule. Three two proton multiplets at  $\delta$  5.36 ( $w_{1/2} = 5.5$  Hz), 5.34 ( $w_{1/2} = 5.6$  Hz) and 5.25 ( $J = 5.5$  Hz) were *cis* oriented vinylic protons of the aliphatic chain. A two proton triplet at  $\delta$  4.16 ( $J = 7.5$  Hz) was accounted to oxygenated methylene  $H_{2-1'}$  protons. A three proton triplet at  $\delta$  0.83 ( $J = 7.5$  Hz) was due to terminal C-23' primary methyl protons. The remaining methylene protons appeared from  $\delta$  2.78 to 1.24.

The <sup>13</sup>C-NMR spectrum displayed signals for lactone carbon at  $\delta$  168.46 (C-14) oxygenated methine carbon at  $\delta$  62.10 (C-1'), arylc carbons from  $\delta$  151.24 to 109.62 methylene and methine carbons between  $\delta$  52.04-22.58 and methyl carbon at  $\delta$  14.09 (C-23'). The <sup>1</sup>H and <sup>13</sup>C-NMR spectral values of the eudesmane moiety were compared with the reported spectral data of these compounds.<sup>[12-14]</sup> On the basis of the foregoing discussion the structure of IR-3 has been formulated as 15-[(13*z*, 18'*z*, 20'*z*)-*n*-tricos-13, 18, 20-trienyl]-eudesmal-4 (11), 6, 12 (13)-trien-8, 14-olide-15-oate. This is a new sesquiterpenic ester.

Compound IR-4, designated as 15-nonadecenyl eudesmalolide, was obtained as a pale yellow crystalline

mass from petroleum ether-chloroform (1:1) eluant. Its IR spectrum displayed distinct absorption bands for  $\gamma$ -lactone ( $1765\text{ cm}^{-1}$ ), ester linkage ( $1735\text{ cm}^{-1}$ ), unsaturation ( $1638\text{ cm}^{-1}$ ) and long aliphatic chain ( $779\text{ cm}^{-1}$ ). On the basis of mass and  $^{13}\text{C}$ -NMR spectra the molecular ion peak of IR-4 was established at  $m/z$  524 consistent to the molecular formula of a sesquiterpenic ester  $\text{C}_{34}\text{H}_{52}\text{O}_4$ . The ion peaks arising at  $m/z$  243 [ $\text{C}_{15}$ -O fission] $^+$  and 281 [ $\text{M}-243$ , O ( $\text{CH}_2$ ) $_{15}$ CH =  $\text{CHC}_2\text{H}_5$ ] $^+$  indicated that n-nonadecenyl alcohol was esterified with a sesquiterpenic acid. Expulsion of ethyl group from the mass unit 281 produced an ion fragment at  $m/z$  281 suggesting the existence of the vinylic linkage at C-16' position. The  $^1\text{H}$ -NMR spectrum of IR-4 displayed signals similar to compound IR-3 indicating exocyclic methylene groups at C-4 and C-12, one vinylic linkage at C-6, an olide ring at C-8 (14) position and carboxylate function at C-15. Two one-proton multiplets at  $\delta$  5.35 and 5.32 with half-width of 5.5 Hz were accounted to the cis-oriented vinylic H-16' and H-17' protons, respectively. A two-proton triplet at  $\delta$  4.14 ( $J = 4.8\text{ Hz}$ ) and a three-proton triplet at  $\delta$  0.83 ( $J = 6.5\text{ Hz}$ ) were attributed to oxygenated methylene  $\text{H}_2$ -1' and primary C-19' methyl protons, respectively. The remaining methine and methylene protons resonated from  $\delta$  2.79 to 1.24. The  $^{13}\text{C}$ -NMR spectrum of IR-4 showed fifteen carbon signals for the sesquiterpenic unit similar to compound IR-3, oxygenated methylene carbon at  $\delta$  62.17 (C-1'), vinylic carbons at  $\delta$  130.23 (C-16') and 127.91 (C-17') and methyl carbon at  $\delta$  14.07 (C-19'). The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data of the eudesmone moiety of IR-4 were compared with the reported values of these sesquiterpenoids.<sup>[12-14]</sup> On the basis of these results the structure of IR-4 has been elucidated as 15-[(16z)-n-monadec-16'-enyl]-eudesmal-4 (11) 6, 12 (13)-trien-8, 14-olide-15-oate. This is a new eudesmanolide ester.

Compound IR-5, named 15-tetracosdienyl eudesmalolide, was obtained as a brown crystalline mass from petroleum ether-chloroform (1:3) eluents. Its IR spectrum showed characteristic absorption bands for  $\gamma$ -lactone ( $1766\text{ cm}^{-1}$ ), ester group ( $1723\text{ cm}^{-1}$ ) and unsaturation ( $1640\text{ cm}^{-1}$ ). The molecular ion peak of IR-5 was established at  $m/z$  592 on the basis of mass and  $^{13}\text{C}$ -NMR spectra, consistent of the molecular formula of a sesquiterpenic ester  $\text{C}_{39}\text{H}_{60}\text{O}_4$ . The ion peaks arising at  $m/z$  243 and 350 [ $\text{M}-243$ ] $^+$  due to fission of the  $\text{C}_{15}$ - $\text{OCH}_2$  linkage indicated that tetracosdienol was esterified with the sesquiterpenic acid. The ion peaks generating at  $m/z$  321 [ $350-\text{C}_2\text{H}_5$ ] $^+$ , 252 [ $\text{C}_{17}$ - $\text{C}_{18}$  fission], O ( $\text{CH}_2$ ) $_{15}$ CH =  $\text{CH}$ ] $^+$ , and 294 [ $\text{C}_{20}$ - $\text{C}_{21}$  fission, O ( $\text{CH}_2$ ) $_{15}$ CH =  $\text{CH}$  ( $\text{CH}_2$ ) $_3$ ] $^+$  suggested vinylic linkages at C-16' and C-21' positions.

The  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR spectral data of the sesquiterpenic unit of IR-5 was similar to that of IR-3. A two proton triplet at  $\delta$  3.65 ( $J = 5.6\text{ Hz}$ ) was assigned to oxygenated methylene  $\text{H}_2$ -1' protons. Four one-proton multiplets at  $\delta$  5.48, 5.36, 5.31 and 5.20 with corresponding half widths of 5.6 Hz, 5.8 Hz, 5.5 Hz and 5.6 Hz were ascribed to the cis-oriented vinylic H-21', H-16', H-17' and H-22', respectively. A three proton triplet at  $\delta$  0.86 ( $J = 6.8\text{ Hz}$ ) was due to C-2H' primary methyl protons. The remaining methine and methylene protons resonated from  $\delta$  2.84 to 1.29. The  $^{13}\text{C}$ -NMR spectrum displayed signals oxygenated carbon at 66.03 (C-1'), vinylic carbons at  $\delta$  130.20 (C-16'), 130.04 (C-17'), 125.05 (C-21') and 128.28 (C-22'), methyl carbon at  $\delta$  14.05 (C-24) together with signals for eudesmalolide.

The  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR spectral values of the sesquiterpenic unit were compared with reported data of eudesmane-type sesquiterpenoids.<sup>[12-14]</sup> On the basis of these results the structure of IR-5 has been characterized as 15-[(16'z), (21'z)-n-tetracos-16', 21'-dienyl]-eudesmal-4 (11) 6, 12 (13)-trien-8, 14-olide-15-oate. This is a new sesquiterpenic ester.

Compound IR-6, named 15-tetracosenyl eudesmalolide, was obtained as a pale yellow crystalline product from chloroform eluents. Its IR spectrum showed characteristic absorption bands for  $\gamma$ -lactone ( $1764\text{ cm}^{-1}$ ), ester function ( $1720\text{ cm}^{-1}$ ) and Unsaturation ( $1645\text{ cm}^{-1}$ ). On the basis of mass and  $^{13}\text{C}$ -NMR spectra, the molecular ion peak of IR-6 was determined at  $m/z$  594 corresponding to the molecular formula of a sesquiterpenic ester  $\text{C}_{39}\text{H}_{62}\text{O}_4$ . The ion peaks arising at  $m/z$  243 and 351 [ $\text{C}_{15}$ - $\text{OCH}_2$ ] $^+$  fission suggested that a sesquiterpenic acid was esterified with  $\text{C}_{24}$  alcohol. The ion frequent arising at  $m/z$  252 [ $\text{C}_{17}$ - $\text{C}_{18}$  fission], O ( $\text{CH}_2$ ) $_{15}$ -CH =  $\text{CH}$ ] $^+$  indicated the location of the vinylic linkage at C-16'. The  $^1\text{H}$ -NMR signals of the eudesmolide unit of IR-6 were similar to that of IR-3 suggesting identical sesquiterpenic unit in IR-3 and IR-6. Two one proton multiplets at  $\delta$  5.33 ( $w_{1/2} = 6.5\text{ Hz}$ ) and 5.17 ( $w_{1/2} = 7.2\text{ Hz}$ ) were assigned to cis-oriented vinylic H-16' and H-17', respectively. A two proton triplet at  $\delta$  ( $w_{1/2} = 6.7\text{ Hz}$ ) was ascribed to oxygenated methylene  $\text{H}_2$ -1'. A three proton triplet at  $\delta$  0.85 ( $J = 7\text{ Hz}$ ) was accounted to the terminal C-24 primary methyl protons. The  $^{13}\text{C}$ -NMR spectrum of IR-6 showed signals for vinylic carbons at  $\delta$  130.19 (C-16') and 113.63 (C-17'), oxygenated methylene carbons between  $\delta$  14.12 (C-24') along with the carbon signals of the sesquiterpenic unit similar to that of IR-3. The  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectral data of IR-6 were compared with the reported data of eudesmalolide compounds.<sup>[12-14]</sup> On the basis of these evidences the structure of IR-6 has been established

as 15-[(16'Z)-n-tetracos-16'-enyl]-endesmal-4 (11), 6, 12 (13)-trien-8,14-olide-15-oate. This is a new eudesmalolide.

## CONCLUSION

On the basis of the above stated evidences, the structure of IR-1 has been elucidated as (5z, 13z)-n-decanyl-n-docos-5, 13-dienoate. This is a new fatty acid ester. Earlier (5E, 13E)-docosdienoic acid from *Hybanthus enneaspermus* whole plant has been identified.

Also on the basis of these results the structure of IR-2 has been characterized as n-Hexadecanyl n-docosanoate and the structure of IR-3 has been formulated as 15-[(13z, 18'z, 20'z)-n-tricos-13, 18, 20-trienyl]-eudesmal-4 (11), 6, 12 (13)-trien-8, 14-olide-15-oate. This is a new sesquiterpenic ester.

The structure of IR-4 as according to the above discussed results has been elucidated as 15-[(16z)-n-monadec-16'-enyl]-eudesmal-4 (11), 6, 12 (13)-trien-8, 14-olide-15-oate. This is a new eudesmanolide ester and IR-5 has been characterized as 15-[(16'z), (21'z)-n-tetracos-16', 21'-dienyl]-eudesmal-4 (11) 6, 12 (13)-trien-8, 14-olide-15-oate. This is a new sesquiterpenic ester.

On the basis of the evidences provided the structure of IR-6 has been established as 15-[(16'z)-n-tetracos-16'-enyl]-eudesmal-4 (11), 6, 12 (13)-trien-8, 14-olide-15-oate. This is a new eudesmalolide.

These compounds have a lot of scope to be studied further and their effects on the living system in relation to the medicinal properties of *I. racemosa* already known.

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