A new lupane-type triterpenoid fatty acid ester and other isolates from *Ophiorrhiza shendurunii*

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Abstract

A new pentacyclic triterpenoid fatty acid ester, lupan-20-ol-3(β)-yl hexadecanoate (**1**), together with lupan-20-ol-3(β)-yl acetate (**2**), olean-18-en-3(β)-yl hexadecanoate (**3**), dotriacontanoic acid (**4**), stigmasterol (**5**), rubiadin (**6**), nonadecanoic acid (**7**), palmitic acid (**8**) and camptothecin (**9**) were isolated from the hexane and chloroform extracts of *Ophiorrhiza shendurunii* from south India. Structures of the isolates were determined by ¹H-, ¹³C-, ¹³C-DEPT, ¹H-¹H COSY, HMBC, HSQC, NOESY NMR, FT-IR, DART-MS, ESI-MS, alkaline hydrolysis, derivatization, GC-MS and HPTLC analyses. *O. shendurunii* hexane and chloroform extracts showed significant activities against *Candida albicans* and *Fusarium oxysporum*. Compounds **1** to **3** showed only moderate antiyeast/antifungal activities.

Keywords: *Ophiorrhiza shendurunii*, pentacyclic triterpenoid fatty acid ester, lupan-20-ol-3(β)-yl hexadecanoate, antifungal activity.

Fig. S1 Spectral data of Lupan-20-ol-3(β)-yl hexadecanoate (**1**)

Key NOESY correlations of lupan-20-ol-3(β)-yl hexadecanoate (**1**).

¹H NMR

OSHe-3 HNMR

¹H NMR EXPANSION 01

OSHe-3 HNMR

¹³C NMR

OSHe-3 CNMR

^{13}C NMR EXPANSION 01

OSHe-3 CNMR

^{13}C NMR EXPANSION 02

OSHe-3 CNMR

^{13}C NMR EXPANSION 03

OSHe-3 CNMR

DEPT 90

OSHe-3 DEPT90

DEPT 135

OSHe-3 DEPT135

DEPT 135 EXPANSION 01

OSHe-3 DEPT135

COSY

OSHe-3 COSY

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NAME          OSHe-3 NMR
EXPNO         5
PROCNO        1
Date_         20130228
Time          19.18
INSTRUM       spect
PROBHD        5 mm F4BBO BB-
PULPROG       cosysep
TD            26248
SOLVENT       CDCl3
NS            1
DS            8
SWH           1984.127 Hz
FIDRES        0.968872 Hz
AQ            0.5161460 sec
RG            40.3
CW            252.000 usec
DE            6.50 usec
TE            296.4 K
D0            0.00000300 sec
d1            1.16248906 sec
d13           0.00000400 sec
d16           0.00020000 sec
IN0           0.00050400 sec

===== CHANNEL f1 =====
NUC1          1H
P0            12.55 usec
F1            12.55 usec
PL1           -2.00 dB
PL1W          17.11860847 W
SF01          400.1310338 MHz

===== GRADIENT CHANNEL =====
GPMAM1        SINE.100
GPM1          10.00 %
P16           1000.00 usec
ND0           1
TD            128
SF01          400.131 MHz
FIDRES        15.500992 Hz
SW            4.959 ppm
F0MODE        QF
SI            1024
SF            400.1300082 MHz
WDW           SINE
SSB           0
LB            0.00 Hz
GB            0
PC            1.40
SI            1024
MC2           QF
SF            400.1300082 MHz
WDW           SINE
SSB           0
LB            0.00 Hz
GB            0
  
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HMBC

OSHe-3 HMBC

HMBC EXPANSION 01

OSHe-3 HMBC

HSQC

OSHe-3 HSQC

HSQC EXPANSION 01

OSHe-3 HSQC

NOESY

NOESY EXPANSION 01

SAIFNM130925A-01(OsHe3) NOSEY

SAIF Cochín

MASS SPECTRUM

Acq. Data Name: 13SEPTEMBER0039E
Internal Sample Id: OSHE3 Dr.B. Sabulal [7744]
Ionization Mode: ESI+
MS Calibration Name: PEG_ESI+_1000_250613
Reduction History: Subtract[Correct Base[5.0%];Average(MS[1] 0.021..0.056);,1.0];Correct Base[5.0%];Average(MS[1] 0.394..0.411)
Experiment Date/Time: 9/9/2013 12:20:44 PM

Orifice1 Volt Sweep: 15V
Acquired m/z Range: 10.0..1000.0

Spec. Record Interval: 0.4[s]
Ring Lens Volt: 5[V]
Time of Maximum: 0.401[min]
Operator Name: admin

GC-MS TIC

File :C:\msdchem\2\EOIL2013\july\ranjith\26071305.D

Operator :

Acquired : 26 Jul 2013 15:47 using AcqMethod ESSENTIALOIL_MANUAL700 M.M

Instrument : Instrument #2

Sample Name: OSHe 3H

Misc Info :

Vial Number: 1

GC-MS MASS SPECTRUM (Methyl ester of hexadecanoic acid)

** Search Report Page 1 of 1 **

Unknown: Scan 4556 (37.932 min): 26071305.D\data.ms
Compound in Library Factor = 199

Hit 1 : Hexadecanoic acid, methyl ester
C17H34O2; MF: 899; RMF: 903; Prob 69.7%; CAS: 112-39-0; Lib: replib; ID: 9000.

Spectral data of other isolated compounds (2 to 9)

Lupan-20-ol-3(β)-yl acetate (**2**): White amorphous solid; m. p. 245°C; IR $\lambda_{\max}$ cm^{-1} : 3494, 2941, 1714, 1455, 1370, 1263, 1190, 1160, 979; HRESI-MS m/z : 509.1899 ($\text{M}+\text{Na}$)⁺, calculated mass for $\text{C}_{32}\text{H}_{54}\text{O}_3\text{Na}$ 509.3970; spectral data (^1H , ^{13}C NMR, correlation data) of (**2**) were comparable with literature data (Yuruker et al. 1998). Compound (**2**), lupan-20-ol-3(β)-yl acetate, has an acetate group at C3 instead of the long chain fatty acid ester side chain in (**1**) (Fig. 1). Spectral data of (**2**) were matching with literature (Yuruker et al. 1998) and also with the terpenoid skeleton of (**1**).

Olean-18-en-3(β)-yl hexadecanoate (**3**): White waxy solid; m. p. 75°C; IR $\lambda_{\max}$ cm^{-1} : 2917, 2850, 1726, 1465, 1454, 1378, 1265, 1247, 1221, 1173, 981, 718; ESI-MS m/z : 663.47 ($\text{M}+\text{Na}$)⁺; spectral data (^1H , ^{13}C NMR, correlation data) of (**3**) were comparable with Ragasa et al. 2012. Compound (**3**), olean-18-en-3(β)-yl hexadecanoate (Fig. 1), has a double bond (C18, C19) and a long chain fatty acid side chain at C3 (Ragasa et al. 2012).

Dotriacontanoic acid (**4**): White amorphous solid; m. p. 95°C; IR $\lambda_{\max}$ cm^{-1} : 2915, 2848, 1737, 1704, 1472, 1462, 1217, 729, 719; DART-MS m/z : 481.36 ($\text{M}+\text{H}$)⁺; spectral data (^1H , ^{13}C NMR, correlation data) of (**4**) were comparable with Parmar et al., 1998.

Stigmasterol (**5**): Compound (**5**) (Fig. 1) was identified by comparison of its IR spectrum, m. p. and NMR data with literature (De-Eknamkul and Potduang 2003).

Rubiadin (**6**): Yellow solid; m. p. 291°C; DART MS: 255.14 ($\text{M}+\text{H}^+$); IR $\lambda_{\max}$ cm^{-1} : 3390, 2921, 2515, 1659, 1619, 1579, 1548, 1503, 1479, 1394, 1335, 1300, 1133, 967, 710; ^{13}C NMR: 161.93 (C1), 119.93 (C2), 163.48 (C3), 108.07 (C4), 126.14 (C5), 134.04 (C6), 134.23 (C7), 127.02 (C8), 187.08 (C9), 183.22 (C10), 130.88 (4a), 132.42 (C8a), 110.03 (C9a), 133.08 (C10a), 8.14 (CH_3), 2.095 (CH_3), 7.154 (C4), 7.810 (C5), 7.931 (C8), 8.189 (C6), 8.206 (C7) (Fig. 1).

Nonadecanoic acid (**7**): White solid; m. p. 68°C; DART MS: 299.06 ($\text{M}+\text{H}^+$); IR $\lambda_{\max}$ cm^{-1} : 2956, 2915, 2848, 1695, 1471, 1429, 1411, 1347, 1330, 1295, 1187, 1099, 934, 720; ^{13}C NMR: 180.76,

34.21, 24.73, 29.79, 29.77, 29.74, 29.69, 29.53, 29.46, 29.33, 29.15, 32.02, 22.77, 14.15; ^1H NMR: 2.34 (t), 1.6 (m), 1.258 (brs), 0.879 (t).

Hexadecanoic acid (Palmitic acid) (**8**): White solid; m. p. 63°C ; DART-MS: 257.19 ($\text{M}+\text{H}^+$); IR λ_{max} cm^{-1} : 2955, 2915, 2848, 1695, 1463, 1429, 1411, 1347, 1330, 1295, 1187, 1099, 934, 720; ^{13}C NMR: 180.41, 34.12, 31.96, 29.72, 29.70, 29.67, 29.39, 29.27, 29.09, 14.70, 22.72, 14.13; ^1H NMR: 2.346 (t), 1.63 (m), 1.25-1.30 (brs), 0.88 (t).

Camptothecin (**9**): Pale yellowish solid; m. p. 275°C ; LC-EI-MS, m/z : 349.52 ($\text{M}+\text{H}^+$); IR λ_{max} cm^{-1} : 3430, 2938, 1737, 1650, 1600, 1579, 1499, 1437, 1349, 1323, 1233, 1197, 1156, 1114, 1040, 1005, 916, 829, 724; ^1H NMR (δ): 5.28 (H-5), 8.66 (H-7), 8.10 (d, H-9, $J = 8.0$), 7.70 (t, H-10, $J = 7.2$), 7.85 (t, H-11, $J = 7.6$), 8.16 (d, H-12, $J = 8.4$), 7.37 (s, H-14), 5.41 (dd, H-17 $J = 16.4, 23.4$), 0.90 (t, H-18, $J = 7.2$), 1.89 (H-19), 6.30 (H-OH); ^{13}C NMR (δ): 152.6 (C2), 145.5 (C3), 50.1 (C5), 129.7 (C6), 131.5 (C7), 128.0 (C8), 128.4 (C9), 127.5 (C10), 130.26 (C11), 129.0 (C12), 148.1 (C13), 96.7 (C14), 150.0 (C15), 119.1 (C16), 156.8 (C16a), 65.3 (C17), 7.7 (C18), 30.7 (C19), 72.4 (C20), 172.2 (C21) (Fig. 1).

References

- De-Eknamkul W, Potduang B. 2003. Biosynthesis of β -sitosterol and stigmasterol in *Croton sublyratus* proceeds via a mixed origin of isoprene units. *Phytochemistry*. 62:389-398.
- Parmar VS, Jain SC, Gupta S, Talwar S, Rajwanshi VK, Kumar R, Azim A, Malhotra S, Kumar N, Jain R, Sharma NK, Tyagi OD, Lawrie SJ, Errington W, Howarth OW, Olsen CE, Singh, S. K., Wengel J. 1998. Polyphenols and alkaloids from *Piper* species. *Phytochemistry*. 49:1069-1078.
- Ragasa CY, Espineli DL, Shen CC. 2012. A new triterpene from *Barringtonia asiatica*. *Nat Prod Res*. 26:1869-1875.
- Yuruker A, Orjala J, Sticher O, Rali T. 1998. Triterpenes from *Rhus taitensis*. *Phytochemistry*. 48:863-866.