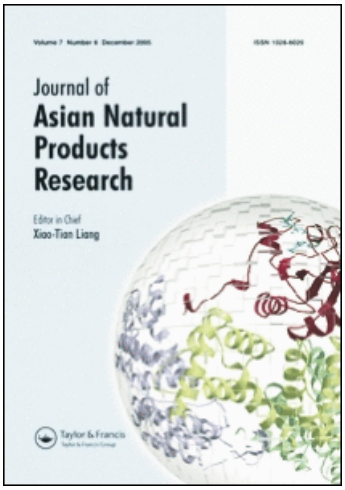


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# New beyerane and isopimarane diterpenoids from Rhizophora mucronata

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# NEW BEYERANE AND ISOPIMARANE DITERPENOIDS FROM *RHIZOPHORA MUCRONATA**

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Chemical examination of the ethyl acetate extract of the roots of *Rhizophora mucronata* collected from Mangalore Coast resulted in the isolation of three more new diterpenoids, rhizophorins C–E (**1**–**3**) in addition to the two, rhizophorin A, (6*R*,11*S*,13*S*)-6,11,13-trihydroxy-2,3-seco-14-labden-2,8-olid-3-oic acid, and rhizophorin B, *ent*-3β,20-epoxy-3α,18-dihydroxy-15-beyerene, recently reported. The structures of the new diterpenoids have been elucidated by a study of their physical and spectral data as 17-hydroxybeyer-15-en-3-one (**1**), 3β,17-diacetoxy-15-beyeren-2-one (**2**) and 3β,6α-diacetoxy-8(14),15-isopimaradien-2-one (**3**).

*Keywords*: Indian mangrove; *Rhizophora mucronata*; Rhizophoraceae; Diterpenoids; Rhizophorins C, D, E; Beyeranes; Isopimarane

*Rhizophora mucronata* Lam (Rhizophoraceae) is one of the two mangrove plants of the *Rhizophora* genus that occur along the tidal shores and creeks of India including the Andaman and Nicobar Islands [1]. Besides being a source of tannins, *R. mucronata* is known to be a powerful astringent and is used in the treatment of hemorrhage, haematuria and angina [2]. The bark of *R. mucronata* is also used as a cure for diabetes [2]. A variety of steroid and triterpenoid derivatives have been reported from the leaves [3,4] and bark [5] of this plant. In our continuing interest on the chemical constituents of the Indian mangrove plants [6,7], we have examined the roots of *R. mucronata* collected from the Mangalore coast of India and reported recently the isolation of two novel diterpenoids, rhizophorin A [8], (6*R*,11*S*,13*S*)-6,11,13-trihydroxy-2,3-seco-14-labden-2,8-olid-3-oic acid and rhizophorin B [9], *ent*-3β,20-epoxy-3α,18-dihydroxy-15-beyerene from the ethyl acetate extract. We report herein the isolation of three new diterpenoids, rhizophorins C, D and E (**1**–**3**) from the same extract. The structures of the three new compounds were established as 17-hydroxybeyer-15-en-3-one, rhizophorin C (**1**); 3β,17-diacetoxy-15-beyeren-2-one, rhizophorin D (**2**); and 3β,6α-diacetoxy-8(14),15-isopimaradien-2-one, rhizophorine E (**3**) by a study of their physical and spectral data.

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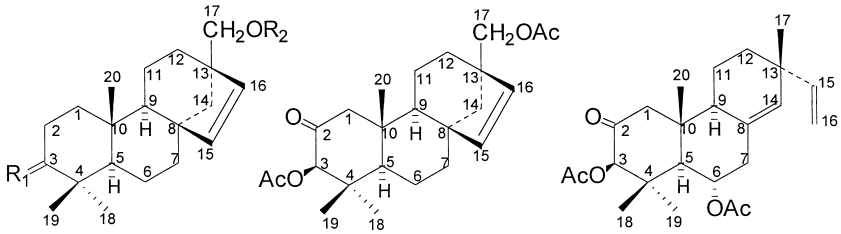


**1** $R_1 = O$ ; $R_2 = H$
**1a** $R_1 = O$ ; $R_2 = Ac$
**1b** $R_1 =$  ; $R_2 = H$

**2** **3**

## EXPERIMENTAL

### General Experimental Procedures

Optical rotations were determined on a Roudalph Autopol-III polarimeter. Melting points were determined on a VEB-Analytic Dreader HMK hot plate and are uncorrected. IR spectra were recorded on a Perkin–Elmer-841 IR spectrometer in $CHCl_3$ solution. UV spectra were recorded on a Milton Roy Spectronic 1201 spectrometer in $CHCl_3$. $^1H$ NMR spectra were measured on a Bruker Advance DRX 300 and JEOL JNM EX-90 spectrometers. $^{13}C$ NMR spectra were measured on a Bruker Advance DRX 300 spectrometer at 75 MHz and JEOL JNM EX-90 spectrometer at 22.5 MHz using $CDCl_3$ as a solvent and tetramethylsilane as an internal reference. Elemental analyses were determined on a Carlo Erba 1108 instrument. Mass spectra were obtained on a JEOL JMS-300 spectrometer.

### Plant Material

The roots of *R. mucronata* were collected from Manglore coast of India in March 1996 and the material was kindly identified by Dr H.R.V. Reddy, College of Fisheries, Mangalore. Voucher specimens (Code: AU1/130) are deposited at the Marine Museums of the School of Chemistry, Andhra University and the National Institute of Oceanography, Goa.

### Extraction and Isolation

The air-dried and powdered plant material (2.5 kg) was exhaustively extracted with $CH_2Cl_2$/MeOH (1:1). Removal of the solvent from the combined $CH_2Cl_2$/MeOH extracts gave a residue (50 g) which was extracted with EtOAc (3 × 500 ml). Removal of the solvent from the EtOAc extract under reduced pressure gave a residue (38 g). This residue was subjected to column chromatography over a column of silica gel (Acme brand, 100–200 mesh, 400 g) using solvents of increasing polarity from *n*-hexane through EtOAc. In all, 250 fractions (750 ml) were collected. The fractions showing similar spots were combined and the residues from therein were subjected to rechromatography over silica gel or silver nitrate (20%) impregnated silica gel columns to yield rhizophorins A-E as given below.

The residue from the column fractions 81–95 (*n*-hexane/EtOAc, 8.5:1.5) furnished rhizophorin C (**1**) (50 mg), the residue from the column fractions 146–162 (*n*-hexane/EtOAc, 7.5:2.5) furnished rhizophorin A (20 mg), the residue from the column fractions 163–180 (*n*-hexane:EtOAc, 7.0:3.0) furnished rhizophorin D (**2**) (30 mg) and rhizophorin E (**3**)

TABLE I $^1$H NMR data of compounds **1**–**3** (chemical shifts in $\delta$ from TMS (multiplicity, $J$ in Hz) in $CDCl_3$)

| *Position* | **1*** | **2*** | **3*** |
|---|---|---|---|
| H-lα | 1.38 m | 2.19 m | 2.55 (d, $J = 12$) |
| H-lbeta; | 1.92 m | 2.38 m | 2.20 (d, $J = 12$) |
| H-2α | 2.55 m | | |
| H-2β | 2.32 m | | |
| H-3 | | 4.94 s | 4.98 s |
| H-5 | 1.35 (d, $J$=5) | 1.62 m | 1.72 m |
| H-6α | 1.34 m | 1.70 m | |
| H-6β | 1.34 m | 1.52 m | 5.14 m |
| H-7α | 1.44 m | 1.50 m | 1.42 m |
| H-7β | 1.75 m | 1.77 m | 1.75 m |
| H-9 | 1.07 m | 1.34 m | 2.18 m |
| H-11α | 1.52 m | 1.50 m | 1.78 m |
| H-11β | 1.52 m | 1.30 m | 1.78 m |
| H-12α | 1.32 m | 1.40 m | 2.35 m |
| H-12β | 1.42 m | 1.40 m | 2.35 m |
| H-14α | 1.02 m | 1.09 m | 5.41 s |
| H-14β | 1.68 m | 1.09 m | |
| H-15 | 5.84 (d, $J = 6$) | 5.74 (d, $J = 6$) | 5.78 (dd, $J = 18, 11$) |
| H-16 | 5.64 (d, $J = 6$) | 5.61 (d, $J = 6$) | 4.95 (d, $J = 18$) |
| | | | 4.92 (d, $J = 11$) |
| $H_2$-17 | 3.50† (ABq, $J = 10.8$) | 3.96† (ABq, $J = 10.8$) | 1.11 s ($H_3$-17) |
| $H_3$-18 | 1.08 s | 1.10 s | 1.11 s |
| $H_3$-19 | 1.05 s | 0.85 s | 0.87 s |
| $H_3$-20 | 0.92 s | 0.75 s | 0.85 s |
| OAc | | 2.17 s | 2.18 s |
| | | 2.08 s | 2.00 s |

* Measured at 300 MHz.
† Quartets centered at the $\delta$ given.



(20 mg), and the residue from the column fractions 181–195 (*n*-hexane/EtOAc, 6.0:4.0) on crystallisation from $CHCl_3$-MeOH gave rhizophorin B (200 mg).

### 17-Hydroxybeyer-15-en-3-one (rhizophorin C) (1)

Colourless needles from MeOH, 50 mg, mp 145–148°C, $[\alpha]_D^{25} = -38.0$ ($c$ 1.5, $CHCl_3$); IR (Nujol) $\nu_{max}$ 3485, 1690, 680 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 300 MHz) data see Table I; $^{13}$C NMR ($CDCl_3$, 75 MHz) data see Table II; EIMS *m/z* 302 $[M]^+$, 284 $[M - H_2O]^+$, 272, 243, 216, 185, 119, 105, 81; anal. C 79.28%, H 9.72%, calcd for $C_{20}H_{30}O_2$, C 79.47%, H 9.93%.

### Acetylation of Rhizophorin C: Formation of the Acetate (1a)

Rhizophorin C (25 mg) was acetylated with a mixture of acetic anhydride (2 ml) and pyridine (2 ml) at room temperature for 24 h. After usual work up, it yielded a monoacetyl derivative **1a** (20 mg), colourless needles from MeOH, mp 126–128°C, $[\alpha]_D^{25} = -40.2$ ($c$ 0.8, $CHCl_3$); IR (Nujol) $\nu_{max}$ 1725, 1240, 1700, 715 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 90 MHz), 0.95, 1.0, 1.10 (3H each, s, Me), 2.10 (3H, s, acetate methyl), 4.0 (2H, ABq, $J = 10.5$ Hz, $H_2$-17), 5.62 (1H, d, $J = 6.0$ Hz, H-15), 5.82 (1H, d, $J = 6.0$ Hz, H-16); $^{13}$C NMR ($CDCl_3$, 75 MHz) data see Table II; EIMS *m/z* 344 $[M]^+$; anal. C 76.50%, H 9.12%, calcd for $C_{22}H_{32}0_3$, C 76.74%, H 9.30%.

### Reduction of Rhizophorin C With $NaBH_4$: Formation of 3α-hydroxybeyer-15-ene (1b)

To rhizophorin C (30 mg) in methanol (1.5 ml) was added sodium borohydride (30 mg) and the solution left for 4 h at room temperature. After work-up in usual way, the reaction product

TABLE II $^{13}C$ NMR data of compounds **1**, **1a**, **1b**, **2**, **3** (chemical shifts in $\delta$ from TMS taken in $CDCl_3$)

| *Carbon No.* | **1*** | **1a†** | **lb†** | **2*** | **3*** |
|---|---|---|---|---|---|
| 1 | 37.9 | 37.8 | 37.6 | 52.5 | 52.0 |
| 2 | 34.4 | 34.2 | 27.4 | 204.0 | 203.2 |
| 3 | 217.3 | 217.2 | 79.0 | 83.9 | 83.5 |
| 4 | 47.6 | 47.5 | 39.0 | 43.6 | 44.3 |
| 5 | 55.8 | 55.7 | 55.6 | 55.1 | 53.9 |
| 6 | 19.9 | 19.7 | 20.0 | 19.6 | 68.8 |
| 7 | 36.6 | 36.4 | 36.5 | 36.3 | 39.7 |
| 8 | 48.4 | 48.2 | 48.8 | 48.5 | 133.4 |
| 9 | 52.5 | 52.1 | 53.2 | 52.4 | 55.2 |
| 10 | 36.9 | 36.8 | 36.8 | 43.3 | 43.2 |
| 11 | 21.0 | 20.9 | 20.5 | 19.9 | 22.1 |
| 12 | 27.6 | 27.8 | 28.0 | 27.7 | 35.1 |
| 13 | 50.1 | 47.8 | 50.4 | 47.8 | 37.8 |
| 14 | 55.4 | 55.7 | 55.8 | 55.7 | 130.2 |
| 15 | 136.3 | 135.9 | 137.0 | 135.5 | 147.4 |
| 16 | 132.6 | 132.1 | 132.2 | 132.5 | 110.7 |
| 17 | 68.4 | 69.4 | 69.0 | 69.3 | 25.8 |
| 18 | 26.2 | 26.2 | 28.2 | 29.6 | 28.9 |
| 19 | 21.8 | 21.8 | 15.2 | 17.6 | 17.4 |
| 20 | 14.7 | 14.6 | 16.0 | 15.6 | 16.1 |
| OAc | | 170.4 | | 170.4 | 170.5 |
| | | 20.8 | | 21.0 | 20.5 |
| OAc | | | | 171.1 | 170.2 |
| | | | | 20.2 | 20.6 |

* Measured at 75 MHz.
† Measured at 22.5 MHz.



was subject to column chromatography (*n*-hexane/acetone, 8.5:1.5), to give compound 3α-hydroxybeyer-15-ene (**1b**), 25 mg, colourless needles from MeOH, mp 190–192°C, $[\alpha]_D^{25} = +25.6$ (*c* 1.0, $CHCl_3$); IR (Nujol) $\nu_{max}$ 3450, 700 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 90 MHz) 0.79, 0.82, 1.10 (3H each, s, Me), 3.50 (2H, ABq, $J = 10.5$ Hz, $H_2$-17), 3.30 (1H, dd, $J = 11, 4$ Hz, H-3), 5.60 (1H, d, $J = 6.0$ Hz, H-15), 5.83 (1H, d, $J = 6.0$ Hz, H-16); $^{13}C$ NMR ($CDCl_3$, 75 MHz) data see Table II; EIMS *m/z* 304 $[M]^+$; anal. C 78.72%, H 10.18%, calcd for $C_{20}H_{32}0_2$, C 78.94%, H 10.52%.

### 3β,17α-Diacetoxy-15-beyeren-2-one (rhizophorin D) (2)

Colourless needles from MeOH, mp 165–168°C, $[\alpha]_D^{25} = -45.1$ (*c* 1.75, $CHCl_3$); IR (Nujol) $\nu_{max}$ 1700, 1740, 1230, 765 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) data see Table I; $^{13}C$ NMR ($CDCl_3$, 75 MHz) data see Table II; EIMS *m/z* 402 $[M]^+$, 360 $[M - COCH_2]^+$, 342, 287, 227, 185, 157, 133, 91, 83; anal. C 71.45%, H 8.32%, calcd for $C_{24}H_{34}O_5$, C 71.64%, H 8.45%.

### 3β,6α-Diacetoxy-8(14),15-isopimaradien-2-one (rhizophorin E) (3)

Colourless oil, $[\alpha]_D^{25} = -26.4$ (*c* 1.7, $CHCl_3$); IR (Nujol) $\nu_{max}$ 1725, 1230, 1630, 1020, 910, 760 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) data see Table I; $^{13}C$ NMR ($CDCl^3$, 75 MHz) data see Table II; EIMS *m/z* 342 $[M - 60]^+$, 327, 285, 267, 185, 135, 119, 81; anal. C 71.50%, H 8.24%, calcd for $C_{24}H_{34}0_5$, C 71.64%, H 8.45%.

## RESULTS AND DISCUSSION

Rhizophorin C (**1**) came as colourless needles from methanol, mp 145–148. Its molecular formula was assigned as $C_{20}H_{30}O_2$ from elemental analysis and intense molecular ion at

*m/z* 302 (98%) in the EIMS. It exhibited keto carbonyl (1690 $cm^{-1}$) and hydroxyl (3485 br $cm^{-1}$) absorptions in its IR spectrum accounting for both the oxygens of the molecule. No conjugation was noticed in its UV spectrum. Its $^{1}H$ and $^{13}C$ NMR spectra revealed that it is a beyerane diterpenoid [10]. Its $^{1}H$ NMR spectrum exhibited two olefinic protons at $\delta$ 5.64 and 5.84 (each d, $J = 6$ Hz) accounting for the characteristic olefinic protons H-15 and H-16 of the beyerene skeleton and two hydroxymethylene protons centered at $\delta$ 3.5 as ABq accounting for the presence of a primary hydroxyl in the molecule. Rhizophorin C on acetylation with pyridine/$Ac_2O$ gave a mono acetate **1a**, $C_{22}H_{32}O_3$, which showed the presence of a keto carbonyl (1700 $cm^{-1}$) and an acetate carbonyl (1725 and 1240 $cm^{-1}$) and the absence of hydroxyl in its IR spectrum. The $^{1}H$ NMR spectrum of the acetate showed, besides other signals, the presence of a primary acetate at $\delta$ 2.1, 3H, s; and two acetoxymethylene protons centered at $\delta$ 4.05 as ABq supported by the acetate carbonyl carbon at $\delta$ 170.4 in its $^{13}C$ NMR spectrum (Table II).

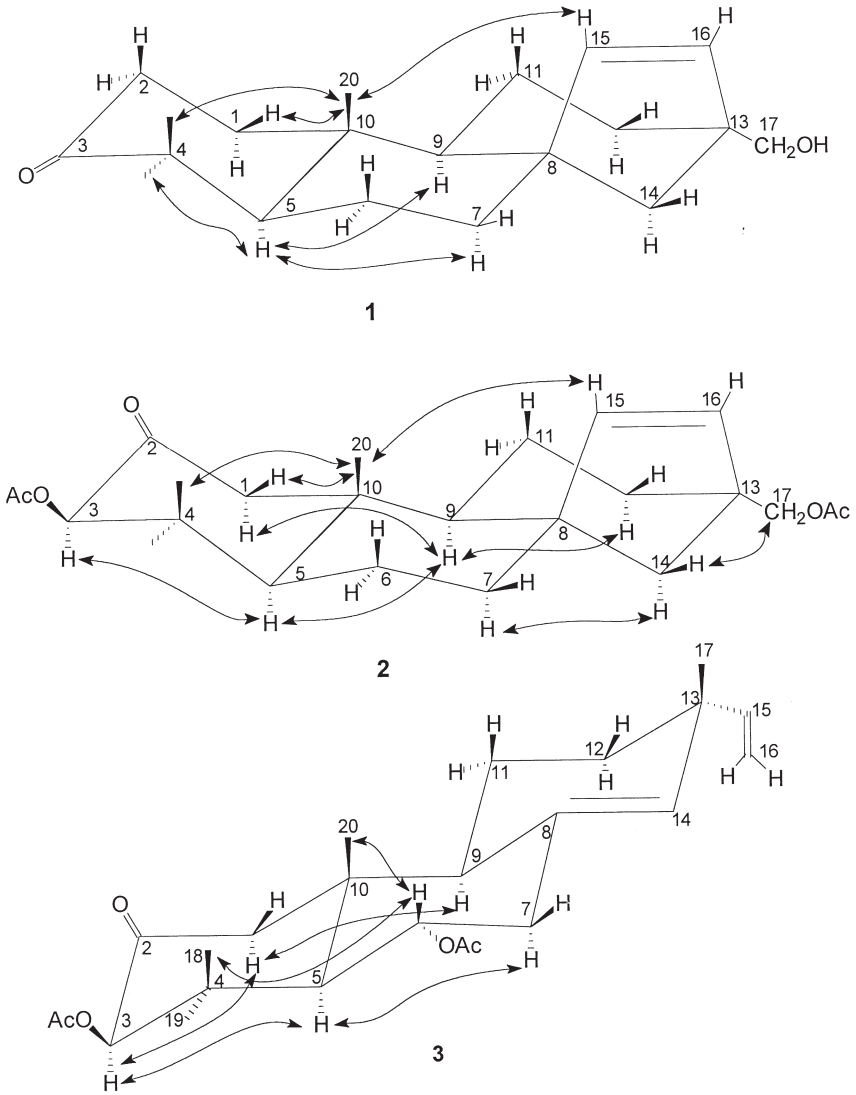


FIGURE 1 NOESY correlations for compounds **1**–**3**.

The $^{13}C$ NMR spectrum of rhizophorin C indicated the presence of 3 methyls, 8 methylenes, 4 methines and 5 quaternary carbons from its DEPT spectrum. The assignment of the chemical shifts of the respective carbons was made by $^{13}C-^{1}H$ (HMQC) and $^{1}H-^{1}H$ COSY correlations. The keto carbonyl signal at $\delta$ 217.3 accounted for a 3-keto diterpenoid [11]. The olefinic carbons of the beyerene C-15 and C-16 appeared at 136.3 (d), 132.6 (d). The remaining problem is to fix which of the four methyls, $H_3$-17, $H_3$-18, $H_3$-19 or $H_3$-20 is in the form of hydroxymethyl. In the absence of a hydroxyl at C-17, the C-14 carbon in beyerenes comes around $\delta$ 60 ppm [12], but in the presence of a hydroxyl the same carbon comes around $\delta$ 55.0 ppm [13]. Since the value of C-14 in rhizophorin C appeared at 55.4, it was concluded that it is having a hydroxyl at C-17. The structure of rhizophorin C could thus be derived as 17-hydroxybeyer-15-en-3-one (**1**).

The location of the functional groups in the molecule was confirmed by the HMBC correlations and the relative stereochemistry at the chiral centers from the NOESY correlations (Fig. 1). The important HMBC correlations observed between the 3-keto carbon ($\delta$ 217.3) and $H_3$-18, $H_3$-19, $H_2$-1 and $H_2$-2; the hydroxymethyl carbon C-17 ($\delta$ 68.4) and H-16 ($\delta$ 5.64) and $H_2$-12; the olefinic carbon C-16 ($\delta$ 132.6) and $H_2$-14, $H_2$-17, H-15 and $H_2$-12 and the olefinic carbon C-15 ($\delta$ 136.3) and $H_2$-14, H-16, $H_2$-7 as well as H-9 are in full agreement with the structure of rhizophorin C (**1**). The important NOESY correlations observed between H-5 and H-9 and the absence of correlation between $H_3$-20 and H-5 and $H_3$-20 and H-9 suggested *trans–trans* relationship between C-5, C-10 and C-10, C-9, respectively. As in other beyeranoids the $H_3$-20 ($\delta$ 0.92) showed correlation with H-15 ($\delta$ 5.84), being close in space, to suggest that the $H_3$-20 and the bridge head at C-8 and C-13 are on the same side with $CH_2OH$-17 on the other side. The foregoing and other NOESY correlations (Fig. 1) confirmed the relative stereochemistry of rhizophorin C as shown in the structure **1**. The absolute configuration of rhizophorin C could not be decided but taken as for beyerane in view of its leavo specific rotation as noticed for all beyeranes [13]. The structure of rhizophorin C could thus be derived as 17-hydroxybeyer-15-en-3-one (**1**).



The structure of rhizophorin C could be further confirmed by reducing its 3-keto group with $NaBH_4$ in $CH_3OH$ to give the 3-hydroxy compound (**1b**), identical in every respect ($^{1}H$ NMR, mp, $[\alpha]_D^{25}$) with the compound isolated earlier from the plant species *Hallichysum clendroideum* [14]. Its $^{13}C$ NMR data have been recorded now (Table II).

Rhizophorin D (**2**) came as colourless needles from methanol, mp 165–168°C. Its molecular formula was assigned as $C_{24}H_{34}O_5$ from elemental analysis and mass ion at *m/z* 402 in the EIMS. It showed multiple carbonyl absorptions between 1740 and 1700 $cm^{-1}$ besides a strong peak at 1230 $cm^{-1}$ in the IR spectrum showing the presence of at least one acetate. Its UV spectrum showed ketonic ($n \rightarrow \pi^*$) absorption at 285 nm. A preliminary study of its $^{1}H$ NMR spectrum showed that rhizophorin D is also a new beyerane derivative related to rhizophorin C. The $^{1}H$ NMR spectrum showed two acetates, one primary ($-CH_2OCOCH_3$; ABq centered at $\delta$ 3.97, 2H, d, $J = 10$ Hz and 2.08, s) and one secondary ($-CHOCOCH_3$ $\delta$ 4.94, 1H, s and $\delta$ 2.17, 3H, s). The characteristic beyerene olefinic protons 15-H and 16-H came at $\delta$ 5.74 and 5.61 (each d, $J = 6$ Hz). All the five oxygens of the molecule could thus be accounted for, four in the two acetates and the remaining in a carbonyl. Its $^{13}C$ NMR spectrum (Table II) showed the presence of 5 methyls, 7 methylenes, 5 methines and 7 quaternary carbon signals from its DEPT spectrum. The chemical shifts of the respective carbons were assigned based on the comparative values from the literature as well as from HMQC, and $^{1}H-^{1}H$ COSY data. The carbon signals at $\delta$ 204.0, 171.1, 170.4 accounted for one keto and two acetate carbonyls, while the signals at $\delta$ 132.5 (d) and 135.5 (d) accounted for the olefinic carbons C-16 and C-15. The appearance of a primary acetate suggested that one of the four methyls of beyerane skeleton is present as acetoxymethyl group, while the secondary acetate might be in one of the rings. The $^{13}C$ value of the keto

carbonyl at $\delta$ 204.0 indicated that the keto group is not at C-3 in which case it should appear around $\delta$ 218.0 as noticed in rhizhophorin C and other 3-keto diterpenoids [11]. The presence of $\alpha$-acetoxymethine proton $\delta$ 4.94 as a singlet suggested that the secondary acetate could be located at C-3 flanked by a keto group at C-2. The $^1$H chemical shifts of $H_3$-18 ($\delta$ 1.10) and $H_3$-19 ($\delta$ 0.85) as noticed in other diterpenoid 3-acetates [12] also supported this. The primary acetate could be located at C-17 keeping in view of the carbon chemical shift of C-14 ($\delta$ 55.7) as noticed in rhizophorin C. The structure of rhizophorin D could thus be derived as 3,17-diacetoxy-15-beyeren-2-one.

The location of the respective functional groups was supported by the HMBC correlations and the relative stereochemistry at the chiral centers from the NOESY correlations (Fig. 1). The C-2 ($\delta$ 204.0) with a carbonyl showed correlations with H-3 and $H_2$-1. The acetate carbonyl at C-3 ($\delta$ 171.1) showed correlations with H-3 and acetate methyl protons. The C-4 ($\delta$ 43.6) showed correlations with $H_3$-18, $H_3$-19, H-3 and H-5 as well. Similarly, the C-3 ($\delta$ 83.9) showed correlations with $H_2$-2, $H_3$-18 and $H_3$-19 confirming beyond doubt the location of the functional groups in ring A. The C-17 ($\delta$ 69.3) with acetate group showed correlations with H-14, H-16 and acetate methyl protons in support of the location of primary acetate at C-13.

With respect to the stereochemistry, the NOESY correlation observed between H-5, and H-9 and the absence of correlation of both of these with $H_3$-20 showed *trans–trans* relationship between C-5, C-10 and C-9, C-10. The correlation between H-5 ($\delta$ 1.62) and H-3 ($\delta$ 4.94) suggested their *cis* relationship to indicate the configuration of H-3 as $\alpha$ and that of the 3-acetate as $\beta$. As in the beyerane derivatives, the $H_3$-20 ($\delta$ 0.75) showed correlation with H-15 ($\delta$ 5.74) suggesting that the bridge head at C-8 and C-13 is *cis* to $H_3$-20 both being $\beta$. This suggests that the 17-acetoxymethyl at C-13 is $\alpha$. These as well as other correlations (Fig. 1) fully supported the beyerane stereochemistry of the molecule. The absolute configuration of rhizophorin D could not be decided but taken as for beyerane in view of its leavo specific rotation as noticed for all beyeranes [13]. The structure of rhizophorin D could thus be derived as 3$\beta$,17-diacetoxy-15-beyeren-2-one (**2**).

Rhizophorin E (**3**) came as oil. Its molecular formula was assigned as $C_{24}H_{34}0_5$ from elemental analysis and mass ion at *m/z* 342 (M $-$ 60) in the EIMS. Its IR spectrum showed close multiple carbonyl absorptions around 1725 cm$^{-1}$, of which at least one is that of an acetate (1230 cm$^{-1}$), besides vinylic absorption at 1630, 1020, 910, 760 cm$^{-1}$. Its UV absorption 256 nm showed conjugation. Its $^1$H NMR spectrum showed the presence of two acetates at $\delta$ 2.0 and $\delta$ 2.18, both representing secondary acetates with the corresponding $\alpha$-methine protons coming at $\delta$ 4.98 (1H, s) and $\delta$ 5.14 (1H, m). The spectrum also exhibited the vinylic protons at $\delta$ 5.78 (1H, dd, $J = 18, 11$ Hz), 4.92 (1H, d, $J = 11$ Hz), and 4.95 (1H, d, $J = 18$ Hz), a trisubstituted olefinic proton at 5.41 (s) and four tertiary methyl groups. A preliminary study of the physical and spectral characteristics of rhizophorin E revealed that it might be a new pimarane or isopimarane diterpenoid diacetate. Four of the five oxygens of the molecule being accounted for in the diacetate the fifth oxygen might be present in the form of a carbonyl or ether linkage.

The $^{13}$C NMR spectrum (Table II) showed that the 24 carbon signals comprised of 6 methyls, 5 methylenes, 6 methines and 7 quaternary carbons. The chemical shifts of the respective carbons were assigned based on the comparative values from the literature as well as from HMQC, and $^1$H–$^1$H COSY data. The $^{13}$C NMR spectrum revealed the presence of a keto carbonyl at $\delta$ 203.2 besides the two acetate carbonyls at $\delta$ 170.2 and 170.5. The four olefinic carbons were noticed at 110.7 (t), 147.4 (d), 130.2 (d) and 133.4 (s) accounting for the vinylic and the trisubstituted double bonds, respectively. Assuming the pimarane or isopimarane skeleton for the molecule, the functional groups, two acetates, one keto and a trisubstituted double bond need to be located in the molecule. The appearance of

trisubstituted olefinic proton at $\delta$ 5.41 as a singlet indicated that it might be attached to $C_8-C_{14}$ double bond [15], for in any other position it would have come as a multiplet. The value of keto carbonyl ($\delta$ 203.2) ruled out the presence of 3-keto group which would have come around $\delta$ 218.0 as in 3-keto diterpenoids [12]. One of the acetates might be located at C-3 considering the $^{13}C$ values of C-l 8, C-19 in related compounds [11]. However, the C-3 carbon appeared around 4 ppm lower field at $\delta$ 83.5 instead of at $\delta$ 79.0 suggesting the presence of carbonyl in its vicinity at C-2. The appearance of the acetoxymethine proton (3-H) at lower field $\delta$ 4.98 and further, as a singlet supported the presence of 2-keto group. The presence of 2-keto group was also supported by the value of C-2 ($\delta$ 203.2) with an acetate at C-3 as reported in 2-keto-3-acetoxy compounds [12]. The location of the second acetate at C-6 as well as other functional groups was supported from the HMBC correlations.

The carbon at $\delta$ 83.5 assigned for C-3 showed HMBC correlation with $H_3$-18, $H_3$-19. Similarly, the C-2 ($\delta$ 203.2) with a keto group showed correlation with H-3 and $H_2$-1. The C-6 carbon ($\delta$ 68.8) with acetate group showed correlation with H-5 and $H_2$-7. One of the olefinic carbons at $\delta$ 133.4 of trisubstituted double bond showed correlation with H-9 and H-6 and the other carbon at $\delta$ 130.2 showed correlation with H-15, H-9 and $H_2$-7 where as the vinylic carbon C-15 ($\delta$ 147.4) showed correlation with $H_2$-16, $H_3$-17 and 14-H. These as well as other correlations observed are consistent with the pimarane or isopimarane skeleton. The value of C-17 at $\delta$ 25.8 suggests that it is isopimarane derivative as the same in a pimarane would have appeared around $\delta$ 29.5 [16]. The structure of rhizophorin E could thus be established as 3,6-diacetoxy-8(14),15-isopimaradien-2-one (**3**).

The stereochemistry at the respective chiral centers could be derived from $^1H-^1H$ NOESY (Fig. 1) correlations, the important of which are the correlations between H-5 and H-9 and the absence of their correlation with $H_3$-20 to show *trans–trans* stereochemistry between C-5, C-10 and C-10, C-9, respectively. The H-5$\alpha$ showed correlation with the $\alpha$-acetoxymethine H-3 giving its configuration as 3-$\alpha$ and thereby the configuration of 3-acetate as $\beta$. The other acetoxymethine proton at $\delta$ 5.14 (H-6) showed correlation with $H_3$-20 and $H_3$-18 suggesting their *cis* relationship to derive the configuration of H-6 as $\beta$ and the 6-acetate as $\alpha$. The absolute configuration of rhizophorin E could not be decided but taken as for isopimarane in view of its leavo specific rotation as noticed for all isopimaranes [15]. The structure of rhizophorin E could thus be derived as 3$\beta$,6$\alpha$-diacetoxy-8(14),15-isopimaradien-2-one (**3**).



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