Decipic acid and 12-acetyl apetalic acid from *Calophyllum decipiens*. Wight

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Triterpenes, stigmasterol, thwaitesii xanthone, apetalic acid, two new chromanones, decipic acid and 12-acetyl apetalic acid are isolated for the first time from the ethyl acetate extract of the bark of *Calophyllum decipiens*, Guttiferae. Structures are elucidated by spectral studies. Apetalic acid shows appreciable amount of radical scavenging activity on comparison with trolox spectra. Apetalic acid showed appreciable amount of radical scavenging activity against (standard) using DPPH free radical. It also shows antibacterial activity against *Mycobacterium tuberculosis* H37Rv at 100 µg/mL using anti-TB drug rifampicin at 0.5 µg/mL.

Keywords: *Calophyllum decipiens*, triterpenes, sterol, xanthone, apetalic acid, decipic acid, 12-acetyl apetalic acid, radical scavenging activity, antibacterial activity

*Calophyllum* species (family Guttiferae) are well known for their bioactivities. *Calophyllum decipiens*, is a big sized tree having very limited distribution in the Western Ghats and in some pockets of the coastal plains of Kerala, India. The tree produces oil bearing seeds. The oil is used for treating skin diseases and also for coating country boats. To the best of our knowledge no phytochemical work has been reported on *Calophyllum decipiens*. Present work concentrates on the isolation and biological evaluation of the secondary metabolites from *Calophyllum decipiens*. Eight compounds have been isolated and identified from the ethyl acetate extract of the bark of the plant using high resolution NMR techniques. Two of them are reported first time. Complete $^1$H and $^{13}$C NMR assignments of the new isolate decipic acid was done with the help of HMQC, HMBC and $^1$H-$^1$H cosy spectra. Apetalic acid showed appreciable amount of radical scavenging and antimicrobial activities.

Experimental Section

The plant material was collected from Chertala, Alapuzha district of Kerala, India during November 2007. Shade dried bark of the plant was powdered to get 500 g of fine powder and was extracted with ethyl acetate in a Soxhlet apparatus. Gummy residue (32 g) was obtained, of which 25 g was chromatographed on a glass column (400 × 80 cm) using silica gel (100-200 mesh) as adsorbent and hexane, chloroform and their mixtures as eluents. IR spectrum was measured using a Beckmann spectrometer. $^1$H NMR spectra were recorded in a Bruker spectrometer at 300 or 500 MHz in CDCl$_3$ with TMS as internal standard. The chemical shifts are recorded in ppm ($\delta$). $^{13}$C NMR spectrum was recorded in 75 or 125 MHz and mass spectra in JEOL JMS600 spectrometer.

Results and Discussion

Compounds 1-5 were identified as friedelan-3-one, $\beta$-amyrin, 3$\beta$-hydroxy olean-5(6)-ene, stigmasterol and thwaitesii xanthone, respectively.

Compound 6 obtained from chloroform fractions, separated as yellow viscous oil and failed to crystallize in any medium. UV-Visible absorptions at 263, 272, 319, and 363(sh) nm which were shifted to 273, 290 and 380 nm with NaOH indicated a chromanone type compound. FAB mass spectrum of 6 gave a molecular ion peak at m/z 388 which was the usual range for the molecular weight of a chromanone acid. IR spectrum consisted of a broad peak at 3500 cm$^{-1}$ characteristic of a H-bonded OH group and prominent absorptions at 1705 and 1647 cm$^{-1}$.

The latter absorptions indicated a $\gamma$-pyranone moiety. $^1$H NMR spectrum (500 MHz) showed resemblances with that of compound 5. Two methyl singlets present at $\delta$ 1.40 and 1.36 showed the presence of one dimethyl substituted pyran ring. Two one proton doublets at $\delta$ 6.6 and 5.5 ($J = 10$ Hz) were assigned to one double bond in the pyran ring. Two methyl doublets at $\delta$ 1.1 and 1.3 were assigned to the gem dimethyl groups of the pyranone ring. In addition to these absorptions, the high field region possessed a number of sharp signals between $\delta$ 0.8 and 2.8 which indicated an aliphatic substitution in one of the rings. The substitution was identified to be in the phenolic ring from the presence of an one proton multiplet at
δ 3.7. Twenty-two signals were present in the $^{13}$C NMR (125 MHz) spectrum of the compound. DEPT 135 spectrum showed the presence of five methyl groups, three methylene groups, five CH groups and nine quaternary carbon atoms which were in good agreement with the $^1$H NMR data. Chemical shifts at δ 157.3, 201.29 and 179.6 confirmed the presence of carbon with phenolic OH group, lactone group and carboxylic acid groups respectively. Comparison of these spectral data with literature showed compound 6 to be apetalic acid$^{10-13}$, a chemo-taxonomic marker compound.

Compound 7 was isolated by repeated column chromatography in silica gel using chloroform-ethyl acetate mixture. Compared to apetalic acid, 7 was present only in small amount in the plant extract (15 mg). FABMS showed the molecular mass as 374. $^1$H NMR and $^{13}$C NMR spectra were in close resemblance with that of apetalic acid. $^1$H NMR (500 MHz) spectrum consisted of methyl signals at δ 0.86 (t, $J = 7.5$Hz), 1.30 (d, $J = 6$ Hz), 1.10 (d, $J = 7.5$Hz), 1.36(s) and 1.45(s). Other characteristic signals were at δ 5.50 and 6.60 as doublets with coupling constant 10 Hz indicating the presence of disubstituted pyran ring with unsaturation at 6 and 7 positions which is common for chromanone type compounds in calophyllum$^{10-13}$. The one proton multiplet observed at δ 3. 71 is also a familiar feature of C-10 substituted chromanones similar to apetalic acid. The number of carbon atoms in the side chain at C-10 was ascertained by the analysis of $^{13}$C NMR, $^1$H-$^1$H COSY (500 MHz) (Figure 1, Table I), HMQC (500/125 MHz) (Figure 1a, Table II) and HMBC (500/125 MHz) (Figure 1a, Table III) spectra.
spectra. NMR signal at HMQC (500/125 MHz) and HMBC (500/125 MHz) C-10 were traced out by the close examination of apetalic acid. The connectivities of the side chain at chromanone ring were found to be identical to that of DEPT 135). All the chemical shifts of the HMBC correlations with carbon signals at 78.00 are evidenced by the mutual correlations of the olefinic proton doublet at δ 6.60 with carbon signals at δ 102.56 (C-5a) and 157.3 (C-5 and C-9a). Presence of gem dimethyl groups at δ 78.00 are evidenced by the mutual correlations of the methyl groups (C-8\textsuperscript{13}Me and C-8\textsuperscript{15}Me) and also with quaternary carbon at δ 78.00 (C-8). Olefinic carbon at 125.5 gave cross peaks with protons at C-8\textsuperscript{13}Me and C-8\textsuperscript{15}Me (δ 1.45 and 1.36). The point of attachment at C-10 was revealed by the cross peak obtained with proton signal at δ 3.71 as a multiplet and carbon signal at δ 108.82(C-10) of the aromatic ring. Signal at δ 3.71 showed correlations with that at δ 38.66 (C-12), 179.46 (C-13), 35.48 (C-14) and a methyl carbon at δ 13.98 (C-15). These correlations clearly indicated the presence of only five carbon atoms in the side chain including a COOH group and its attached to the chromanone ring as in apetalic acid. Molecular ion peak of 7 was found to be at 374 by the FABMS with a base peak at m/z 373(M-1H)+. Hence compound 7 has 14 mass units lesser than apetalic acid and possess a five carbon side chain attached to C-10 of apetalic acid and is named as decipic acid. It is the first report of this compound. Apetalic acid has a six carbon side chain attached to C-10 of apetalic acid skeleton is isolated from Calophyllum pinetorium\textsuperscript{11}.

Compound 8 was isolated as a red gummy material from chloroform-ethylacetate fractions. IR spectrum displayed peaks corresponding to OH group (3414 cm\textsuperscript{-1}) and pyranone ring (1712 and 1633 cm\textsuperscript{-1}). \textsuperscript{13}C NMR spectrum had 21 signals with 9 quaternary carbon atoms, 5 CH, 2CH\textsubscript{2} and 5 CH\textsubscript{3} carbons (\textsuperscript{13}C DEPT 135). All the chemical shifts of the chromanone ring were found to be identical to that of apetalic acid. The connectivities of the side chain at C-10 were traced out by the close examination of HMQC (500/125 MHz) and HMBC (500/125 MHz) spectra. NMR signal at δ 4.50 (1H, dq) showed HMBC correlations with carbon signals at δ 16.20, 44.19 and 9.27. Proton signal at δ 2.54 (1H, dq) gave cross peaks with carbon signals at δ 9.27, 16.20, 76.71, 101.2 and 201.12. These correlations helped in positioning the pyranone ring of compound 7. The presence of pyran ring fused to an aromatic ring having a phenolic group was evidenced by HMBC correlations of the olefinic proton doublet at δ 6.60 with carbon signals at δ 102.56 (C-5a) and 157.3 (C-5 and C-9a). Presence of gem dimethyl groups at δ 78.00 are evidenced by the mutual correlations of the methyl groups (C-8\textsuperscript{13}Me and C-8\textsuperscript{15}Me) and also with
(3H, t) indicated the end methyl group of an aliphatic side chain as in compounds 6 and 7. 13C NMR spectrum furnished 23 signals. All the signals for the chromanone ring of 8 were matching with that of apetalic acid and decipic acid except one signal. This major difference was the replacement of signal at δ 179.6 corresponding to the carboxylic acid group in 6 and 7 which by a signal at 201.0. This indicated the presence of acetyl group in 8 instead of a COOH group in apetalic acid. FABMS gave a molecular ion peak at m/z 386 confirming the above structure. Hence 8 is 12-acetyl apetalic acid.

Radical scavenging activity

Radical scavenging activity of apetalic acid was measured by DPPH (1, 1-diphenyl-2-picrylhydrazyl) method using Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) as standard. Standard solutions of DPPH in alcohol were treated with solutions of the samples and the activity was assessed by measuring the fall in absorbance at 517 nm spectrophotometrically. Percentage inhibition and TEAC were calculated using the relations

% inhibition, \( T = \frac{(A_{\text{solvent}} - A_{\text{compound}})}{A_{\text{solvent}}} \times 100 \)

% inhibition of sample/ % inhibition of trolox

Apetalic acid 6 showed inhibition of 22.64 % and trolox equivalent antioxidant activity (TEAC) of 0.46 which indicate the high antioxidant capacity of apetalic acid.

Antimycobacterial activity

Antimycobacterial study of the compound 6 was conducted with Mycobacterium tuberculosis H37Rv
in 96 well microtitre plates. **Rifampicin** is used as the standard antituberculosis drug. Change of colour from blue to pink would indicate growth while lack of colour change would suggest inhibition of growth. Apetalic acid showed activity at 100 µg/mL. Thwaitesii xanthone was inactive.

**Experimental Section**

**Decipic acid, 7**: MF. C_{21}H_{26}O_{6}. Reddish yellow oil, M.Wt. 374. IR (KBr): 3510 (OH), 1700 (C=O), 1641 cm\(^{-1}\) (γ-pyranone); \(^{1}H\) NMR and \(^{13}C\) NMR (see Tables I and II respectively).

**12-Acetyl apetalic acid, 8**: MF. C_{23}H_{30}O_{5}. Reddish yellow oil. M.Wt. 386. IR (KBr): 3414 (OH), 1713 (C=O), 1634 cm\(^{-1}\) (γ-lactone); \(^{1}H\) NMR (CDCl\(_3\), 500 MHz): δ 4.10 (1H, dq, J = 6.5, 3.5 Hz, H-2), 2.35 (1H, dq, J = 7.2 Hz, 3.5 Hz, H-3), 6.38 (1H, d, J = 10 Hz, H-6), 5.78 (1H, d, J = 10 Hz, H-7), 3.80 (1H, m, H-11), 2.3 (2H, m, H-12a and 12b), 1.50 (1H, m, H-14a), 1.70 (1H, m, H-14b), 1.2 (2H, m, H-15), 0.88 (3H, t, H-16), 1.32 (2-Me, d, J = 6.5 Hz), 1.11 (3-Me, d, J = 6.5 Hz), 1.46 (s, 8'-Me), 1.36 (s, 8''-Me), 2.05 (3H, s, 12-Ac); \(^{13}C\) NMR (CDCl\(_3\), 125MHz): δ 76.71 (C-2), 44.10 (C-3), 201.29 (C-4), 101.31 (C-4a), 151.32 (C-5), 102.20 (C-5a), 115.62 (C-6), 125.50 (C-7), 78.11 (C-8), 28.21, 28.11 (C-8' and C-8''), 157.32 (C-9a), 108.76 (C-10), 159.81 (C-10a), 16.20 (2-Me), 9.30 (3-Me), 30.48 (C-11), 38.42 (C-12), 201.00 (C-13), 35.43 (C-14), 20.81 (C-15), 14.11 (C-16).

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**References**