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Five diterpenoids (agallochins A–E) from the mangrove plant *Excoecaria agallocha* Linn☆

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Abstract

Chemical examination of the hexane extract of the roots of *Excoecaria agallocha* Linn collected from the Godavari estuary resulted in the isolation of altogether eleven diterpenoids of which five (**1–5**) are new. The structures of the new diterpenoids have been elucidated by a study of their physical and spectral (UV, IR, ^{1}H, ^{13}C, DEPT, ^{1}H–^{1}H COSY, NOESY, HMQC, HMBC and MASS) data as 3-oxo-*ent*-13epi-8(13)-epoxy-15-chloro-14-hydroxylabdane (**1**), *ent*-15-chloro-13,14-dihydroxylabd-8(9)-en-3-one (**2**), *ent*-15-chloro-labd-8(9)ene-3α,13,14-triol (**3**), *ent*-11β-hydroxy-8(14),15-isopimaradien-3-one (**4**), 8,13-epoxy-3-nor-2,3-seco-14-epilabden-2,4-olide (**5**). The six known diterpenoids have been characterised respectively as *ent*-3-oxo-13-epi-manoyl oxide (**6**), *ent*-3β-hydroxy-13-epi-manoyl oxide (**7**), (13*R*,14*S*)-*ent*-8α,13;14,15-diepoxy-13-epi-labdan-3-one (**8**), *ent*-16-hydroxy-3-oxo-13-epi-manoyl oxide (**9**), *ent*-15-hydroxylabda-8(17),13*E*-dien-3-one (**10**), labda- 8(17),13*E*-diene-3β,15-diol (**11**) by a comparative study of their spectral data with the literature values.

Keywords: Indian mangrove; *Excoecaria agallocha*; Euphorbiaceae; New diterpenoids; Agallochins A–E; New labdanes; New nor-labdane; Isopimarane

1. Introduction

The plants of *Excoecaria* genus (family: Euphorbiaceae) comprise of nearly 40 species which are distributed throughout tropical Africa, Asia and north west Australia (Wills, 1973; Wiriyachitra et al., 1985; Karalai et al., 1994). They are well-known as extreme skin irritants and tumor promoters (Erickson et al., 1995). *Excoecaria agallocha* Linn is distributed on the seashores and edge mangroves, sometimes cultivated for wind-and sea-breaks in tropical Africa and East Asia. The leaves and latex of *E. agallocha* have been used as a dart poison and fish poison in India (Prakash et al., 1983), New Caledonia (Ohhigashi et al., 1974) and Malaysia (Kawashima et al., 1971). The bark and wood of this tree have been used in traditional medicine in Thailand for flatulence (Erickson et al., 1995). The resinous wood including the latex of the tree known as "Okinawa-jinko" has been used as a substitute for the incense of agarwood (Jinko) (Metcalfe, 1933; Varma et al., 1965; Nakanishi et al., 1984). The piscicidal constituent of the twigs and bark of the *E. agallocha,* native to Okinawa has been characterised as the daphnane diterpene ester, excoecariatoxin (Ohhigashi et al., 1974). The same diterpene ester was isolated from the latex of *E. agallocha* along with some related compounds in Thailand. The other constituents isolated from *E. agallocha* include triterpenes (Hui and Sung, 1968; Kawashima et al., 1971; Anjaneyulu et al., 1993), hydrocarbons and fatty acids forming surface waxes of woods (Sil et al., 1983) and a cinnamoyl piperidine alkaloid in the stem wood which has been encountered so far in the plants of *Piper* genus (Prakash et al., 1983). A novel phorbol ester, an anti-HIV principle has been recently isolated from the leaves and stems of *E. agallocha* collected in Australia (Wiriyachitra et al., 1985; Karalai et al., 1994). From the resinous wood of *E. agallocha* collected in Okinawa T. Konishi and his co-workers have reported very recently in a series of papers (Konishi et al.,1996a,b,c; 1998a,b, 1999), the isolation and structural elucidation of a large number of new diterpeniods of different skeletons such as manoyl oxides, labdanes, beyeranes and kauranes

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along with some known derivatives. In primary screening, some of the diterpenes isolated have been found to possess anti-tumor promoting activity in vivo against mouse-tumor (Konishi et al., 1998c).

In our continuing interest (Anjaneyulu and Kameswara Rao, 1997; Anjaneyulu et al., 1997, 1998a,b; Anjaneyulu et al., 1991) on the bioactive substances from the marine fauna and flora of Indian sea waters, we report herein the chemical examination of the mangrove plant *E. agallocha* collected from the Godavari Estuary (Bhiravapalem, Corangi forest 16° 58′ N Latitude and 82° 15′ E Longtitude).

2. Results and discussion

The root powder of the plant material was exhaustively soxhleted with *n*-hexane. The residue from the combined *n*-hexane extracts was chromatographed over a column of Si gel eluting with solvents of increasing polarity from hexane to hexane-ethylacetate mixtures. The residues from the column fractions on further purification resulted in the isolation of five new diterpenoids agallochins A–E (**1**–**5**) in addition to six (**6**–**11**) known diterpenoids, *ent*-3-oxo-13-epi-manoyl oxide (**6**), *ent*-3β-hydroxy-13-epi-manoyl oxide (**7**), (13*R*,14*S*)-*ent*-8α,13;14,15-diepoxy-13-epi-labdan-3-one (**8**), *ent*-16-hydroxy-3-oxo-13-epi-manoyl oxide (**9**), *ent*-15-hydroxylabda-8(17),13*E*-dien-3-one (**10**), labda- 8(17),13*E*-diene-3β,15-diol (**11**). Of the new diterpenoids two are labdanes (**2** and **3**), two are manoyl oxide derivatives (**1** and **5**) and one an isopimarane (**4**). Their structures have been established by a study of their physical and spectral (UV, IR, ^{1}H and ^{13}C NMR, DEPT, ^{1}H–^{1}H COSY, NOESY, HMQC, HMBC and MS) data respectively as 3-oxo-*ent*-13-epi-8(13)-epoxy-15-chloro-14-hydroxylabdane (**1**), *ent*-15-chloro-13,14-dihydroxylabd-8(9)en-3-one (**2**), *ent*-15-chloro-labd-8(9)ene-3α,13,14-triol (**3**), *ent*-11β-hydroxy-8(14),15-isopimaradien-3-one(**4**), 8,13-epoxy-3-nor-2,3-seco-14-epilabden-2,4-olide (**5**).

Agallochin A (**1**) came as colourless needles from MeOH, mp 145–148°C and its molecular formula was fixed as $C_{20}H_{33}O_3Cl$ from elemental analysis and mass ions *m*/*z* at 356 $[M]^+$ and 358 $[M+2]^+$ in the ratio 3:1 in its E.I. mass spectrum. The presence of a 6-membered keto carbonyl (1690 cm^{-1}), hydroxyl (3450 cm^{-1}), and ether absorption (1070 cm^{-1}) accounting for all the three oxygens and a halogen atom (758 cm^{-1}) in the molecule was noticed in the I.R. spectrum. In most of the 3-keto diterpenoids reported here as well as elsewhere the six-membered keto carbonyl in I.R. spectrum is coming slightly at lower frequency, may be, due to transannular conjugation. It was readily recognised as a 8,13-epoxy labdane diterpenoid from its ^{1}H and ^{13}C NMR spectral data. Its ^{1}H and ^{13}C NMR spectral data were, in fact, very close to the data of ent-3-oxo-13-epimanoyl oxide (**6**) isolated from the same species by earlier workers (Konishi et al., 1996a) and in the present investigation as well. The main difference between agallochin A (**1**) and **6** lies in the fact that in place of a vinyl group in the former an α-chloro hydroxy system is present in the latter. The two chloromethylene protons were found at 3.45 (*dd*) and 3.58 (*dd*) and the carbinolic methine proton at 3.84 (*dd*). The ^{13}C NMR chemical shifts of carbonyl carbon at δ 217.0 (C-3) and the oxygenated carbons at 75.4 (C-8), 74.8(C-13) and 77.0 (C-14) as well as of other carbons and the ^{1}H NMR data were found to be consistent with the epimanoyl oxide structure (**1**) proposed for the molecule. The chemical shifts of the respective carbons were assigned by comparison of its HMQC, ^{1}H–^{1}H COSY data with the literature data for compound (**6**). The location of the functional groups was supported by the HMBC (Table 2 and Fig. 2) for example, the C-3 showed correlation with 2-Ha, 2-He, 1-Ha, 1-He, 18-H_3 and 19-H_3 and the C-14 (δ 77.0) bearing secondary hydroxyl showed correlation with 15-H_2 protons, 16-H_3, 12-H_a, 12-H_e and the chloromethylene carbon at δ 46.3 showed correlation with only the carbinolic proton 14-H at δ 3.84 as expected. The relative stereochemistry of the molecule could be established from the ^{1}H–^{1}H NOESY data (Table 2 and Fig. 2) which showed essential correlations, amongst others, such as 20-H_3 with 17-H_3 and 17-H_3 with 14-H to suggest ent-labdane skeleton for agallochin A and to deduce its structure as 3-oxo-*ent*-13epi-8(13)-epoxy-15-chloro-14-hydroxylabdane (**1**). Although agallochin A (**1**) has not been reported from this species by the earlier workers, its possible biogenic precessor i.e. (13*R*,14*S*)-*ent*-8α,13;14,15-diepoxy-13-epi-labdan-3-one (**8**) has been isolated by us as well as the earlier workers (Konishi et al., 1996b) from the same species. The 15-chloro-14-hydroxy isomer was also reported by the earlier workers as a dimethyl ester of 2,3-seco derivative (**12**). The absolute configuration at C-14 in agallochin A could not be decided, but was reasonably assumed to be 14-*R* in view of the NOESY correlations noticed between 14-H and 17-H_3, 14-H and 16-H_3 as noticed in the seco derivative (**12**).

Agallochin B (**2**) came as colourless needles from MeOH, mp 157–159°C and its molecular formula was fixed as $C_{20}H_{33}O_3Cl$ from elemental analysis and mass ions *m*/*z* at 356 $[M]^+$ and 358 $[M+2]^+$ in the E.I. mass. It was recognised as a new diterpenoid of labdane skeleton from its ^{1}H and ^{13}C NMR spectral data. The presence of a six membered keto carbonyl (1685 cm^{-1}), hydroxyl (3430 cm^{-1}), unsaturation (1620 cm^{-1}) and halogen (760 cm^{-1}) was evident from its IR spectrum. It did not show conjugation in its UV spectrum. The ^{13}C NMR showed all the 20 carbons which were analysed from the DEPT spectrum to consist of five methyls, seven methylenes, two methines and six quaternary carbons. The presence of a carbonyl at 217.3 was taken for the

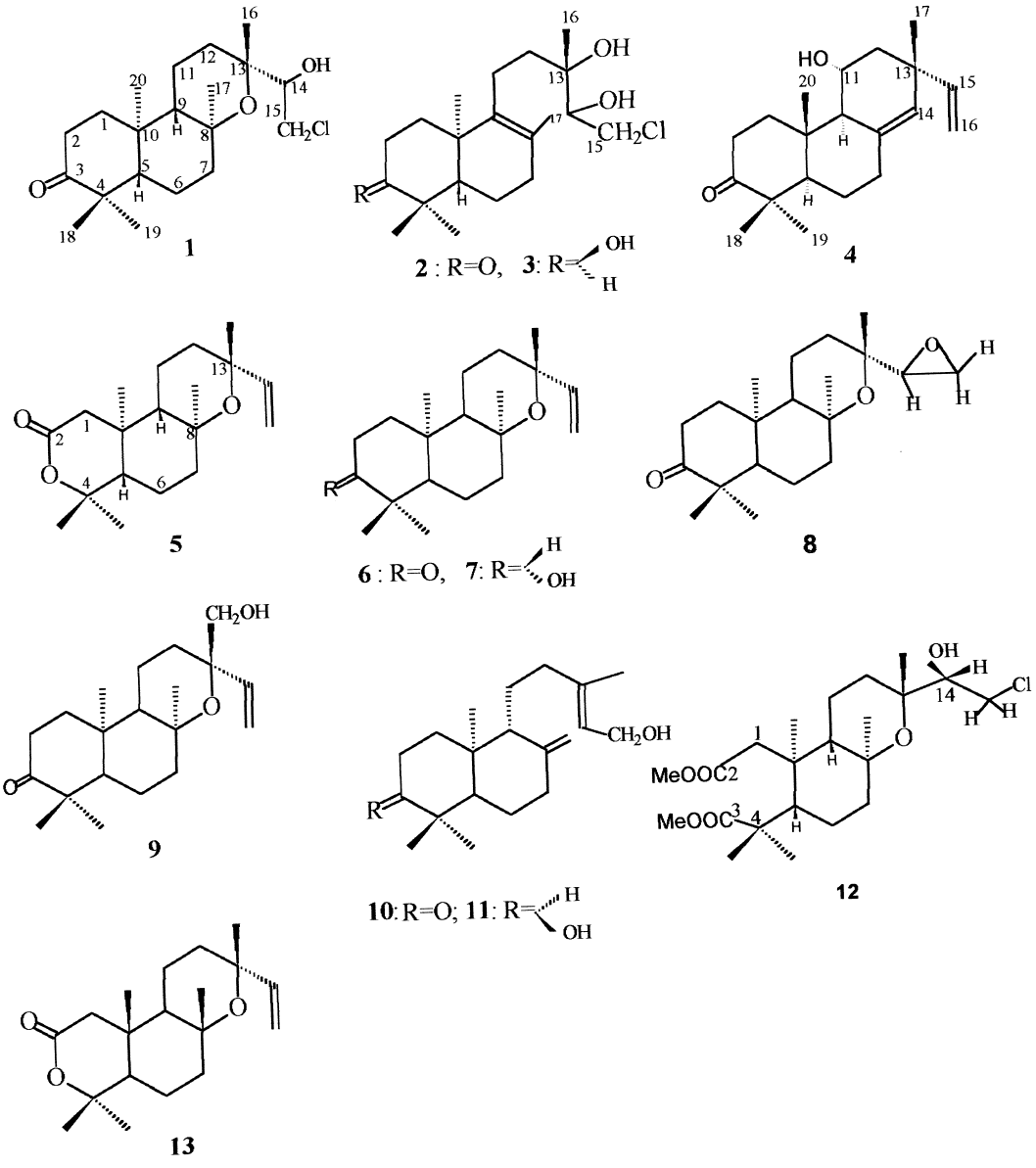

Fig. 1.

keto group at C-3 as found as in other 3-keto diterpenoids and two carbons at 127.3 (*s*), 137.5 (*s*) for a tetra substituted double bond. The two oxygenated carbons at 73.8 (*s*) and 76.3 (*d*) accounted for the carbons carrying tertiary and secondary hydroxyls with the carbinolic methine proton coming at δ 3.63 (1H, *dd*, $J=2$, 10 Hz, 14-H). The value at 47.1 accounted for the chloromethelene carbon with the corresponding methylene protons coming at δ 3.56, 3.73 each as a doublet. The molecule thus accounting for all the oxygens and two degrees of unsaturation (found in a carbonyl and a double bond) must be bicyclic and a derivative of labdane. Of the five methyls one was found to be olefinic methyl (δ 1.58) with the remaining tertiary. The chemical shifts of the respective carbons of agallochan B were assigned by a comparative study of its spectral data with those of *ent*-15-hydroxy-labda-8(17),13*E*-dien-3-one (**10**) isolated from the Okinawan species (Konishi et al., 1996a) and also in the present investigation and also by its HMQC and ^{1}H–^{1}H COSY data. The location of the functional groups was also confirmed by HMBC (Table 3 and Fig. 2), for example the C-3 keto carbon (217.3) showed correlation with 18-H_3, 19-H_3, 2-H_2 and 1-H_2, the C-13 (δ 73.8) with carbinolic methine proton at δ 3.63 (H-14), 16-H_3 and 12-H_2, the C-14 (δ 76.3) carrying secondary hydroxyl with the chloromethylene protons 15-H_2, 16-H_3 and 12-H_2 and so also the chloromethylene carbon (δ 47.1) with 14-H. Similarly, the olefinic carbons at 137.5 and 127.3 showed the respective correlations as noted in Table 3. The ^{1}H–^{1}H NOESY spectrum of the

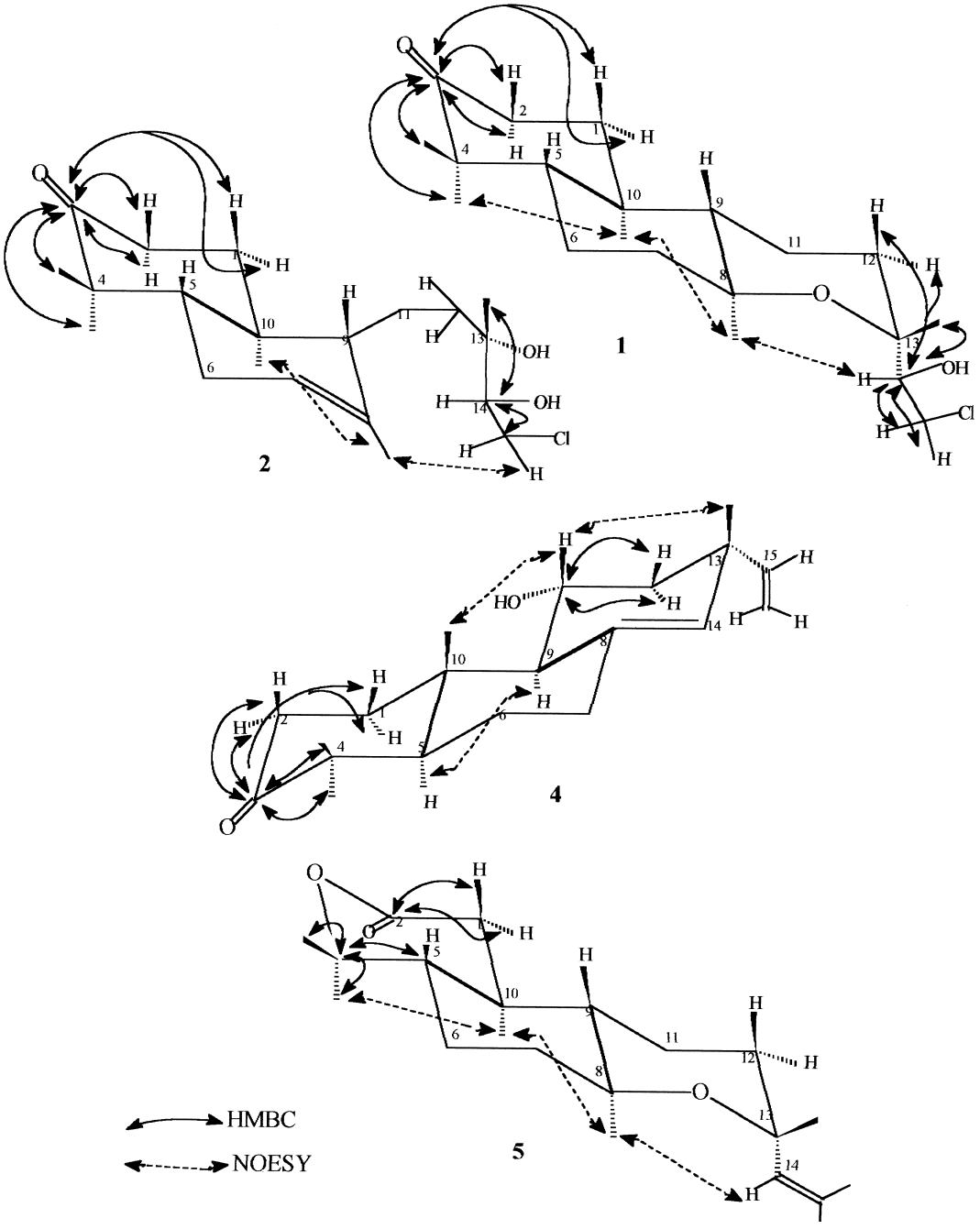

Fig. 2. Some important HMBC and NOESY correlations of **1**, **2**, **4**, **5**.

compound showed several correlations (Table 3 and Fig. 2) where, however, the absence of correlation between 20-H_3 and 5-H showed their *trans* relationship. Although the absolute configuration at C-13 and C-14 needs elaboration, the structure of the molecule could thus be derived as *ent*-15-chloro-13,14-dihydroxy-labd-8(9)-en-3-one (**2**).

Agallochin C (**3**), colourless oil, was also found to be a new chlorolabdane diterpenoid as agallochin B and its molecular formula was fixed as $C_{20}H_{35}O_3Cl$ from elemental analysis and the mass ions m/z 358 and 360 in its E.I. mass spectrum. Unlike agallochin B, it did not show the carbonyl frequency in its IR spectrum but only hydroxylic absorption (3450 cm^{-1}), unsaturation (1635 cm^{-1}), and halogen (730 cm^{-1}). The ^1H and ^{13}C NMR spectral data of agallochin C were very similar to those of agallochin B except for the difference in their functional groups, the former being a trihydroxy compound while the latter a dihydroxy keto derivative. This was evident from the absence of keto carbonyl (C-3) in agallochin C in whose place appeared an additional oxygenated carbon at δ 79.6(*d*) carrying a secondary

hydroxyl. The appearance of 3-H as a *dd* with coupling constants 11 and 5 suggested its 3α-axial configuration. The structure of agallochin C could thus be derived as *ent*-15-chloro-labd-8(9)ene-3α,13,14-triol (**3**). The absolute configuration at C-13 and C-14 need elaboration as in agallochin B.

Agallochan D (**4**) came as colourless needles from MeOH, mp 145–148°C and its molecular formula was

Table 1
^{13}C NMR data[a] and assignments of compounds **1**–**12**

Carbon no.	(1)b	(2)b	(3)b	(4)b	(5)b	(6)c	(7)c	(8)c	(9)c	(10)c	(11)c	(12)b
1	37.8	35.3	35.8	38.2	45.8	38.2	37.7	37.9	38.1	37.6	37.1	40.6
2	33.7	34.3	28.4	34.7	170.3	33.8	27.3	33.7	33.7	34.7	27.9	171.5
3	217.0	217.3	79.6	216.3	–	217.1	78.8	216.9	217.0	217.0	78.8	179.5
4	47.3	47.1	40.2	47.7	85.6	47.2	38.8	47.3	47.2	47.7	39.1	45.9
5	54.3	51.4	51.8	55.3	52.4	54.7	55.3	54.7	54.6	55.2	54.7	46.1
6	20.8	21.7	22.3	23.5	22.1	20.8	19.6	20.8	20.7	25.1	24.0	21.9
7	42.8	33.4	34.5	35.6	41.5	42.2	43.0	42.0	42.0	38.3	38.3	42.2
8	75.4	127.3	127.1	134.9	74.9	75.4	75.6	74.0	76.1	147.2	147.0	75.4
9	54.9	137.5	139.7	58.6	55.9	57.7	58.3	56.5	57.5	55.2	56.1	48.3
10	36.6	38.7	39.6	37.6	36.2	36.4	36.6	36.4	36.4	39.2	39.4	41.5
11	15.1	19.9	19.4	65.9	15.9	16.4	15.9	16.8	15.8	22.3	22.0	15.6
12	31.2	39.4	39.5	43.8	34.5	34.9	34.9	31.0	28.5	37.9	38.2	30.2
13	74.8	73.8	74.7	38.4	73.6	73.6	73.4	71.5	76.4	140.0	140.0	74.5
14	77.0	76.3	77.0	128.5	147.0	147.4	147.6	57.5	143.8	123.5	123.2	77.4
15	46.3	47.1	47.9	148.4	110.0	109.7	109.6	47.2	113.6	59.4	59.4	46.4
16	24.7	21.5	22.2	110.6	32.5	32.6	32.6	29.1	69.7	16.3	16.3	24.2
17	24.1	19.5	19.9	26.6	23.0	23.4	23.8	23.5	23.4	107.5	106.7	24.3
18	26.5	26.7	28.8	25.8	32.5	26.7	28.0	26.7	26.7	26.0	28.3	27.9
19	21.0	21.4	16.1	22.1	24.3	20.8	15.2	20.9	20.8	21.6	15.4	23.6
20	14.9	19.4	20.8	15.2	16.2	15.4	16.0	15.2	15.5	14.0	14.5	29.1

[a] Chemical shifts in δ from TMS taken in $CDCl_3$: a, 75 MHz; b, 22.5 MHz.

Table 2
^{1}H NMR and ^{13}C NMR assignments and HMBC, NOESY and COSY correlations of agallochin A (**1**)[a]

Carbon no.	^{1}H (δ)	^{13}C (δ)	HMBC	NOESY	COSY
C-1	1.83 *m*	37.8	2-H_2, 5-H, 20-H_3	20-H_3	1-H_a
	1.45 *m*				
C-2	2.58 *m*	33.7	1-H_2	20-H_3, 1-H	1-H_a,2-H_a
	2.42 *m*			1-H	
C-3		217.0	2-H_2, 1-H_2, 18-H_3, 19-H_3		
C-4		47.3	5-H, 18-H_3, 19-H_3		
C-5	1.48 *m*	54.3	6-H_2, 7-H_2, 9-H, 1-H_2, 20-H_3, 18-H_3, 19-H_3		
C-6	1.62 *m*	20.8	7-H_2, 5-H	5-H, 19-H_3	6-H_a
	1.42 *m*				
C-7	1.46 *m*	42.8	6-H_2, 9-H, 17-H_3	6-H	6-H_a
	1.87 *m*				
C-8		75.4	9-H, 11-H_2, 7-H_2, 6-H_2, 17-H_3		
C-9	1.53 *m*	54.9	12-H_2, 11-H_2, 1-H_2, 7-H_2, 5-H, 20-H_3, 17-H_3		
C-10		36.6	1-H_2, 2-H_2, 5-H, 20-H_3, 9-H, 11-H_2		
C-11	1.48 *m*	15.1	12-H_2, 9-H		
	1.60 *m*				
C-12	2.01 *m*	31.2	11-H_2, 9-H, 16-H_3	9-H	12-H_a
	1.58 *m*				
C-13		74.8	14-H, 15-H_2, 11-H_2, 12-H_2, 16-H_3		
C-14	3.84 (*dd*, *J* = 2, 8 Hz)	77.0	15-H_2, 16-H_3, 12-H_2	15-H_2, 12-H_a, 17-H_3, 16-H_3	15-H_2
C-15	3.45 (*dd*, *J* = 9.5, 8 Hz)	46.3	14-H	16-H_3	
	3.58 (*dd*, *J* = 9.5, 2 Hz)			16-H_3	
C-16	1.09 *s*	24.7	14-H, 12-H_2		
C-17	1.35 *s*	24.1	7-H_2, 9-H	20-H_3	
C-18	1.09 *s*	26.5	19-H_3, 5-H		
C-19	1.03 *s*	21.0	18-H_3, 5-H	20-H_3	
C-20	0.92 *s*	14.9	1-H_2, 5-H, 9-H		

[a] Chemical shifts in δ from TMS (multiplicity) in $CDCl_3$.

Table 3
^{1}H NMR and ^{13}C NMR assignments and HMBC, NOESY and COSY correlations of agallochin B (**2**)[a]

Carbon no.	^{1}H (δ)	^{13}C (δ)	HMBC	NOESY	COSY
C-1	2.05 *m*	35.3	2-H_2, 5-H, 20-H_3	20-H_3	20-H_3
	1.72 *m*				20-H_3
C-2	2.53 *m*	34.3	1-H_2	1-H_a, 19-H_3	1-H_e, 2-H_a
	2.46 *m*			18-H_3	1-H_a
C-3		217.3	2-H_2, 1-H_2, 18-H_3, 19-H_3		
C-4		47.1	5-H, 18-H_3, 19-H_3		
C-5	1.68 (*d*, J= 5 Hz)	51.4	6-H_2, 7-H_2, 9-H, 1-H_2, 20-H_3, 18-H_3, 19-H_3		18-H_3
C-6	2.00 *m*	21.7	5-H	19-H_3	
	2.12 *m*			7-H_a, 5-H_a, 17-H_3	
C-7	1.95 *m*	33.4	6-H_2, 5-H, 17-H_3	5-H_a	
	2.02 *m*				7-H_a
C-8		127.3	6-H_2, 11-H_2, 7-H_2, 17-H_3		
C-9		137.5	12-H_2, 11-H_2, 1-H_2, 7-H_2, 5-H, 20-H_3, 17-H_3		
C-10		38.7	1-H_2, 2-H_2, 5-H, 20-H_3, 11-H_2, 6-H_2		
C-11	1.48 *m*	19.9	12-H_2		
	1.52 *m*				20-H_3
C-12	1.56 *m*	39.4	11-H_2, 16-H_3		
	1.62 *m*				11-H, 12-H_a
C-13		73.8	15-H_2, 11-H_2, 12-H_2, 16-H_3		
C-14	3.63 (*dd*, J=2, 10 Hz)	76.3	15-H_2, 16-H_3, 12-H_2	16-H_3, 17-H_3	
C-15	3.56 (*dd*, J=10.8,10 Hz)	47.1	14-H	16-H_3	
	3.73 (*dd*, J=10.8, 2 Hz)			16-H_3, 17-H_3	15-H_a
C-16	1.16 *s*	21.5	14-H, 12-H_2		
C-17	1.58 *s*	19.5	7-H_2	20-H_3	
C-18	1.06 *s*	26.7	19-H_3, 5-H		
C-19	1.02 *s*	21.4	18-H_3, 5-H		
C-20	1.02 *s*	19.4	1-H_2, 5-H		

[a] Chemical shifts in δ from TMS (multiplicity) in $CDCl_3$

fixed as $C_{20}H_{30}O_2$, from elemental analysis and the molecular ion m/z $[M]^+$, 302, EIMS. It was recognised as a new diterpenoid from its ^{1}H NMR and ^{13}C NMR spectral data. The presence of keto carbonyl (1690 cm^{-1}), hydroxyl (3440 cm^{-1} br) and olefinic unsaturation (1670, 995, 900, 860 cm^{-1}) was noticed in its IR spectrum, but no conjugation in its UV spectrum. Its ^{13}C NMR spectrum showed all the 20 carbons which were analysed as four methyls, six methylenes, five methines, five quaternary carbons from its DEPT spectrum. The value at δ 216.3 accounted for 3-keto group and the lone oxygenated carbon at δ 65.9(*d*) showed the presence of a secondary hydroxyl with the carbinolic proton coming at δ 4.00 as *dd* (1H, *dd*, J=12, 6 Hz, 11-H). Four olefinic carbons were noticed, two of a vinyl group at δ 110.6 and 148.4 with the corresponding characteristic protons coming at δ 4.89 (1H, *d*, J=10, Hz, 16-H), 4.96 (1H, *d*, J= 18, Hz, 16-H), 5.83 (1H, *dd*, J= 18, 10 Hz, 15-H) and two more of a trisubstituted double bond at δ 128.5 and 134.9 with the corresponding olefinic proton coming as a singlet at δ 5.34. All the four methyls present were found to be quaternary, each coming as a singlet at δ 0.98, 1.02, 1.04 and 1.06, respectively. The molecular formula requiring six double bond equivalence, three of which being accounted for, one in a carbonyl and two in double bonds, should be tricyclic.

The spectral data of agallochin D resemble very much those of a pimarane or isopimarane skeleton. The chemical shifts of the respective carbons were assigned based on the data from HMQC, ^{1}H–^{1}H COSY and literature data. The location of the keto carbonyl at C-3 was also supported by the values of the chemical shifts of the tertiary methyls at C-4 [δ_c 25.8 (C-18); δ_c 22.1 (C-19)]. The presence of 3-keto group was also supported by HMBC (Table 4 and Fig. 2) data, where the C-3 showed correlations with 2H_a, 2H_e; 1H_a, 1H_e; 18-H_3 and 19-H_3. If the vinylic group is taken in its common position at C-15, the trisubstituted double bond might be located at 5(6), 7(8), 9(11) or 8(14) positions. In ^{13}C NMR the chemical shift values of this double bond carbons (128.5 and 134.9) might correspond to the values of 8(14) double bond (128.5, 137.3) as noticed in (−)-isopimara-8(14),15-diene (Touche et al., 1997), but certainly not of 5(6) double bond (approximately δ 140 and 120 ppm) as noticed in steroid 5-enes or 9(11) double bond (δ 148.6, 113.5 ppm) as noticed in isopimara-9(11),15-diene-3β,19-diol (Nishiya et al., 1991) or of 7(8) double bond (δ 119.7, 136.8 ppm) as noticed in 9-epi-*ent*-pimara-7,15-diene-2α,19-diol (Chamy et al., 1995). The presence of trisubstituted olefinic proton attached to this double bond as a singlet at δ 5.34 fixes the double bond at 8(14) excluding all other possibilities where it would have come as a multiplet. The location of this

Table 4
^{1}H NMR and ^{13}C NMR assignments and HMBC, NOESY and COSY correlations of agallochin D (**4**)[a]

Carbon no.	^{1}H (δ)	^{13}C (δ)	HMBC	NOESY	COSY
C-1	1.76 (*dt*, *J* = 14, 6 Hz)	38.2	2-H_2, 5-H, 9-H		20-H_3
	2.22 (*dt*, *J* = 14, 6 Hz)			20-H_3	1-H_a
C-2	2.63 (*dt*, *J* = 16, 6 Hz)	34.7	1-H_2	1-H_a, 19-H_3	2-H_a,1-H_e,1-H_a
	2.32 (*dt*, *J* = 16, 6 Hz)			1-H_e	1-H_a
C-3		216.3	2-H_2, 1-H_2, 18-H_3, 19-H_3		
C-4		47.7	2-H_2, 5-H, 6-H_2, 18-H_3, 19-H_3		
C-5	1.53 *m*	55.3	6-H_2, 7-H_2, 9-H, 1-H_2, 20-H_3, 18-H_3, 19-H_3		
C-6	1.6 *m*	23.5	5-H, 7-H_2		
	1.48 *m*				
C-7	2.35 *m*	35.6	6-H_2, 5-H, 14-H, 9-H		6-H_a,7-H_a
	2.02 *m*			9-H_a, 6-H_e, 5-H_a	
C-8		134.9	6-H_2, 11-H, 7-H_2, 9-H		
C-9	1.75 *m*	58.6	12-H_2, 11-H, 1-H_2, 7-H_2, 5-H, 20-H_3, 14-H	12-H_2, 5-H_a	
C-10		37.6	1-H_2, 2-H_2, 5-H, 20-H_3, 11-H, 6-H_2, 9-H		
C-11	4.0 (*dd*, *J* = 12, 6 Hz)	65.9	12-H_2, 9-H	1-H_e, 12-H_2, 20-H_3, 17-H_3	12-H, 9-H
C-12	1.62 *m*	43.8	11-H, 14-H, 15-H, 17-H_3		
C-13		38.4	15-H, 11-H, 12-H_2, 16-H_2, 14-H, 17-H_3		
C-14	5. 34 *s*	128.5	15-H, 17-H_3, 12-H_2, 7-H_2, 9-H	17-H_3, 7-H_e	9-H_a
C-15	5.83 (*dd*, *J* = 18,10)	148.4	14-H, 16-H_2, 12-H_2, 17-H_3	12-H_2, 17-H_3	16-H_2
C-16	4.96 (*d*, *J* = 18 Hz)	110.6		17-H_3	16-H_a
	4.89 (*d*, *J* = 10 Hz)			17-H_3	
C-17	1.04 *s*	26.6	15-H, 12-H_2, 14-H		
C-18	1.06 *s*	25.8	19-H_3, 5-H		
C-19	1.02 *s*	22.1	18-H_3, 5-H		
C-20	0.98 *s*	15.2	1-H_2, 5-H, 9-H		

[a] Chemical shifts in δ from TMS (multiplicity) in $CDCl_3$.

double bond was also supported by the HMBC data (Table 4 and Fig. 2) for example the olefinic carbon C-14 (128.5) showed correlation with 15-H, 17-H_3, 12-H_2, 7-H and 9-H and similarly the other olefinic carbon C-8 (134.9) showed correlation with 6-H_2, 11-H, 7-H_2, and 9-H. The secondary hydroxyl was located at C-11 taking its ^{13}C value into consideration in comparison with other 11-hydroxy diterpenoids and also by the ^{1}H chemical shift of the carbinolic proton at C-11 as a *dd* (coupling with 9-H and one of the vicinal protons at C-12 and probably the other proton making no coupling with a dihedral angle of 90° in a ring C quasy chair form) and also by HMBC data where the C-11 (65.9) showed correlation with 9-H and 12-H_a, H_e.

With respect to the stereochemistry of the molecule, it was related to an isopimarane skeleton by the correlations found in the ^{1}H–^{1}H NOESY data (Table 4 and Fig. 2). The presence of correlation between 5-H and 9-H and the absence of correlation between 20-H_3 with 5-H as well as 9-H indicated *trans–trans* configuration at C-5, C-10 and C-10, C-9 ring junctions. The 9α-H did not show correlation with the carbinolic 11β-H showing their *trans* relationship to fix, incidentally, the stereochemistry of 11-hydroxyl as α. The 11β-H showed correlation with 17-H_3 as well as 20-H_3 showing their *cis*-relationship indicating β-configuration for 17-H_3 which in turn is *trans* to 9-α H suggesting an isopimarane skeleton for the molecule. The structure and relative stereochemistry of agallochin D has thus been fixed as *ent*-11α-hydroxy-8(14),15-isopimaradien-3-one (4).

Agallochin E (**5**) came as colourless needles from MeOH, m.p. 140–42°C, Its molecular formula was fixed as $C_{19}H_{30}O_3$ from elemental analysis and by the mass ions *m*/*z* $[M]^+$ 306 followed by very intense $[M\text{-}15]^+$ peak at *m*/*z* 291 (72%). The molecular formula, ^{1}H and ^{13}C NMR spectral data suggested it to be a new nor 8,13-epoxylabdane diterpenoid. Its IR spectrum showed strong δ-lactonic absorption at 1730 cm^{-1}, multiple ether absorption peaks around 1090 $cm^{-1}$and vinylic absorption (990, 910 cm^{-1}). The ^{1}H NMR spectrum showed five tertiary methyls but four of them, unusual in the diterpenoids, connected to oxygenated carbons at δ1.14, 1.25, 1.32 and 1.43. The three vinylic protons were noticed at δ 6.00 (1H, *dd*, *J* = 11,18 Hz, 14-H), δ 5.00 (1H, *d*, *J* = 18 Hz, 15-H), δ 4.97 (1H, *d*, *J* = 11 Hz, 15-H) as noticed in epimanoyl oxide and other similar derivatives (**6**). The ^{13}C NMR spectrum showed all the nineteen carbon peaks which were analysed as five methyls, six methelenes, three methines, five quaternary carbons from its DEPT spectrum. It showed three oxygenated carbons, two at δ 74.9 and 73.6 accounting for C-8, C-13 of 8,13-epoxylabdane skeleton and the third coming at δ 85.6(*s*) as a quaternary carbon, which in all probability, might be C-4 carrying the methyls 18-H_3 and 19-H_3. This fact incidentally suggested that the oxygen at C-4 might be part of a lactone ring, which, in

all probability, might be formed from a hydroxy acid derived from ring-A *seco* derivative losing a carbon subsequently as CO_2. The tricyclic nature of the molecule was also derived by the fact that only two out of the five degrees of unsaturation were found, one in the lactone carbonyl and the other in vinylic double bond. The ^{13}C NMR spectrum amongst the other carbons confirmed the presence of a lactone carbonyl carbon at δ 170.3 and the vinylic carbons at 110.0 and 147.0. The structure of agallochan E in all probability could be 8,13-epoxy-3-nor-2,3-seco-14-epilabden-2,4-olide (**5**). That the lactone is part of ring A and it is 3-nor derivative has been deduced by HMBC data (Table 5 and Fig. 2). The lactone carbon C-2 (δ 170.3), C-3 being lost, showed correlations only with 1-H_a and 1-H_e as expected from the structure. The oxygenated carbon C-4 at δ 85.6 showed correlation with 18-H_3 and 19-H_3 besides 5-H. The other correlations (cf. Table 5) noticed in the spectrum are in full support of the structure.

The relative stereochemistry at the chiral centres has been deduced by the NOESY spectrum (Table 5 and Fig. 2). The 20-H_3 showed NOESY correlation with 17-H_3 showing their *cis* relationship and the same did not show correlation with either 5-H or 9-H showing the overall stereochemistry between the A, B and C rings as *trans–trans–trans*. The 17-H_3 showed correlation with 14-H of the vinyl group showing their *cis* disposition thus relating the molecule to epimanoyloxy skeleton to derive its structure as 8,13-epoxy-3-nor-2,3-*seco*-14-epilabden-2,4-olide (**5**) or 2-oxo-3-oxa epimanoyl oxide. A search in literature revealed that an isomeric δ-lactone (**13**) belonging to manoyloxide was isolated from the plant *Dacrydium colensoi* (Grant et al., 1965) whose physical (no $[\alpha]_D^{25}$) and spectral (no ^{13}C data) data are available partly. A comparative study of the available data of **5** and **13** revealed that they are different and **5** is a new compound whose structure and stereochemistry have been unequivocally established by all its spectral (including HMBC) data.

Ent-3-oxo-13-epi-manoyl oxide (**6**) $C_{20}H_{32}O_2$, colourless needles, mp 122–124°C, mass ions CIMS: *m/z* 305 $[M+H]^+$, 290 $[M+H–CH_3]^+$(100%); *ent*-3β-hydroxy-13-epi-manoyl oxide (**7**), $C_{20}H_{34}O_2$, colourless needles, mp 82–84°C, mass ions CI–MS: *m/z* 307 $[M+H]^+$; (13R,14S)-*ent*-8α,13;14,15-diepoxy-13-epi-labdan-3-one (**8**), $C_{20}H_{32}O_3$, colourless needles, mp 145–146°C, mass ions CIMS at *m/z* 321 $[M+H]^+$, and *m/z* 303 $[M+H–H_2O]^+$; *ent*-16-hydroxy-3-oxo-13-epi-manoyl oxide (**9**), $C_{20}H_{32}O_2$, colourless needles, mp 134–136°C, mass ions CIMS at *m/z* 321 $[M+H]^+$ and *ent*-15-hydroxy-labda-8(17),13E-dien-3-one (**10**), $C_{20}H_{32}O_2$, colourless needles, mp 117–118°C, mass ions EIMS *m/z* 304 $[M]^+$ were readily characterised by comparison of their physical and spectral data with the data recorded for these

Table 5
^1H NMR and ^{13}C NMR assignments and HMBC, NOESY and COSY correlations of agallochin E (**5**)[a]

Carbon no.	^1H (δ)	^{13}C (δ)	HMBC	NOESY	COSY
C-1	2.66 (*d*, *J*=16 Hz)	45.8	20-H_3	20-H_3, 11-H_a	1-H_a
	1.93 (*d*, *J*=16 Hz)			5-H_a, 9-H	20-H_3
C-2		170.3	1-H_2		
C-3		–			
C-4		85.6	5-H, 18-H_3, 19-H_3		
C-5	1.6 *m*	52.4	6-H_2, 7-H_2, 9-H, 1-H_2, 20-H_3, 18-H_3, 19-H_3	18-H_3, 9-H_a	
C-6	1.6 *m*	22.1	7-H_2, 5-H		5-H
	1.46 *m*				
C-7	1.48 *m*	41.5	5-H, 6-H_2, 9-H, 17-H_3		
	1.84 *m*			17-H_3	7-H_a, 6-H_e
C-8		74.9	9-H, 11-H_2, 7-H_2, 6-H_2, 17-H_3		
C-9	1.26 *m*	55.9	12-H_2, 11-H_2, 1-H_2, 7-H_2, 5-H, 20-H_3, 17-H_3		
C-10		36.2	1-H_2, 5-H, 20-H_3, 9-H, 11-H_2, 6-H_2		
C-11	1.56 *m*	15.9	12-H_2, 9-H	16-H_3	
	1.42 *m*			20-H_3	
C-12	1.52 *m*	34.5	11-H_2, 14-H, 16-H_3	9-H_a	
	2.27 *m*			11-H_a	12-H_a, 16-H_3
C-13		73.6	14-H, 11-H_2, 12-H_2, 16-H_3		
C-14	6.00 (*dd*, *J*= 11, 18 Hz)	147.0	15-H_2, 16-H_3, 12-H_2	15-H_2, 17-H_3, 16-H_3	12-H_a
C-15	4.94 (*d*, *J*= 11 Hz)	110.0		12-H	
	5.00 (*d*, *J*= 18 Hz)				
C-16	1.14 *s*	32.5	14-H		
C-17	1.25 *s*	23.0	7-H_2, 9-H	20-H_3	
C-18	1.43 *s*	32.5	19-H_3, 5-H		
C-19	1.32 *s*	24.3	18-H_3, 5-H	20-H_3	
C-20	0.89 *s*	16.2	1-H_2, 5-H, 9-H		

[a] Chemical shifts in δ from TMS (multiplicity) in $CDCl_3$.

compounds, which have, in fact, been isolated from the Okinawan species of *E. agallocha* earlier (Konishi et al., 1996a,b).

Labda-8(17),13*E*-diene-3β,15-diol (**11**) came as colourless needles, mp 158–159°C. Its molecular formula was fixed as $C_{20}H_{34}O_2$ from the elemental analysis and mass ions CIMS *m/z* 307 $[M+H]^+$, 289 $[M+H–CH_3]^+$. The spectral characteristics of this compound were found to be nearly the same as for compound (**10**) described above except for the absence of carbonyl at C-3 and in which place a secondary hydroxyl was noticed suggesting that this might be dihydro derivative of the former. The carbinolic methine proton was noticed at δ 3.25 as a *dd* ($J=5$, 11 Hz) representing the 3α-H. Its ^{13}C NMR spectrum did not show the carbonyl carbon and in its place showed an oxygenated carbon at δ 78.8 (*d*) accounting for C-3. It was reported as a $NaBH_4$ reduction product of compound (**10**) (Konishi et al., 1996a) although this was not isolated as such form this species. Its enantiomeric diol was, however, reported from the same species earlier (Braun and Breitenbach, 1977; Forster et al., 1985). The optical rotation and spectral data of the diol isolated now agreed with the data of $NaBH_4$ reduction product to characterise it as (−)labda-8(17),13*E*-diene-3β,15-diol. The isolation of this enantiomeric diol thus forms its first report from nature.

3. Experimental

3.1. General

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. Optical rotations were determined on a Roudalph Autopol-III Polarimeter. Elemental analyses were determined on a Carlo Erba 1108 instrument. Mass spectra were obtained on a JEOL JMS-300 spectrometer.

3.2. Plant material

The roots of *E. agallocha* were collected from Corangi Mangrove forest near Bhiravapalem of Godavari Estuary (16° 58′ N latitude and 82° 15′ E longitude) in March 1998 and was identified by Professor B. Kondala Rao, Dept. of Marine Living Sources, Andhra University, Visakhapatnam. Voucher specimens (Code: AU1/160) have been deposited at the Marine Museums of School of Chemistry, Andhra University and National Institute of Oceanography, Goa.

3.3. Extraction, isolation and characterization

The air-dried and powdered plant material (4 kg) was exhaustively extracted with *n*-hexane. Removal of the solvent from the combined *n*-hexane extracts under reduced pressure gave a residue (35 g). This residue was subjected to column chromatography over a column of silica gel (Acme brand, 100–200 mesh, 350 g) using solvents of increasing polarity from *n*-hexane through EtOAC. In all 250 fractions (500 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 11 pure compounds (**1**–**11**).

The residue from the column fractions 52–75 (*n*-hexane: EtOAc; 9.5:0.5) on further purification by passing over a silica gel column furnished compound **6** (200 mg).

The residue from the column fractions 76–88 (*n*-hexane: EtOAc; 9.0:1.0) on further purification by passing over a silica gel column furnished compound **7** (60 mg).

The residue from the column fractions 89-114 (*n*-hexane: EtOAc; 8.75:1.25) on further purification by passing over a column of silica gel impregnated with silver nitrate (20%) furnished compound **8** (40 mg) and compound **1** (30 mg).

The residue from the column fractions 115–142 (*n*-hexane:EtOAc; 8.5:1.5) on further purification by passing over a silica gel column impregnated with silver nitrate (20%) furnished compound **2** (60 mg) and compound **3** (35 mg).

The residue from the column fractions 143–167 (*n*-hexane:EtOAc; 8.25:1.75) on further purification by passing over a silica gel column in CH_2Cl_2–EtOAC medium furnished compound **4** (40 mg) and compound **9** (30 mg).

The residue from the column fractions 168–192 (*n*-hexane:EtOAc; 8.0:2.0) on further purification by passing over a silica gel column impregnated with silver nitrate (20%) furnished compound **5** (35 mg) and compound **10** (30 mg).

The residue from the column fractions 193–212 (*n*-hexane:EtOAc; 7.5:2.5) on crystallisation from benzene gave compound **11** (40 mg).

3.3.1. Agallochin A (*1*)-3-oxo-ent-13epi-8(13)-epoxy-15-chloro-14-hydroxylabdane

Colourless needles from MeOH, mp 145–148°C, $[\alpha]_D^{25}$ −38.0° ($CHCl_3$, *c* 1.5). IR γ_{max} cm^{-1}: 3450 (OH), 1690 (C=O), 1070 (ether), 758 (halogen). Found C 67.20%, H 9.12%, $C_{20}H_{33}O_3Cl$ requires C67.41%, H 9.26%. EIMS *m/z*: 358 $[M+2]^+$, 356 $[M]^+$, 276, 258, 240, 200, 150, 108, 80. ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$): see Table 2.

*3.3.2. Agallochin B (**2**)- ent-15-chloro-13,14- dihydroxy-labd-8(9)-en-3-one*

Colourless needles from MeOH, mp 157–159°C, $[\alpha]_D^{25}$ −45.1° (*c* 1.75, $CHCl_3$). IR γ_{max} cm^{-1}: 3430 (OH), 1685 (C=O), 1620 (unsaturation), 760 (halogen). Found C 67.12%, H 9.08%, $C_{20}H_{33}O_3Cl$ requires C 67.41%, H 9.26%. EIMS *m/z*: 358 $[M+2]^+$, 356 $[M]^+$, 277, 259, 218, 205, 161, 119, 107. ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$): see Table 3.

*3.3.3. Agallochin C (**3**)- ent-15-chloro-labd-8(9)ene-3α, 13,14-triol*

Colourless oil, $[\alpha]_D^{25}$ −26.4° (*c* 1.7, $CHCl_3$). IR γ_{max} cm^{-1}: 3450 (OH), 1635 (vinyl), 730 (halogen). Found C 66.90%, H 9.52%, $C_{20}H_{35}O_3Cl$ requires C 67.03%, H 9.77%. EIMS *m/z*: 360 $[M+2]^+$, 358 $[M]^+$, 340, 325, 307, 289, 271, 189, 133, 119. ^1H NMR (300 MHz, $CDCl_3$) δ 0.79, 0.95, 0.99, 1.18, 1.57 (3H each, *s*, Me), 3.23 (1H, *dd*, *J*=11, 5 Hz, 3-H), 3.75 (1H, *dd*, *J*=11, 2 Hz, 15-H), 3.66 (1H, *dd*, *J*=10, 2 Hz, 14-H), 3.59 (1H, *dd*, *J*=10, 11 Hz, 15-H) and ^{13}C NMR (75 MHz, $CDCl_3$): see Table 1.

*3.3.4. Agallochin D (**4**)- ent-11β-hydroxy-8(14),15-iso-pimaradien-3-one*

Colourless needles, mp 145–148°C, $[\alpha]_D^{25}$ +45.3° (*c* 1.9, $CHCl_3$). IR γ_{max} cm^{-1}: 3440 (OH), 1690 (C=O), 1670, 995, 900, 860 (unsaturation). Found C 79.04%, H 9.64%, $C_{20}H_{30}O_2$ requires C 79.47%, H 9.93%. EIMS *m/z*: 302 $[M]^+$, 287 $[M-CH_3]^+$, 269, 227, 159, 133, 105. ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$): see Table 4.

*3.3.5. Agallochin E (**5**)- 8,13-epoxy-3-nor-2,3-seco-14-epilabden-2,4-olide*

Colourless needles, mp 140–142°C, $[\alpha]_D^{25}$ −101.2° (*c* 1.6, $CHCl_3$). IR γ_{max} cm^{-1}: 1730 (C=O), 1090 (ether), 990, 910 (vinyl). Found C 74.12%, H 9.34%, $C_{19}H_{30}O_3$ requires C 74.50%, H 9.80%. EIMS *m/z*: 306 $[M]^+$, 291 $[M-CH_3]^+$, 273 $[M-CH_3-H_2O]^+$, 231, 221, 208, 177, 161, 108, 81, 44. ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$): see Table 5.

*3.3.6. Ent-3-oxo-13-epi-manoyl oxide (**6**)*

Colourless needles from MeOH, mp 122–24°C. $[\alpha]_D^{25}$ −36.0° (*c* 0.36, $CHCl_3$). IR γ_{max} cm^{-1}: 1700 (C=O), 1630, 900 (vinyl), 1065 (ether). CIMS *m/z*: 305 $[M+H]^+$, 290 $[M+H-CH_3]^+$, 206, 149. ^1H NMR (90 MHz, $CDCl_3$) δ 0.85 (3H, *s*, H-20), 1.00 (3H, *s*, H-19), 1.10 (3H, *s*, H-18), 1.19 (3H, *s*, H-16), 1.25 (3 H, *s*, H-17), 4.92 (1H, *d*, *J*= 11.0 Hz, H-15), 4.98 (1H, *dd*, *J*= 18.0 Hz, H-15), 6.15 (1H, *dd*, *J*=11.0, 18.0 Hz, H-14) and ^{13}C NMR (22.5 MHz, $CDCl_3$): see Table 1.

*3.3.7. Ent-3β-hydroxy-13-epi-manoyl oxide (**7**)*

Colourless needles from MeOH, mp 82–84°C. $[\alpha]_D^{25}$ −15.2° (*c* 1.3, $CHCl_3$). IR γ_{max} cm^{-1}: 3360 (OH), 1620, 960 (vinyl), 1080 (ether). CIMS *m/z*: 307 $[M+H]^+$, 292 $[M+H-CH_3]^+$. ^1H NMR (90 MHz, $CDCl_3$) δ 0.78 (3H, *s*, H-20), 0.78 (3H, *s*, H-19), 0.97 (3H, *s*, H-18), 1.10 (3H, *s*, H-16), 1.22 (3 H, *s*, H-17), 3.22 (1H, *ddd*, *J*=5.5, 5.5, 11.0 Hz, H-3), 4.92 (1H, *d*, *J*= 11.0 Hz, H-15), 4.97 (1H, *d*, *J*= 18.0 Hz, H-15), 6.00 (1H, *dd*, *J*=11.0, 18.0 Hz, H-14) and ^{13}C NMR (22.5 MHz, $CDCl_3$): see Table 1.

*3.3.8. (13R,14S)-Ent-8α,13;14,15-diepoxy-13-epi-labdan-3-one (**8**)*

Colourless needles from MeOH, mp 145–46°C. $[\alpha]_D^{25}$ −26.0° (*c* 0.5, $CHCl_3$). IR γ_{max} cm^{-1}: 1690 (C=O), 1100 (ether). CIMS *m/z*: 321 $[M+H]^+$, 303 $[M+H-CH_3]^+$, 285, 277, 263, 257, 244, 220, 207. ^1H NMR (90 MHz, $CDCl_3$) δ 0.87 (3H, *s*, H-20), 1.02 (3H, *s*, H-19), 1.11 (3H, *s*, H-18), 1.20 (3H, *s*, H-16), 1.35 (3 H, *s*, H-17), 2.45 (1H, *dd*, *J*=7.5, 15.5Hz, H-2), 2.50 (1-H, *dd*, *J*=7.5, 15.5 Hz, H-2), 2.80 (1H, *dd*, *J*= 3.0, 5.0 Hz, H-15), 2.84 (1H, *dd*, *J*= 4.0, 5.0 Hz, H-15), 2.93 (1H, *dd*, *J*=3.0, 4.0 Hz, H-14) and ^{13}C NMR (22.5 MHz, $CDCl_3$): see Table 1.

*3.3.9. Ent-16-hydroxy-3-oxo-13-epi-manoyl oxide (**9**)*

Colourless needles from MeOH, mp 134–36°C. $[\alpha]_D^{25}$ −20.2° (*c* 0.3, $CHCl_3$). IR γ_{max} cm^{-1}: 3470 (OH), 1680 (C=O), 1430,950, 900 (vinyl), 1100 (ether). CIMS *m/z*: 321 $[M+H]^+$, 290 $[M+H-CH_3]^+$. ^1H NMR (90 MHz, $CDCl_3$) δ 0.92 (3H, *s*, H-20), 0.99 (3H, *s*, H-19), 1.05 (3H, *s*, H-18), 1.28 (3H, *s*, H-17), 2.50 (1H, *dd*, *J*=9.0, 14.0 Hz, H-2), 5.12 (1H, *dd*, *J*= 10.0, 11.0 Hz, H-15), 5.24 (1H, *dd*, *J*= 18.0 Hz, H-15), 6.00 (1H, *dd*, *J*=11.0, 18.0 Hz, H-14) and ^{13}C NMR (22.5 MHz, $CDCl_3$): see Table 1.

*3.3.10. Ent-15-hydroxy-labda-8(17),13E-dien-3-one (**10**)*

Colourless needles from MeOH, mp 117–18°C. $[\alpha]_D^{25}$ −9.2° (*c* 0.8, $CHCl_3$). IR γ_{max} cm^{-1}: 3450 (OH), 1670 (C=O),3060,1640,780, 880 (exocylic, vinyl). EIMS *m/z*: 304 $[M]^+$. ^1H NMR (90 MHz, $CDCl_3$) δ 0.90 (3H, *s*, H-20), 1.02 (3H, *s*, H-19), 1.12 (3H, *s*, H-18), 1.70 (3H, *s*, H-16), 2.40 (2H, *m*, H-2), 4.20 (2H, *d*, *J*= 9.0 Hz, H-15), 4.60 (1H, *s*, H-17), 4.95 (1H, *s*, H-17), 5.50 (1H, *t*, *J*=9 Hz, H-14) and ^{13}C NMR (22.5 MHz, $CDCl_3$): see Table 1.

*3.3.11. Labda 8(17),13E-diene-3β,15-diol (**11**)*

Colourless needles from MeOH, mp 158–59°C. $[\alpha]_D^{25}$ −24.3° (*c* 0.5, $CHCl_3$). IR γ_{max} cm^{-1}: 3450 (OH), 3070, 1630, 885 (exocylic, vinyl). Found C 78.11%, H 10.92%, $C_{20}H_{34}O_2$ requires C 78.43%, H 11.11%. CIMS *m/z*: 307 $[M+H]^+$, 289 $[M+H-H_2O]^+$, 271, 248, 191, 135, 123. ^1H NMR (90 MHz, $CDCl_3$) δ 0.70 (3H, *s*, H-20), 0.80 (3H, *s*, H-19), 1.00 (3H, *s*, H-18), 1.70 (3H, *s*, H-16), 3.25 (1H, *d*, *J*=6 Hz, H-3), 4.12 (2H, *d*, *J*= 6.0 Hz, H-15), 4.58 (1H, *s*, H-17), 4.90 (1H, *s*, H-17), 5.40 (1H, *t*, *J*=9 Hz, H-14) and ^{13}C NMR (22.5 MHz, $CDCl_3$): see Table 1.

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