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Spectral characterization, stereochemical assignments and thermal rearrangements of naturally occurring furanogermacranes and furanoelemanes

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ABSTRACT

Furanosesquiterpenoids, abundantly available in secondary plant metabolites show inspiring biological activities and some of them are utilized as pharmaceuticals. The aim of the present study was structural elucidation and stereochemical assignments of furanosesquiterpenoids form a new source viz. Himalayan Lauraceae. Compounds 1-5 were isolated from the essential oils of two Lauraceae species and their structures were unambiguously assigned by IR, EI-MS, ^{1}H-NMR, ^{13}C-NMR, DEPT, ^{1}H-^{1}H-COSY, HMQC, HMBC and NOE techniques. Thermal rearrangement was noticed between furanogermacranes and furanoelemanes. Though, gas chromatographic analysis revealed some ambiguous observations due to heat sensitive compounds in plant extracts yet thermal rearrangements would eventually lead to the discovery of new skeletons of biological importance.

Keywords: Furangermacranes; furanoelemanes; Cope rearrangement; Spectral characterization; Stereochemical assignment

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INTRODUCTION

Germacranes, a group of naturally occuring sesquiterpenes are considered as important intermediates in the biosynthesis of several classes of furanosesquiterpenoids [1]. Due to their non rigid structure, sensitivity to acidic conditions and elevated temperatures, germacranes undergo rearrangements and transformations to a large variety of products [1,2]. Furan containing compounds, abundantly available in secondary plant metabolites show inspiring biological activities and some of them are utilized as pharmaceuticals [3]. The Lauraceae or Laurel family is well known for the distribution of sesquiterpene furans along with other compounds [4-15]. As a part of the chemical investigation of active constituents of Himalayan laurels, present study deals with the structural, stereochemical and thermal aspects of furanosesquiterpenoids isolated from the essential oils of two species. Literature survey revealed a few similar reports from *Commiphora* and *Curcuma* species from different regions where one or more of these compounds were detected [16-22]. This is the first report on the structural elucidation and stereochemical assignments of five furanosesquiterpenoids form a new source *viz*. Himalayan Lauraceae along with the Cope rearrangements of furanogermacranes to furanoelemanes.

MATERIALS AND METHODS

2.1. Plant Materials

The arboreous Laurels *viz. Lindera pulcherrima* (Nees.) Benth. ex Hook. f. and *Neolitsea pallens* (D. Don), syn. *Litsea umbrosa* var. *consimilis* (Nees) Hook f., syn. *L. consimilis* Nees., collected (September 2010), from the temperate forests (1800m) of Pithoragarh district of Uttarakhand, were identified from Botanical Survey of India, Dehradun (BSD 101366 and BSD 3418 respectively) and voucher specimen were preserved in the phytochemistry laboratory, Kumaun University, Nainital.

2.2. Extraction of essential oils

The fresh aerial parts (5 kg each) of both the species were chopped and steam distilled separately (2h) using copper still fitted with spiral glass condensers. The distillates (10 L) were saturated with NaCl and the oils were extracted with *n*-hexane and dichloromethane. The oils were dried over sodium sulphate and percentage yield was calculated on the basis of dry weight of plant materials (0.5 and 0.9 for *Lindera pulcherrima* and *Neolitsea pallens* respectively). Finally the oils were stored in tightly closed vials in the dark at 4^{o}C until used for further analysis.

2.3. Isolation of compounds

The essential oils (5 mL each) were fractionated by column chromatography on silica gel CC (230-400 mesh, Merck, 600 × 25 cm column) packed with hexane, and eluted with hexane followed by gradient elution by Et_2O/hexane (5%-20%). The isolated compounds were further purified by Waters' HPLC using μ-Porosil column (250 mm × 7.8 mm), 2.0 mL/min flow rate, RI detector in an attenuation of 32X at 3000 psi using 5%-15% Et_2O in hexane. Compounds **1**, **3**, **4, 5** were isolated from *L. pulcherrima*, while **2** was isolated from *N. pallens*.

2.4. Gas chromatography/Mass spectrometry

The essential oils were analyzed using a gas chromatograph (Shimadzu GC-17 A/QP-5000) fitted with a capillary column (DB-5, 30m × 0.25mm internal diameter, film coating 0.25μm). The temperature program used was 60 ^{o}C-210 ^{o}C @ 3 ^{o}C/min using helium as carrier gas. The injector temperature was set at 200 ^{o}C. GC/MS was done using Thermo quest Trace GC 2000 interfaced with Finnigan MAT PolarisQ Ion Trap Mass Spectrometer fitted with Rtx-5 non polar fused silica capillary column (30m × 0.25mm internal diameter). The column temperature was programmed 60 ^{o}C-210 ^{o}C @ 3 ^{o}C/min using helium as carrier gas at 1.0ml/min. The injection temperature was 210 ^{o}C, ion source temperature 200 ^{o}C, MS transfer line temperature 275 ^{o}C, injection size 0.1μl, split ratio 1:40. MS were taken at 70 eV with mass range of m/z 40-450 amu.

2.5. IR and NMR spectral analysis

The IR spectra of the isolated compounds were taken in the Perkin Elmer FTIR (Spectrum bx) by salt plate method. ^{1}H and ^{13}C-NMR spectra were recorded in $CDCl_3$ on a Bruker-Avance DRX 500 MHz and 125 MHz instrument using TMS as internal standard. Chemical shifts were referenced to the residual solvent signal ($CDCl_3$ δ_H 7.26, δ_C 77.0). Multiplicities of ^{13}C-NMR spectra were assigned by DEPT experiments. Standard NMR FT pulse sequences were employed for COSY, HMQC, HMBC and NOE experiments.

RESULTS AND DISCUSSION

3.1. Structural elucidation

The sesquiterpenoid character of compounds **1-5** (Fig 1) is evident by their mass (m/z ranging from 216 to 232) and ^{13}C-NMR (15 carbons in each) spectral data. The mass spectral fragmentation of **1-5** showed two set of base peaks; one at m/z 108 for **2**, **4** and **5** and the other at m/z 122 for **1** and **3** with a difference of m/z 14 which is due to one more oxygen in latter (Scheme 1). The common signal in ^{1}H-NMR spectrum of **1-5** is that of aromatic proton coupled to β-methyl (*J*=1.0-1.2 Hz) characteristic of trisubstituted furan moiety in the skeleton. IR spectrum further supported the mass spectral observations by exhibiting carbonyl absorptions for **1-3** which were absent in **4** and **5**. Therefore, **4** and **5** contain the sole oxygen of the furan moiety while **1-3** contain additional oxygen in the sesquiterpene skeleton. The structural assignment of **1-5** was done by homonuclear (^{1}H-^{1}H COSY) and heteronuclear (HMQC, HMBC) NMR experiments (Fig 2a).

Furanodienone (1)
IR v_{max} cm^{-1} 2930, 2865, 1675, 1651, 1533, 1440, 1403, 1381, 1255, 1239, 1104, 1018, 895, 843, 752, 737. MS (70 eV, m/z) $C_{15}H_{18}O_2$, m/z 230.15, base peak m/z 122 (Scheme 1). ^{1}H-NMR spectrum of **1** (Table 1) showed two vinyl hydrogens at δ 5.13, 5.78, two vinyl methyl signals at δ 2.01, 1.38 corresponding to the substituted germacrane skeleton along with a downfield doublet at δ 7.06 long range coupled (1.0 Hz) to the β-methyl (δ 2.09) of the furan ring. Vinyl protons are coupled to the upfield methylene hydrogens as evident by their signal broadening and by ^{1}H-^{1}H COSY correlations. The most downfield chemical shift in the ^{13}C-NMR spectrum (Table 2) was due to the conjugated carbonyl carbon at δ 189.0. HMQC correlations were used to associate the proton signals to the corresponding carbon signals while HMBC correlations were used to assign the long range heteronuclear couplings (Fig 2a).

Furanogermenone (2)
IR v_{max} cm^{-1} 2926, 2871, 1713, 1605, 1563, 1454, 1418, 1374, 1135, 1097, 1045, 988, 885, 761. MS (70 eV, m/z) $C_{15}H_{20}O_2$, m/z 232.16, base peak m/z 108 (Scheme 1). ^{1}H-NMR spectrum of **2** (Table 1) showed a broad triplet for vinyl hydrogen at δ 5.16 with vicinal coupling to methylene protons in the germacrane skeleton. The downfield doublet at δ 7.07 long range coupled (1.0 Hz) to the β-methyl at δ 1.86 is the characteristic signal of trisubstituted furan ring. Further, ^{1}H-NMR spectrum exhibited an upfield doublet at δ 1.10 (7.2 Hz) for a methyl group attached to methine carbon which is observed by ^{1}H-^{1}H COSY and further confirmed by DEPT and HMQC correlations. ^{13}C-NMR spectrum showed the most downfield signal at δ 213.6 assigned to unconjugated carbonyl group (Table 2). Further, HMBC correlations were used to assign its correct position (Fig 2a).

Curzerenone (3)
IR v_{max} cm^{-1} 3083, 2964, 2929, 2872, 1668, 1563, 1428, 1384, 1350, 1259, 1104, 1070, 1035, 998, 916, 738. MS (70 eV, m/z) $C_{15}H_{18}O_2$, m/z 230.15, base peak m/z 122 (Scheme 1). ^{1}H-NMR spectrum of **3** (Table 1) showed a total of five protons in the olefinic region having a downfield chemical shift for one proton at δ 5.75 *dd* (*J*=17.0, 10.4 Hz) characteristic of *cis-trans* olefinic coupling followed by a signal at δ 4.71-4.98 integrated for four protons, indicated the presence of two terminal methylene groups in the molecule. ^{1}H-^{1}H COSY correlations further confirmed these observations. With the help of DEPT experiment and HMQC correlations, the sole proton at δ 2.97 was found to be attached to the methine having no further ^{1}H-^{1}H COSY correlation. These observations led to the conclusion that germacrane skeleton is absent in **3**. However, the presence of α-H and β-Me at δ7.05 and δ2.15 respectively along with mass and ^{13}C –NMR spectral data showed the furanosesquiterpenoid nature of the molecule. With the combination of DEPT, HMQC and HMBC experiments, it was concluded that the ring has a furanoelemane skeleton. Two olefinic methylene signals in the DEPT spectrum at δ112.7 and δ115.3 confirmed their terminal assignment (Table 2). The carbonyl carbon was observed at δ193.2. HMQC correlations were used to assign the protons attached to the corresponding carbons while HMBC correlations were used to assign the long range ^{1}H-^{13}C heteronuclear couplings (Fig 2a).

Isofuranogermacrene (4)
IR v_{max} cm^{-1} 2964, 2929, 2872, 1769, 1563, 1428, 1384, 1259, 1070, 916, 738. MS (70 eV, m/z) $C_{15}H_{20}O$, m/z 216.18, base peak m/z 108 (Scheme 1). ^{1}H-NMR spectrum of **4** (Table 1) showed similar spectral pattern to **3** with two more protons in aliphatic region. Among five olefinic protons, a downfield signal for one proton at δ 5.91 *dd* (*J*=17.0, 10.4 Hz) characteristic of *cis-trans* olefinic coupling followed by δ 4.86-5.02 integrated for four protons indicates the presence of two olefinic methylene groups in the molecule. The furanoid signals were observed at δ7.05 and δ1.91 for α-H and β-Me respectively. With the help of DEPT, ^{1}H-^{1}H- COSY and HMQC correlations, the proton at δ 2.68 (*d*) was found to be correlated with upfield protons indicating the absence of oxo group in the elemane skeleton which was also evident by mass and IR spectral data. The DEPT spectrum showed two olefinic methylene signals at δ112.2 and δ110.7 exhibiting their terminal nature (Table 2). Directly attached and long range coupled heteronuclear correlations were further assigned by HMQC and HMBC experiments respectively (Fig 2a).

Furanodiene (5)
IR v_{max} cm^{-1} 2930, 2865, 1533, 1440, 1403, 1381, 1255, 1239, 1104, 1018, 942, 895, 870, 843, 795, 779, 752, 737. MS (70 eV, m/z) $C_{15}H_{20}O$, m/z 216.18, base peak m/z 108 (Scheme 1). ^{1}H-NMR spectrum of **5** (Table 1) showed the downfield doublet at δ 7.08 coupled to the β-methyl of the furan ring. Two vinyl hydrogens were observed at δ 4.93 and 4.68 coupled to the upfield methylene protons as evident by ^{1}H-^{1}H COSY correlations. Further, the presence of

two vinyl methyl signals at δ 1.55 and 1.24 confirmed the presence of two trisubstituted double bonds in the germacrane moiety. DEPT spectra showed four upfield methylene and two olefinic methine signals in the germacrane ring (Table 2). HMQC spectrum was used to correlate the proton signals to the corresponding carbon signals while HMBC correlations were used to assign the long range heteronuclear couplings (Fig 2a).

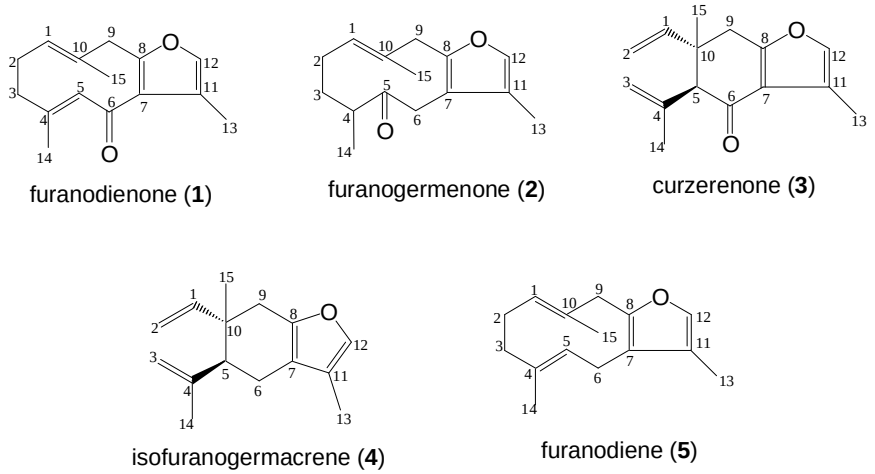
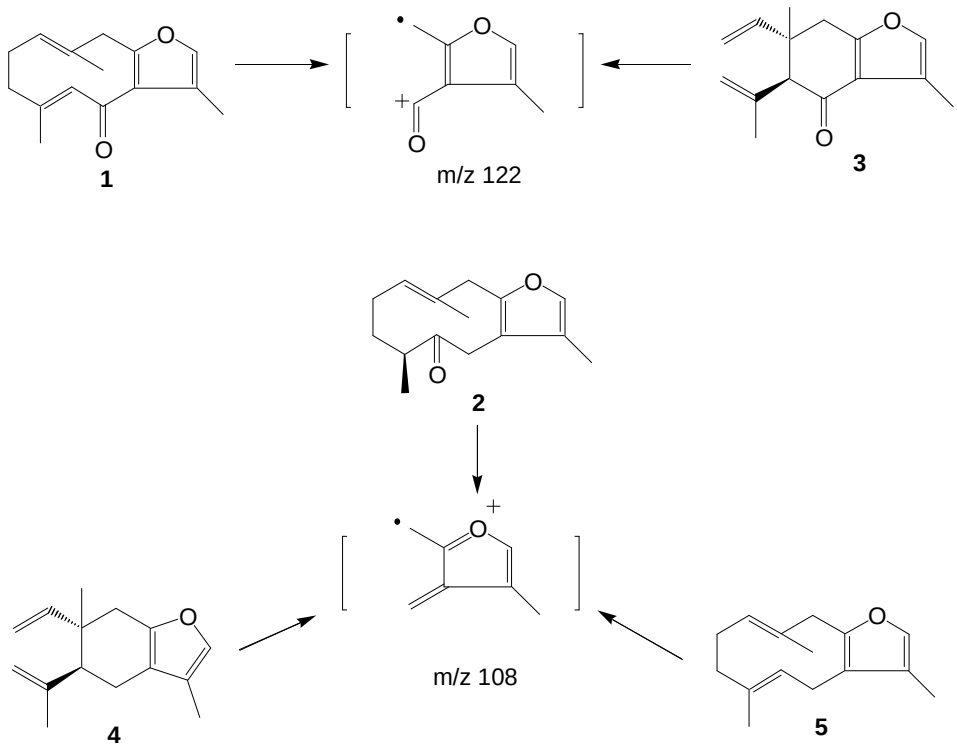


Figure 1. Structure of furanosesquiterpenoids from Himalayan Laurels



Scheme 1. Mass fragmentation of 1-5

Table 1. ^{1}H-NMR data of 1-5

H	**1**	**2**	**3**	**4**	**5**
1	5.13 (*br dd*, 6.4, 4.0 Hz)	5.16 (*br t*)	5.75 (*dd*, 17.0, 10.4 Hz)	5.91 (*dd*, 17.0, 10.4 Hz)	4.93 (*m*)
2	2.17 (*dt* 11.6, 3.4) 2.30 (*m*)	1.95 (*m*)	4.71-4.98 (*m*)	5.02 (*dd*, 16.8, .1.0 Hz) 4.98 (*dd*, 10.4, 1.0. Hz)	2.01 (*m*), 2.19 (*m*)
3	1.87 (*dt*, 10.8, 4.0), 2.47 (*td*, 11.2, 3.8)	1.67 (*m*)	4.71-4.98 (*m*)	4.86, 4.74 (*d*, 1.0. Hz)	1.77 (*dt*, 5.0 Hz), 2.23 (*td*, 3.4 Hz)
4	-	2.51 (*m*)	-	-	-
5	5.78 (*br s*)	-	2.97 (*s*)	2.68(*d*, 1.6. Hz)	4.68 (*dd*, 8.0, 5.8 Hz)
6	-	3.18, 3.34 (*ABq*, 16.0 Hz)	-	2.42 (*dd*, 1.2, 1.6. Hz)	2.98, 3.05 (*br d*, 7.2 Hz)
7	-	-	-	-	-
8	-	-	-	-	-
9	3.65 (*br s*)	3.29 (*m*)	2.80 (*ABq*, 17.6 Hz)	2.33(*br s*)	3.41, 3.50 (*d*, 16.2 Hz)
10	-	-	-	-	-
11	-	-	-	-	-
12	7.06 (*br s*)	7.07 (*br s*)	7.05 (*br s*)	7.05 (*br s*)	7.08 (*d*, 1.2 Hz)
13	2.09 (*d*, 1.0 Hz)	1.86 (*d*, 1.0 Hz)	2.15 (*br s*)	1.91 (*br s*)	1.89 (*d*, 1.4 Hz)
14	2.01 (*br s*)	1.10 (*d*, 7.2 Hz)	1.80 (*br s*)	1.74 (*s*)	1.55 (*s*)
15	1.38 (*br s*)	1.72 (*br s*)	1.20 (*s*)	1.10 (s)	1.24 (*s*)

s singlet; d doublet; t triplet; br s broad singlet; m multiplet; dd doublet of doublet; dt doublet of triplet; td triplet of doublet. coupling constants (Hz) are in bracket

Table 2. ^{13}C-NMR and DEPT spectral data of 1-5

C	**1**		**2**		**3**		**4**		**5**	
	^{13}C	**DEPT**	**^{13}C**	**DEPT**	**^{13}C**	**DEPT**	**^{13}C**	**DEPT**	**^{13}C**	**DEPT**
1	129.9	CH	134.5	CH	145.1	CH	146.5	CH	134.6	CH
2	25.8	CH_2	22.7	CH_2	115.3	CH_2	112.2	CH_2	29.7	CH_2
3	41.2	CH_2	36.2	CH_2	112.7	CH_2	110.7	CH_2	24.7	CH_2
4	145.1	qC	47.3	CH	140.5	qC	148.9	qC	127.9	qC
5	131.8	CH	213.6	qC	63.7	CH	51.8	CH	128.5	CH
6	189.0	qC	27.3	CH_2	193.2	qC	24.0	CH_2	41.0	CH_2
7	121.6	qC	113.8	qC	119.6	qC	116.1	qC	118.4	qC
8	156.1	qC	149.0	qC	165.0	qC	147.5	qC	150.0	qC
9	39.8	CH_2	38.4	CH_2	33.1	CH_2	35.8	CH	40.4	CH_2
10	135.0	qC	131.7	qC	42.4	qC	39.6	qC	129.1	qC
11	123.2	qC	121.8	qC	118.6	qC	119.0	CH_2	120.9	qC
12	137.5	CH	136.5	CH	139.0	CH	136.9	qC	139.3	CH
13	9.7	CH_3	9.5	CH_3	9.7	CH_3	8.8	CH_3	10.3	CH_3
14	18.7	CH_3	18.3	CH_3	25.0	CH_3	23.1	CH_3	16.8	CH_3
15	15.4	CH_3	16.8	CH_3	22.6	CH_3	18.9	CH_3	16.7	CH_3

3.2. Stereochemical assignment

Intramolecular nuclear Overhauser effect (nOe) experiments were used to determine the configurational assignment in **1**-**5**. The observation of nOe's involves a consideration of the intramolecular spin-lattice relaxation paths for the various protons in the molecule. UV spectral observation of cyclodeca-1,5-dienes exhibited anomalous behaviour due to transannular conjugation of the double bonds across the ring. Molecular models imply that the double bond planes must be approximately perpendicular to the ring plane. The double bonds may have either a parallel or crossed spatial arrangement. Thus these molecules can have four different conformations since these are substituted at C-4 and C-10, however, stereochemistry of Cope rearrangement and *trans* annular additions suggest a crossed orientation [23]. The *cis-trans* configuration of the trisubstituted double bonds in **1** and **5,** were determined by intramolecular nOe. Irradiation of 14 and 15 methyl signals exhibited no effect in the intensity of 5H and 1H signals respectively, indicating the ring to be *trans-trans* cyclodeca-1,5-diene. Significant increasement in the intensity of

H-1 upon irradiation of H-5 further determined the ring conformation where both the methyl groups are *syn* to each other. Another nOe enhancement was observed for H-9 and H-2 upon irradiation of H-1 (Fig 2b). The enhanced intensity of H-1 was noticed upon irradiation of H-9 and H-6 in **2**. Further**,** irradiation of 15-methyl did not affect the signal intensity of H-1 which is the evidence of *trans* geometry of 1, 10 double bond. Not much is obvious about the absolute configuration at C-4, however, literature reported it to be *S* [17]. When the molecule is conformationally mobile, the occurrence of a nOe is not sufficient evidence for the time-independent proximity of two protons. The only allowed conclusion is that two sets of nuclei are located in each other's vicinity for a period of time [23]. Further nOe studies were done in order to determine the absolute configuration at the two stereocentres in **3** and **4**. Irradiation of 5-H did not affect the intensity of 15-methyl and vice versa, therefore due to this *trans* fusion the absolute configuration is 5*S* 10*S* (Fig 2b).

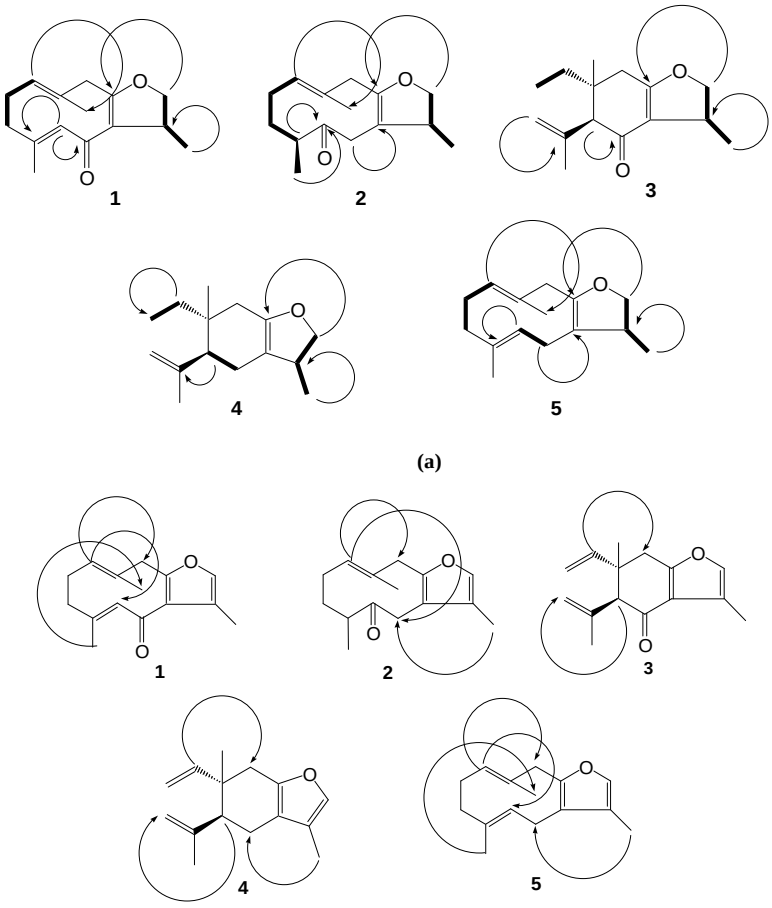
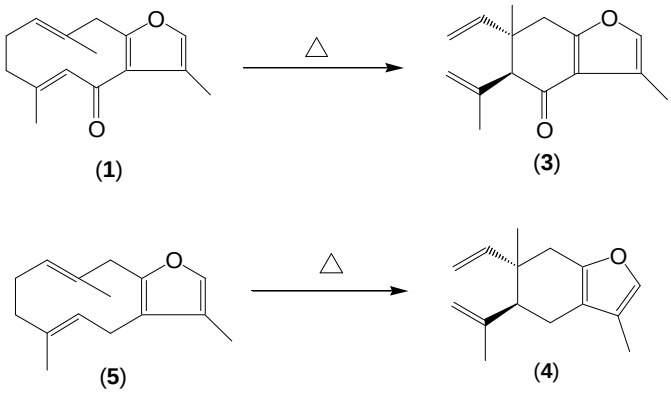


Figure 2. a) Homonuclear (^{1}H-^{1}H COSY; thick line) and heteronuclear (^{1}H-^{13}C HMBC; arrow) correlations b) Stereochemical correlations (NOE; arrow)

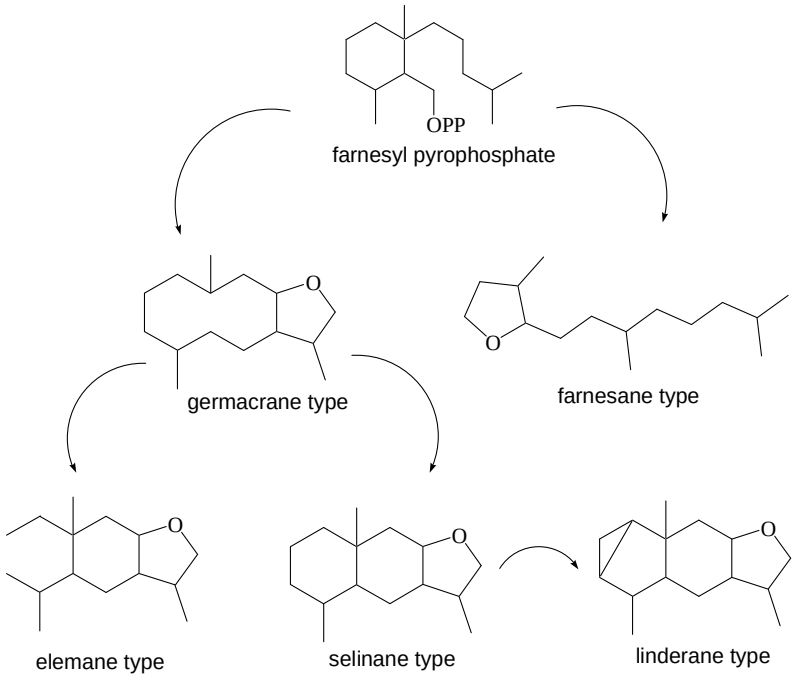
3.3. Thermal rearrangements

Gas chromatographic analysis of extracts or essential oils commonly shows the co-existence of germacrene and its thermal artifact elemane. Sometimes, presence of a particular elemane type serves as a reliable indicator of its precursor germacrane. Thus, in cases where germacrane is present in trace amounts the corresponding elemane allows accurate prediction because the elemane type is usually more abundant [1]. The isolation of germacranes from natural sources is often complicated by the sensitivity of these sesquiterpenes to acidic conditions and elevated temperatures. During distillation and GC analysis these are known to undergo facile Cope rearrangement to elemanes [2]. Similarly, furanogermacranes undergo Cope rearrangement to furanoelemanes [24].

In the present study, gas chromatographic analysis showed sharp peaks for **3** and **4** preceded by significantly smaller hump shaped peaks having a retention time similar to that of **1** and **5** respectively. This GC behaviour is typically attributed to the occurrence of Cope rearrangement on the column. GC analysis showed a major peak at retention time 35 min and a hump shaped peak at 38 min, both accounting for **3** and **1** respectively. Another sharp peak at retention time 31 and the corresponding hump shaped peak at 36 min, both accounting for **4** and **5** respectively. Furanodienone and furanodiene changed into curzerenone and isofuranogermacrene, respectively (Scheme 2). The retention times of these compounds were further confirmed by online mass spectral identification followed by repeated injections of pure isolates under similar chromatographic conditions.



Scheme 2. Cope rearrangement; furanogermacranes and furanoelemanes



Scheme 3. Biosynthesis of furanogermacranes and furanoelemanes

The structure and configuration of **1-5** were further confirmed by NMR spectroscopy. DFT calculations showed furanodiene to be 0.56 kcal-mol^{-1} lower in energy than curzerene and most of the essential oil containing furanodienone and curzerenone showed higher concentrations of former over latter [12-15]. DFT total electronic energies of furanodiene and curzerene do not account for this behaviour, but post –HF MP2 calculations show curzerene to be 3.07 kcal mol^{-1} more stable than furanodiene. Furanodienone generally predominates curzerenone in the essential oil compositions. The energies of the furanodienone to curzerenone conversion (DFT, ΔHr = 2.35 kcal

mol^{-1}; MP2 ΔHr = 4.45 kcal mol^{-1}) were consistent with these experimental results [25]. Therefore, it may be concluded that the optimization of GC-MS conditions should be based on resolutions and stabilities of the analytes because volatile oils usually contain heat sensitive components which may degrade and result in wrong observations during GC analysis. However, chemical properties of the volatile oil components are unknown in most cases and pure compounds are difficult to be obtained for the related study.

3.4. Biosynthesis of furanogermacranes and furanoelemanes

One of the interesting features of chemical composition of laurels is the occurrence of a large number of sesquiterpene furans possessing farnesane, germacrane, elemane, selinane and linderane skeletons [5,6]. Based on the biosynthetic pathway of these compounds (Scheme 3) it may be considered that evolutionary lower plant species are only capable of carrying out a limited number of biosynthetic steps while evolutionary more advanced species can perform the complete biosynthetic sequence. From these considerations, the species containing furanofarnesanes can be regarded as being evolutionary less advanced than that containing furanolinderane skeleton [4-6]. Although the elemane type sesquiterpene furans may be the artifacts produced from germacrane type sesquiterpene furans, yet the occurrence of furanoelemanes (**3**, **4**) and furanogermacranes (**1**, **2**, **5**) in the Himalayan lauraceae species supports for the presence of furansesquiterpenes as a chemosystematic feature of this family.

CONCLUSION

The stereostructure of five furanosesquiterpenoids were assigned along with the noticeable facile Cope rearrangement between furanogermacranes and furanoelemanes. Though, gas chromatographic analysis resulted some wrong observations due to heat sensitive compounds in plant extracts yet thermal rearrangements would eventually lead to the discovery of new skeletons of biological importance.

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