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Antitubercular diterpenoids from *Vitex trifolia*

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ABSTRACT

A new halimane diterpenoid, 13-hydroxy-5(10),14-halimadien-6-one (**1**) and two new labdane diterpenoids, 6α,7α-diacetoxy-13-hydroxy-8(9),14-labdadien (**2**) and 9-hydroxy-13(14)-labden-15,16-olide (**3**), were isolated for the first time, along with fifteen known compounds, from the hexane soluble fraction of methanolic extract of *Vitex trifolia* leaves. The structures of these new diterpenoids were elucidated by spectral analysis. Their relative configurations were established using analysis of NOESY correlations and coupling constants observed in ^{1}H NMR. Compounds **2, 3** and another known diterpenoid, isoambreinolide (**4**) were evaluated for antitubercular activity. **3** and **4** exhibited antitubercular activity (MIC = 100 and 25 μg/ml) against *Mycobacterium tuberculosis* H37Rv in BACTEC-460 assay.

Introduction

Vitex trifolia L. (*syn. Vitex rotundifolia*), a member of the family Verbenaceae, is known to produce a variety of diterpenoids (Alam et al. 2002; Kiuchi et al. 2004; Ono et al. 2002, 2001a, 2001b, 2000, 1999, 1997, 1998a) and iridoids (Ono et al. 1998b, 1997) that display antioxidant (Ono et al. 1998a), tracheospasmolytic (Alam et al. 2002), cytotoxic (Li et al. 2005) and trypanosidal (Kiuchi et al. 2004) activities. The plant species is a deciduous shrub native to Southeast Asia, Micronesia, Australia and East Africa. This plant can be commonly found along the banks of water bodies like canals, rivers and ponds and hence locally known as "Neer Nochi" ("Neer" means water). The leaves of *V. trifolia* are consumed to improve memory, relieve pain, remove bad taste in mouth and cure fever (Kirtikar and Basu 1991). The leaf extract is anti-cancerous while fruits are good for amenorrhea (Ghani 1998). The flowers of *V. trifolia* mixed with honey are used in fevers accompanied by vomiting and severe thirst (Bhattacharjee and De 2005). The aerial parts of this plant are useful in the treatment of diabetes (Pullaiah and Naidu 2003). The methanol extract of this plant has been reported to possess strong antioxidant activity (Ono et al. 1998b). As part of our ongoing efforts to isolate novel bioactive naturals from Indian medicinal plants (Pandey et al., 2005; Tiwari et al., 2008), leaves of *V. trifolia* were subjected to phytochemical investigation and led to the identification of a new halimane type (**1**) and two new labdane type (**2** and **3**; Fig. 1) diterpenoids along with 15 known compounds.

All isolates were identified using a combination of ^{1}H and ^{13}C NMR spectroscopy and LC–MS analyses. The ^{1}H, ^{13}C NMR and NOESY data allowed the establishment of the relative configuration of the new compounds. This work is the product of our continuing efforts to explore traditional drugs used in ayurvedic system of medicine (India) for unexplored biological activities (Shukla et al. 2012; Yadav et al. 2013). Although there is no information on the antitubercular activity of this plant or its constituent, but still researches on antitubercular activity of diterpenoids and their potent MIC values have designated immense prospects in this field (Salinas et al. 2010; Singh et al. 2010; Gordien et al. 2009). Terpenoids because of their lipophilic nature could more easily penetrate the wax layer of the tubercular bacteria (Sikkema et al. 1995, 1994). Thus, in the present study we elucidated the structure of three diterpenoids and assessed their antitubercular activity.

Materials and methods

General experimental procedures

Optical rotations were measured on a HORIBA polarimeter (SEPA-300). ^{1}H NMR spectras were recorded in $CDCl_3$ solutions using a Bruker Avance spectrometer at 75 (^{13}C) and 300 MHz (^{1}H) and chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. The HREIMS was recorded on a JEOL-Accu-TOF JMS-T100LC mass spectrometer having a DART source (direct analysis in real time) Column chromatography was carried out using silica gel (Merck, 60–120 mesh). HPLC separation was done using a Shimadzu LC-8A semi preparative HPLC, equipped with a reverse phase semi preparative column (RP-18, Supelcosil; 25 cm, 21.2 mm, 10 μm; particle size) and a PDA detector.

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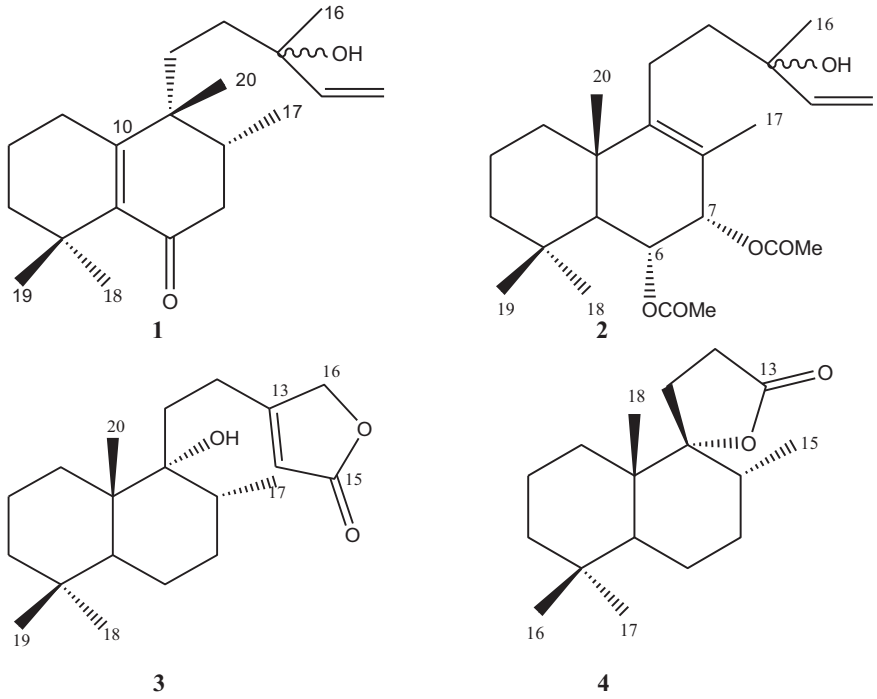

Fig. 1. Chemical structures of compounds 1–4.

Plant material

Leaves of *V. trifolia* were obtained locally from Lucknow. Identification and authentication was done by Botany and Pharmacognosy Department of our Institute (CIMAP voucher specimen number 9417).

Preparation of the crude plant extracts

Powdered leaves of *V. trifolia* (2.7 kg) were extracted with cold methanol (7 LX 24 h) at room temperature and the extract was concentrated under reduced pressure. The methanolic extract (500 g) was suspended in water (1 l) and partitioned with *n*-hexane (fr. A, 50 g), chloroform (fr. B, 23 g) and *n*-butanol (fr. C, 200 g).

Isolation of compounds

Fr. A was chromatographed on silica gel (60–120 mesh, 2 kg, column dimension, 2 m × 8 cm) eluting with *n*-hexane, gradually increasing the polarity with ethyl acetate to give fractions: fr. A1 (5 l, *n*-hexane), fr. A2–A5 (each 5 l, *n*-hexane–ethylacetate, 19:1), fr. A6- A8 (each 5 l, *n*-hexane–ethylacetate, 23:2), fr. A9–A12 (each 5 l, *n*-hexane–ethylacetate, 22:3), fr. A13- A15 (each 5 l, *n*-hexane–ethylacetate, 17:3) and fr. A17–A20 (each 5 l, *n*-hexane–ethylacetate, 41:9). Fr. A13–A20 were pooled on the basis of similar TLC profile and further purified by preparative HPLC (Solvent system, acetonitrile:water; 70:30, flow rate 15 ml/min) to obtain compound **1** (20 mg), **2** (50 mg) and **3** (40 mg). Fraction A3 (8 g) was chromatographed on silica gel (60–120 mesh) eluting with *n*-hexane–ethylacetate to give 20 fractions (0.25 l each, fr. A3-1 to A3–20). Fr. A3-3 (*n*-hexane–ethylacetate, 49:1) was crystallized (hexane-acetone) to obtain compound **4.** Fr. A4 was crystallized to obtain α amyrin while fr. A7 was crystallized to obtain ursolic acid.

Fr. B (40 g) was chromatographed on silica gel (230–400 mesh) eluting with *n*-hexane–ethylacetate to give 15 fractions (each 5 L, B1–B15). Fr. B1 (*n*-hexane–ethylacetate, 19:1) was crystallized with chloroform–methanol to obtain *p*-methoxy benzoic acid. Fr. B5 (*n*-hexane–ethylacetate, 4:1) was purified further by preparative TLC (solvent system, chloroform–methanol, 9:1) to obtain *p*-hydroxy benzoic acid. Fr. B8 (*n*-hexane–ethylacetate, 3:1) and B10 (*n*-hexane–ethylacetate, 13:7) were crystallized to obtain vitexicarpin and chrysoplenol D, respectively.

Fr. C was chromatographed on silica gel (60–120 mesh) eluting with chloroform–methanol to obtain 15 fractions (each 5 l, C1–C15). Fr. C1 (pure chloroform) on crystallization gave 2-hydroxy, 3-methoxy benzoic acid, while fr. C2 (chloroform–methanol, 49:1) afforded corosolic acid. Fr. C3 (chloroform–methanol, 19:1) was purified further by preparative TLC to obtain 2,3 dihydroxy benzoic acid (solvent system, chloroform–methanol, 4:1), Fr. C4 was crystallized (chloroform–methanol) to obtain agnuside. Fr. C5–C8 (Chloroform-methanol, 9:1 and 17:3) were pooled and purified by preparative HPLC (methanol–water 2:3) to afford negundoside and 6′*p*-hydroxymussaenosidic acid and vitexin. Fr. C-9 and C10 (chloroform–methanol, 4:1 and 3:1, respectively) were pooled and purified by preparative HPLC (methanol–water 1:4) to obtain mussaenosidic acid.

*Compound **1** (13-hydroxy-5(10),14-halimadien-6-one)*

white amorphous solid; $[\alpha]_D^{25} + 4.73$ (c 0.08, $CHCl_3$); UV (MeOH) λ_{max} 251 nm; IR (KBr) ν_{max} 3443, 2932, 1659, 1458, 1366 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) see Table 1; HRESIMS *m*/*z* 305.17219 $[M+H]^+$-(calcd for $C_{20}H_{33}O_2$).

*Compound **2** (6α,7α-diacetoxy-13-hydroxy-8(9),14-labdadiene)*

Viscous mass; $[\alpha]_D^{25} + 40.17$ (c 0.1, CHC_{l3}); IR (KBr) ν_{max} 3467, 2964, 2931, 1738, 1368, 1248, 1230, 1028 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) see Table 1; HRESIMS *m*/*z* 407.27978 $[M+H]^+$ -(calcd for $C_{24}H_{39}O_5$).

Table 1
NMR spectroscopic data (300 MHz, $CDCl_3$) for compound **1–3**.

Position	13-Hydroxy-5(10),14-halimadien-6-one (**1**)		6α,7α-Diacetoxy-13-hydroxy-8(9),14-labdadien (**2**)		9-Hydroxy-13(14)-labden-15,16-olide **(3)**	
	δ_C, Type	δ_H (*J* in Hz)	δ_C, Type	δ_H (*J* in Hz)	δ_C, Type	δ_H (*J* in Hz)
1	29.3, CH_2	a 2.19 (m) b 2.19 (m)	26.3, CH_2	a 2.00 (m) b 2.00 (m)	32.3, CH_2	a 1.70 (m) b 1.70 (m)
2	19.5, CH_2	a 1.63 (m) b 1.63 (m)	19.9, CH_2	a 1.60 (m) b 1.60 (m)	19.0, CH_2	a 1.30 (m) b 1.50 (m)
3	41.6, CH_2	a 1.48 (m) b 1.48 (m)	39.0, CH_2	a 1.45 (m) b 1.45 (m)	42.1, CH_2	a 1.15 (m) b 1.30 (m)
4	34.0, C	–	35.0, C	-	33.7, C	–
5	139.8, C	–	36.9, CH	1.99 (d, 12.6)	47.0, CH	a 1.50 (m)
6	198.8, C	–	73.2, CH	4.84 (dd, 3.3, 12.6)	22.7, CH_2	a 1.40 (m) b 1.60 (m)
7	44.4, CH_2	a 2.19 (m) b 2.47 (dd, 5.4, 17.4)	66.6, CH	5.58 (d, 3.3)	31.7, CH_2	a 1.55 (m) b 1.55 (m)
8	36.8, CH	1.97 (m)	133.0, C	–	37.1, CH	1.70 (m)
9	41.6, C	–	141.9, C	–	77.0, C	–
10	162.5, C	–	43.3, C	–	43.6, C	–
11	29.7, CH_2	a 1.43 (m) b 1.49 (m)	28.5, CH_2	a 1.40 (m) b 1.40 (m)	32.6, CH_2	a 1.75 (m) b 1.85 (m)
12	36.9, CH_2	a 1.46 (m) b 1.46 (m)	39.8, CH_2	a 1.43 (m) b 1.43 (m)	22.0, CH_2	a 2.43 (m) b 2.43 (m)
13	73.6, C	–	73.5, C	–	135.6, C	–
14	145.1, CH	5.86 (dd, 17.4, 10.8)	144.9, CH	5.82 (dd, 17.4, 10.8)	144.2, CH	7.08 (brs)
15	112.6, CH_2	a 5.08 (d, 17.4) b 5.19 (d, 10.8)	112.5, CH_2	a 5.04 (d, 17.1) b 5.17 (d, 10.8)	175.0, C	–
16	28.5, CH_3	1.28 (s)	29.7, CH_3	1.25 (s)	70.6, CH_2	4.74 (brs)
17	16.1, CH_3	0.94 (d, 6.9)	28.2 CH_3	0.90 (s)	16.5, CH_3	0.89 (d, 5.4)
18	24.6, CH_3	1.14 (s)	29.8, CH_3	1.05 (s)	34.1, CH_3	0.85 (s)
19	29.0, CH_3	1.24 (s)	29.8, CH_3	1.05 (s)	22.4, CH_3	0.81 (s)
20	28.4, CH_3	1.19 (s)	11.5, CH_3	0.87 (s)	16.8, CH_3	0.87 (s)
21			171.2, C	–		
22			21.3, CH_3	2.02 (s)		
23			171.2, C	–		
24			21.8, CH_3	2.02 (s)		

*Compound **3** (9-hydroxy-13(14)-labden-15, 16-olide)*

Viscous mass; $[\alpha]_D^{25} - 4.53$ (c 0.1, $CHCl_3$); UV (MeOH) λ_{max} 276 nm; IR (KBr) ν_{max} 3546, 1751, 1647, 1459, 1073, 1062 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) see Table 1; HRESIMS *m/z* 321.24298 $[M+H]^+$-(calcd for $C_{20}H_{33}O_3$).

Antimycobacterial assay

Mycobacterium tuberculosis H37Rv (ATCC 27294) used in this screening was obtained from National JALMA Institute for Leprosy and other Mycobacterial Diseases (ICMR), Agra, India and maintained on Löwenstein-Jansen media slant at 37 °C. After 21 days of incubation bacterial cells were scraped from slants and transferred in 1.0 ml of BACTEC diluting fluid and made complete homogenized suspension by vortexing with glass beads (2 mm diameter). The suspension was allowed to stand for a few minutes to permit sedimentation of the bacterial clumps if any. The turbidity of the homogenous suspension was adjusted to McFarland standard 1.0 with diluting fluid. A BACTEC 12B vial (Becton–Dickinson) was injected with 0.1 mL of this suspension. This vial was used as primary inoculum after the growth index (GI) reached a value of about 500 (approximately 1×10^6 cfu/ml).

Briefly, 0.1 ml of bacterial suspension from the primary inoculum culture vial (GI 500) was injected into test compound-containing vials using 1.0 ml insulin syringe. To comply with 1% proportion method, 0.1 ml of primary inoculum was added to 9.9 ml BACTEC diluting fluid to obtain 1:100 dilutions. From this 0.1 ml was injected into two 12B media vials and used as control. Vials were incubated at 37 °C, and the GI was recorded every 24 hours in a BACTEC 460 TB instrument (Becton–Dickinson). Once the GI of the control vial (1:100) reached 30 then the GI values of the test (compound containing) vials were compared with that of control vials based on difference in growth (ΔGI). The result was interpreted as follows: If the difference (called as ΔGI) of current GI from previous day GI in the case of drug containing vials is lower than the ΔGI of 1:100 control vial for the same period then the test compound is termed as active against MTB or otherwise inactive.

Two-fold serial dilution technique was used to assess the minimum inhibitory concentration (MIC) of a test compounds. Only broth culture was used as a positive control and media as a negative control.

Result and discussion

The dried leaves of *V. trifolia* were extracted with methanol at room temperature. After concentration to dryness the methanolic extract was dissolved in water and partitioned with hexane, chloroform and butanol. The hexane soluble part upon fractionation by a combination of column chromatography on silica gel and C-18 reverse phase preparative HPLC led to the isolation of three new compounds together with isoambrienolide (Ono et al. 2001a), ursolic acid (Seebacher et al. 2003) and α-amyrin (Dekebo et al. 2002). The structure of **4** has been elucidated by comparing the physical data with reported one. Column chromatography of chloroform fraction led to the isolation of two previously described flavonoids, vitexicarpin (Ahmed et al. 1989) and chrysoplenol-D (Dayrit et al. 1987) and two benzoic acid derivatives *p*-hydroxy benzoic acid and *p*-methoxy benzoic acid (Pouchert and Behnke 1993) while the butanol fraction afforded eight compounds

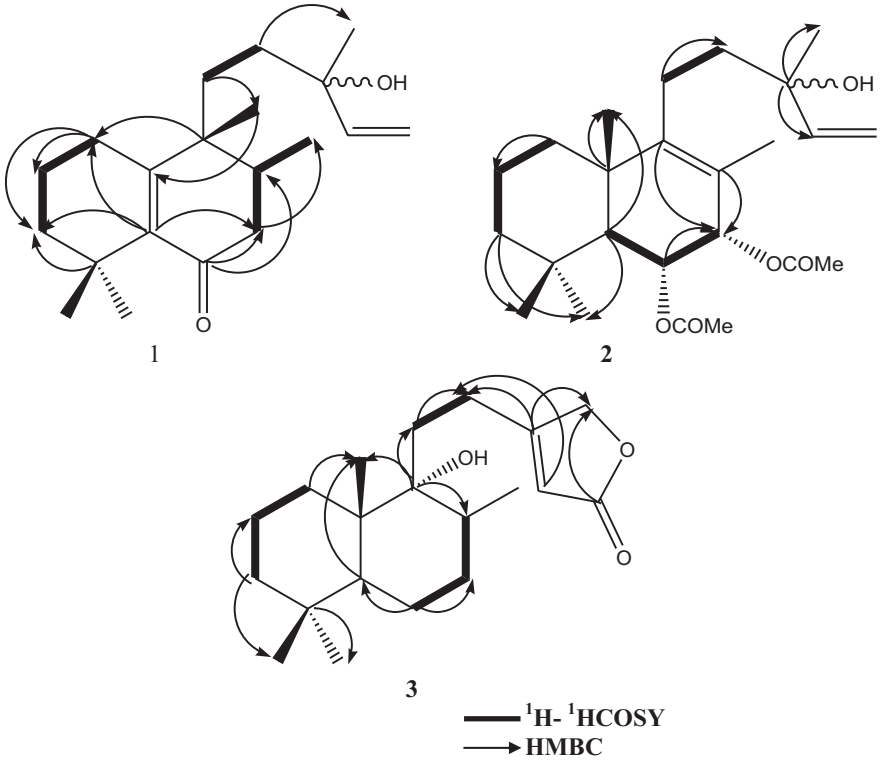

Fig. 2. HMBC and COSY correlations of compounds 1–3.

agnuside (Dayrit and Lagurin 1994), negundoside (Sehgal et al. 1982), 6′p-hydroxybenzoyl mussaenosidic acid (Dayrit and Lagurin 1994), mussaenosidic acid (Damtoft et al. 1984), 2-hydroxy,3-methoxy benzoic acid, 2,3-dihydroxy benzoic acid (Pouchert and Behnke 1993), corosolic acid (Park et al. 2002) and vitexin (Peng et al. 2008). Known compounds have been identified by comparing NMR, mass and other physical properties with reported data.

Compound **1** was obtained as white amorphous solid. Its HRES-IMS showed the protonated molecular ion at m/z 305.17219 (calc. for $C_{20}H_{33}O_2$), a molecular formula indicative of five degrees of unsaturation. The IR spectra of **1** displayed absorption bands at 1659 cm^{-1} (carbonyl) and 3443 cm^{-1} (OH). The ^{13}C NMR ($CDCl_3$) of **1** showed the resonances of 20 carbons consisting of five methyls at δ 16.1, 24.6, 28.4, 28.5 and 29.0, six methylenes at δ 19.5, 29.3, 29.7, 36.9, 41.6 and 44.4, one methine at δ 36.8 and six quaternary carbons at δ 34.0, 41.6 including one carbonyl at 198.8, two sp^2 carbons at 162.5 and 139.8 and one hydroxyl carbon at 73.6. Signals at δ 145.1 and 112.6 confirmed the presence of a vinyl group. Taking into account five degrees of unsaturation, compound **1** should include two rings. The ^{1}H NMR spectrum displayed four methyl singlets at δ1.14, 1.19, 1.24 and 1.28, one methyl doublet at δ 0.94 and three vinylic protons at δ 5.19, 5.08 and δ 5.86 (Table 1). Detailed analysis of ^{1}H–^{1}H COSY and HSQC spectra established the presence of three sub-structures: a (—CH_2—CH_2—CH_2—), b (—CH_2—CH_2) and c (—CH_2—CH_2—) that are marked with bold lines in Fig. 2 and further assembled from the cross peaks observed in the HMBC spectrum. The carbonyl signal at δ 198.8 was assigned as an α–β unsaturated C-6 ketone because of the correlation with protons at δ 2.19 (H-7a), 2.47 (H-7b) and 1.97 (H-8). Similarly the hydroxyl was assigned to C-13 due to correlation with the proton at δ 1.46 (H-12). Out of two double bonds one was assigned at ring A and B junction, as both double bond carbons were quaternary and HMBC correlation of δ 139.8 with H-7, H-1and H_3-19 and δ 162.5 with H-1, H-2 and H_3-20. A double bond at ring A and B junction is of common occurrence in some previously reported compounds of this plant (Kiuchi et al. 2004; Ono et al. 2002). H_3-20 was migrated to C-9 as it correlated with H-11a, H11b, H_3-17 and C-10 correlated with H_3-20. This structure was confirmed by analysis of 1D and 2D NMR spectra of **1** in $CDCl_3$. The relative configuration of **1** was determined by analysis of difference NOE spectra, in which correlations were observed between H_3-20 and H-7b, H_3-17 and H_2-11, H_3-17 and H-7a. The coupling constant values also supported the above proposed structure.

Compound **2** was obtained as yellowish viscous mass. The molecular formula was determined to be $C_{24}H_{38}O_5$ by HR ESIMS as it showed an $[M+H]^+$ peak at 407.27978. The IR spectrum displayed diagnostic absorption bands for hydroxyl (3467 cm^{-1}) and ester moieties (1738 cm^{-1}). The ^{1}H NMR spectrum of **2** indicated signals due to five tertiary methyl groups (δ 1.25, 0.90, 1.05, 1.05. 0.87), one vinylic group (δ 5.82, 5.04, 5.17) and two acetate methyls. Since the ^{13}C NMR spectrum of **2** gave 24 carbon signals including four olefinic carbons (δ 133.0, 141.9, 144.9, 112.5) one oxygenated quaternary carbon (δ 73.5) and two acetate methyl carbons (δ 21.3, 21.8), It was established to be a diterpene having two acetyl, one hydroxyl and one vinylic group with a quaternary double bond. In ^{1}H–^{1}H COSY spectrum of compound **2** correlations between H-14/H_2-15, H-6/H-7,H-5, $COCH_3$, H_2-1/H_2-2, H_3-20, H-5/H_3-20 were observed. On the basis of these COSY relations three sub-structures were established that are marked as bold lines in Fig. 2. HMBC correlations between C-14/H-15, C-6/H-7, C-4/H-6, C-8/H-7, C-2/H_2-1, C-10/H_3-20 were diagnostic in putting these sub-structures to assemble whole molecule. Correlation between C-5/H_3-18, H_3-19, C-20/H_2-1, C-9/H_2-1, H-7, H_2-11were also observed. Attachment of acetate groups was confirmed as C-6 and C-7 on the basis of HMBC correlations. A COSY relation between H-6 and H_3-22 and H-7 and H_3-24 further confirmed the position of acetyl groups. As a result of coupling of the signal at δ 171.2 to H-6 and H-7, this peak seemed to be a single signal for two acetate carbonyls. Furthermore in the HMBC spectrum it became obvious, that C-8 is connected to C-9 by a double bond because of the correlation to H-11, H-7, H-17

Table 2
Antimycobacterial activity of compounds **2**–**4** against M. tuberculosis H37Rv strain by BACTEC assay.

S. no.	Compound no.	MIC (μg/ml)
1	**2**	>100
2	**3**	100
3	**4**	25
4	Rifampicin	2.0
5	Stretomycin	2.0

and H-1. In the side chain, connections between C-13/H-14 and C-13/H-16 were also noticed.

Relative configuration of compound **2** was based on coupling constant and NOESY experiment. Large coupling constant of H-5 and H-6 (12.6 Hz, J_{ax-ax}) confirmed the diaxial orientation of these protons while small coupling constant of H-6 and H-7 (3 Hz, J_{ax-eq}) established their orientation as axial and equatorial, respectively In addition NOESY correlation was not found between H-7 and H-5, thereby further confirming that H-7 is equatorial. This observation confirmed that H-6 and H-7 both are β oriented and thus acetates attached to C-6 and C-7are α oriented. Thus, compound **2** has been established as 6α, 7α -diacetoxy-13-hydroxy-8(9), 14-labdadiene (Fig. 2).

Compound **3** was obtained as semi solid mass. The HRESIMS of **3** indicated a molecular ion peak at 321.24298 $[M+H]^+$ indicating its molecular formula to be $C_{20}H_{32}O_3$. The IR spectrum of compound **3** exhibited strong absorption bands due to hydroxyl group (3546 cm^{-1}) and an α–β unsaturated lactone (1751 and 1654 cm^{-1}) (Ono et al. 1998b). The ^1H NMR spectrum showed the signals of three tertiary methyl groups at δ 0.87, 0.85 and 0.81 and one secondary methyl group at δ 0.89. ^1H signals of H-14 (δ 7.08) and H-16 (δ 4.74) appeared as singlet confirming the substitution of butenolide ring to be β. The ^{13}C NMR spectrum gave 20 carbon signals including one α–β unsaturated lactone at δ 135.6, 144.2, 70.6 and 175.0, one quaternary carbinol group at δ 77.0 and four methyls at δ 16.5, 34.1, 22.4, 16.8. In ^1H NMR spectrum a singlet was observed for H-14 which indicated β substitution (Ono et al. 2001a) of lactone ring. Had it been α, a more complex pattern for H-14 would have been observed. The relative configuration of **3** was assigned with the help of difference NOE spectrum and comparison of chemical shifts and coupling constant values with related compounds (Fig. 2).

Antitubercular activities of compound **2**, **3** and **4** were evaluated against *M. tuberculosis* H37Rv strain in BACTEC-460 assay. The results were calculated and expressed as minimum inhibitory concentrations (MIC) in μg/ml (Table 2). Compound **3** exhibited antitubercular activity at MIC 100 μg/ml and **4** possessed antitubercular activity at MIC 25 μg/ml respectively, Compound **2** was inactive at 100 μg/ml concentration. Although plants are excellent source of diverse molecules, there is no plant based drug for the treatment of tuberculosis. Insufficient quantities of compounds in plant extracts for detection in whole cell assays and the lack of follow up studies for their modes of action and in vivo studies be the reasons of failure of plant based TB treatment, but now improved purification techniques for phytomolecules using modern technologies are opening new perspectives for natural product drug development. The present study has brought out a new halimane diterpenoid, 13-hydroxy-5(10),14-halimadien-6-one (**1**) and two new labdane diterpenoids, 6α,7α-diacetoxy-13-hydroxy-8(9),14-labdadien (**2**) and 9-hydroxy-13(14)-labden-15,16-olide (**3**), molecule for the first time from hexane extract of *Vitex trifolia* leaves. Compound **3** and **4** endowed with antitubercular activity. Although lactones are toxic, but further structural modification of active natural molecules may lead to the possibility of developing potential anti tubercular drug.

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