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Seco diterpenoids from *Excoecaria agallocha* L.☆

Ammanamanchi S.R. Anjaneyulu*, Vadali Lakshmana Rao

School of Chemistry, Andhra University, Visakhapatnam-530 003, India

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Abstract

Chemical examination of the ethyl acetate solubles of the CH_3OH:CH_2Cl_2 (1:1) extract of the roots of *Excoecaria agallocha* L. collected from Godavary estuary resulted in the isolation of three more new diterpenoids, agallochins M–O (**1–3**). The structures of the new diterpenoids were elucidated by a study of their physical and spectral data as methyl *ent*-13-*epi*-8,13-epoxy-4,6α-dihydroxy-3,4-secolabd-14-en-3-oate (**1**), methyl *ent*-13-*epi*-8,13-epoxy-2,3-secolabd-14-en-2,11-olid-3-oate (**2**), and methyl *ent*-17-hydroxy-3,4-secokaura-4(19),15-dien-3-oate (**3**).

Keywords: Indian mangrove; *Excoecaria agallocha* L.; Euphorbiaceae; New seco diterpenoids; Agallochins M–O

1. Introduction

We have reported the isolation of a large number of new and known diterpenoids from the *n*-hexane extract (Anjaneyulu and Lakshmana Rao, 2000) and from the ethyl acetate solubles of the CH_3OH:CH_2Cl_2 (1:1) extract (Anjaneyulu et al., 2002) of the roots of Indian mangrove plant *Excoecaria agallocha* L. (family: Euphorbiaceae).

A careful examination of the more polar chromatographic fractions from the same column resulted in the isolation of three new seco diterpenoids agallochins M–O (**1–3**) and their structural elucidation.

2. Results and discussion

Agallochin M (**1**) was isolated as colorless oil, and its molecular formula was established as $C_{21}H_{36}O_5$ from elemental analysis and (+) ve ion FABMS, $[M+H-H_2O]^+$ at *m*/*z* 351 . It exhibited hydroxyl, ester carbonyl, vinyl and ether absorptions in the IR spectrum. It was recognised as a new 8,13-epoxylabdane diterpenoid by a study of its ^1H and ^{13}C NMR spectral data. The presence of vinyl and carbomethoxyl groups were evident from the spectra. The presence of all the five methyls (Tables 1 and 2) of a labdane skeleton intact suggested that it might be a ring A seco derivative to accommodate for the ester functionality. The ^{13}C NMR spectrum showed five oxygenated carbons, two at δ 74.4 (*s*), 73.4 (*s*) accounting for the C-8 and C-13 of epimanoyloxy structure (Anjaneyulu and Lakshmana Rao, 2000a), one at δ 51.7 (*q*) for carbomethoxyl and two more carbons at δ 71.5 (*s*) and at δ 68.0 (*d*). The appearance of the gem dimethyl groups 18-H_3 and 19-H_3 deshielded at δ 1.43 and 1.39 suggested the presence of tertiary hydroxyl at C-4, i.e a 3,4-secolabdanoid.

The location of tertiary hydroxyl at C-4 was also supported by the HMBC correlation (full data in supplementary material) between C-4 (δ 75.7) and 18-H_3, 19-H_3 and 5-H. The carbon bearing secondary hydroxyl at δ 68.0 (d) showed HMBC correlation with 5-H and 7-H_2 to locate the hydroxyl at C-6. The structure of agallochin M could thus be established as 13-*epi*-8,13-epoxy-4,6-dihydroxy-3,4-secolabd-14-en-3-oate (**1**).

The important NOESY correlations, in particular, between 5-H and 9-H, 17-H_3 and 20-H_3, 14-H and 17-H_3, 6-H and 17-H_3 and 20-H_3 established the relative stereochemistry. The appearance of hydroxymethine 6-H as td ($J=10.8$, 4.2 Hz) supported its axial configuration leaving the hydroxyl as equatorial. A secolabdanoid without the hydroxyl at C-6, excoecarian H, has been recently reported from the same species of Okinawan origin (Konishi et al., 1998a). The ^{13}CNMR

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* Corresponding author. Tel.: +91-891-754871x214 and 236; fax: +91-891-755547 and 0891-570365.

E-mail addresses: anjaneyuluasr@yahoo.com or orgchem@satyasaionline.net.in (A.S.R. Anjaneyulu).

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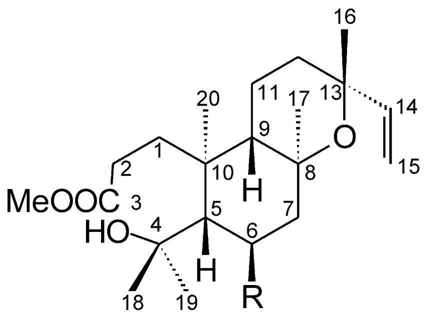

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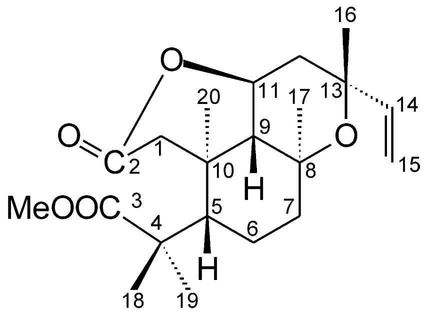
4. R = H



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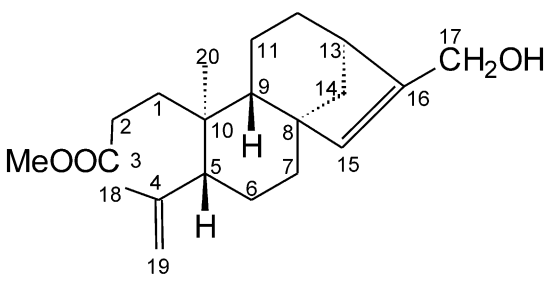

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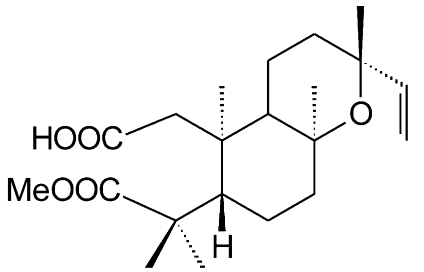

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Table 1
^{1}H NMR data of compounds **1**–**3**[a]

Position	**1**[b]	**2**[b]	**3**[b]
1α	2.18 *m*	2.56 (*d*, *J* = 16.0)	1.70 *m*
1β	1.74 *m*	2.32 (*d*, *J* = 16.0)	1.62 *m*
2α	2.16 *m*	–	2.05 *m*
2β	2.46 *m*	–	2.30 *m*
5	1.64 (*d*, *J* = 10.8)	1.18 *m*	1.95 (*dd*, *J* = 12.0, 2.4)
6α	4.05 (*td*, *J* = 10.8, 4.2)	1.42 *m*	1.40 *m*
6β	–	1.70 *m*	1.68 *m*
7α	2.04 (*dd*, *J* = 11.4, 4.2)	1.84 *m*	1.60 *m*
7β	1.48 *m*	1.64 *m*	1.48 *m*
9	1.30 *m*	1.56 *m*	1.16 (*d*, *J* = 7.8)
11α	1.46 *m*	4.63 (*td*, *J* = 12.0, 4.0)	1.65 *m*
11β	1.46 *m*	–	1.58 *m*
12α	1.42 *m*	2.72 (*dd*, *J* = 13.0, 4.0)	1.50 *m*
12β	2.20 *m*	1.60 *m*	1.60 *m*
13	–	–	2.57 *m*
14α			
	5.98 (*dd*, *J* = 18.0, 10.8)	6.00 (*dd*, *J* = 18.0, 11.0)	1.42 *m*
14β	–	–	2.04 *m*
15α	(*d*, *J* = 10.8)	5.00 (*d*, *J* = 11.0)	5.37 *s*
15β	4.97 (*d*, *J* = 18.0)	5.09 (*d*, *J* = 18.0)	–
16	1.14 *s*	1.23 *s*	–
17	1.25 *s*	1.25 *s*	4.19 *s*
18	1.43 *s*	1.23 *s*	1.72 *s*
19	1.39 *s*	1.19 *s*	4.85 *s*
			4.64 *s*
20	0.79 *s*	1.07 *s*	1.01 *s*
COOMe	3.68 *s*	3.70 *s*	3.63 *s*

[a] Measured at 300 MHz (the chemical shifts of the respective protons between 1.0 and 2.5 were taken from HMQC data).

[b] Chemical shifts in δ from TMS (multiplicity, J in Hz) in $CDCl_3$.

spectral data for excoecarin H methylester (**4**) (Table 2) were found to be very close to those of agallochin M(1) except for perceptible difference in the values of carbons C-6, C-5 and C-7. In the latter these carbons appeared deshielded respectively by ≅δ 44, 5 and 9 as a consequence of the presence of a hydroxyl at C-6 and its effect on the α- and β-carbons. Agallochin M was also considered as an *ent*-derivative in view of its levo specific rotation (Konishi et al., 1998b; Habtemariam et al., 1991) to derive its structure as *ent*-13-*epi*-8,13-epoxy-4,6α-dihydroxy-3,4-secolabd-14-en-3-oate (**1**).

Agallochin N (**2**) was isolated as colorless needles from MeOH, and its molecular formula was established as $C_{21}H_{32}O_5$ from elemental analysis and (+) ve ion FABMS, $[M+H]^+$ at *m/z* 365. It was also recognised as a new 13-*epi*-8,13-epoxy-secolabdanoid by a study of its ^1H and ^{13}C NMR spectral data (Tables 1 and 2). The presence of two carbonyl functionalities, one as a methyl ester and the other a δ-lactone, was evident from the spectral data, suggesting that agallochin N (**2**) could be a ring A 2,3-secolabdanoid with the possibility that either C-3 is a methyl ester and C-2, a lactone carbonyl or vice versa. The ester carbonyl at δ 178.6 showed HMBC correlations (full details in supplementary material) with 18-H_3, 19-H_3 and 21-H_3 supporting the presence of C-3 as methyl ester. Similarly, C-2 (δ 169.2) showed HMBC correlation only with 1-H_2. The lactone was found to be a secondary lactone with the oxygenated carbon resonating at δ 71.7 (*d*) and the α-methine at δ 4.63 as *td* (*J* = 12.0, 4.0 Hz). The δ-lactone could, therefore, be between C-2 and C-11 or C-2 and C-6. The C-12 signal in a labdanoid resonates around δ 45 in the presence of an oxygen at C-11 (Konishi et al., 1996) and at δ 34 in the absence of oxygen at C-11 (Anjaneyulu and Lakshmanarao, 2000). Similarly, C-7 resonates around δ 46.0 in the presence of oxygen at C-6 and at δ 42.0 (Anjaneyulu and Lakshmana Rao, 2001) in the absence of an oxygen at C-6. These carbons C-7 and C-12 in agallochin N (**2**) appeared at δ 41.7 and 41.6 to support a lactone bridge between C-2 and C-11. The large coupling constants of 11-H (*td*, *J* = 12.0, 4.0) suggested its axial configuration. Agallochin N thus appears to be the first secolabdanoid with a δ-lactone between C-2 and C-11. In the absence of a true model compound, the ^{13}CNMR spectral data of agallochin N (**2**) can be made with a 2,3-secolabdane (**5**) isolated from the same species (Konishi et al., 1998b). Compound (**5**) unlike in (**2**) does not possess the hydroxyl at C-11 and incidentally no δ-lactone between C-2 and C-11. A comparison of the ^{13}CNMR of these two (Table 2) showed that the ^{13}C values of C-11, C-12 and C-9 in **2** appeared deshielded by ≅δ 55, 7 and 5 as excepted for the presence of oxygen at C-11. A significant deshielding (≅δ 6) noticed on the value of C-1 in **2** might be due to presence of δ-lactonic structure in it.

Table 2
^{13}C NMR data of compounds **1–5**[a,b]

Carbon No.	**1**	**4**	**2**	**5**	**3**
1	33.5	33.6	47.4	41.3	34.0
2	28.3	28.7	169.2	175.7	28.6
3	174.2	175.5	178.6	180.1	174.6
4	75.7	75.5	45.1	46.0	147.5
5	55.8	50.8	54.6	48.0	50.3
6	68.0	23.7	21.3	21.8	25.3
7	51.3	42.2	41.6	41.6	37.6
8	74.4	76.0	75.3	75.7	48.5
9	50.3	51.1	55.3	50.6	38.5
10	39.7	40.8	37.8	41.5	41.0
11	16.2	15.7	71.7	16.7	18.7
12	34.8	34.9	41.7	34.6	25.3
13	73.4	73.3	75.5	73.4	41.0
14	147.0	147.4	146.5	147.4	43.7
15	109.9	109.8	110.7	109.7	135.2
16	32.9	32.6	32.5	32.4	146.2
17	23.9	23.2	22.8	23.4	61.2
18	32.1	34.2	27.8	28.1	23.3
19	28.2	27.3	22.5	23.4	113.5
20	19.9	20.3	17.1	19.6	21.7
COOMe	51.7	51.7	52.0	52.1	51.6

[a] Chemical shifts in δ from TMS taken in $CDCl_3$ measured at 75 MHz.

[b] The assignments of chemical shifts to the respective carbons was made by comparative literature data and corroborated by HMQC and ^1H–^1H COSY data.

The NOESY correlations between 5-H and 9-H, 17-H_3 and 20-H_3, 14-H and 17-H_3, 11-H and 17-H_3 and 20-H_3 established the relative stereochemistry. The absolute configuration of agallochin N was assumed as an *ent*-derivative in view of its levo specific rotation (Konishi et al., 1998b; Habtemariam et al., 1991). Its structure was thus methyl *ent*-13-*epi*-8,13-epoxy-2,3-secolabd-14-en-2,11-olid-3-oate (**2**).

Agallochin O (**3**) was isolated as colorless oil, and its molecular formula was established as $C_{21}H_{32}O_3$ from elemental analysis and (+) ve FABMS, $[M+H–H_2O]^+$ at *m/z* 315. A preliminary analysis of its ^1H and ^{13}C NMR spectral data suggested it to be a new secokaurenoid. The presence of a carbomethoxyl, isopropenyl, and hydroxymethyl groups and a trisubstituted double bond were evident from the ^1H and ^{13}C NMR spectral data (Tables 1 and 2). The ^{13}C values of agallochin O (**3**) agreed very well with respect to the carbons of rings B, C and D with 17-hydroxy-15-kaurenoids (Lopes et al., 1990). A structure with C-3 as carbomethoxyl and an isopropenyl group at C-5. was supported by the HMBC correlations (full data in supplementary material) between the carbomethoxyl carbon at δ 174.6 and 21-H_3, 2-H_2 and 1-H_2; between C-4 and 18-H_3, 5-H and 6-H_2; and between C-19 and 5-H and 18-H_3 and other correlations.

The NOESY correlations observed between 5-H and 9-H, 9-H and 15-H and 20-H_3 and 14-H_2 as well as other correlations were in full agreement with the kaurenoid

Table 3
HMBC correlations noticed in **1**, **2** and **3**

Carbon No.	Compound **1**		Compound **2**		Compound **3**	
	^{13}C (δ)	^{1}H	^{13}C (δ)	^{1}H	^{13}C (δ)	p^{1}H
C-1			47.4	9-H, 20-H_3		
C-2			169.2	1-H_2		
C-3	174.2	21-H_3, 2-H_2, 1-H_2	178.6	21-H_3, 18-H_3, 19-H_3	174.6	21-H_3, 2-H_2, 1-H_2,
C-4	75.7	18-H_3, 19-H_3, 5-H			147.5	18-H_3, 5-H, 6-H_2,
C-5	55.8	7-H_2, 18-H_3, 19-H_3, 20-H_3	54.6	9-H, 1-$H_2$18-H_3, 19-H_3, 20-H_3	50.3	19-H_2, 9-H, 7-H_2, 1-H_2, 20-H_3
C-6	68.0	7-H_2, 5-H				
C-7	51.3	17-H_3 5-H, 9-H				
C-8	74.4	7-H_2, 17-H_3	75.3	9-H, 17-H_3, 6-H_2 7-H_2	48.5	15-H, 13-H, 14-H_2, 9-H, 11-H_2 7-H_2, 6-H_2
C-9	50.3	1-H_2, 7-H_2 17-H_3, 20-H_3	55.3	12-H_2, 1-H_2		
C-10						
C-11			71.7	12-H_2, 9-H, 7-H_2, 17-H_3, 20-H_3		
C-12						
C-13	73.4	11-H_2, 16-H_3	75.5	15-H_2, 16-H_3, 12-H_2		
C-14	147.0	15-H_2, 16-H_3	146.5	15-H_2 12-H_2		
C-15					135.2	17-H_2, 13-H 7-H, 14-H_2 9-H
C-16					146.2	15-H, 17-H_2 14-H_2, 13-H
C-17					61.2	15-H
C-18						
C-19					113.5	5-H, 19-H_3
C-20						

stereochemistry. Agallochin O was also suggested to be an *ent*-derivative to derive, its structure as *ent*-17-hydroxy-3,4-secokaura-4(19),15-dien-3-oate 3 in view of its levo specific rotation (Konishi et al., 2000).

Several new diterpenoids ranging from labdanes, beyeranes to kauranes have been reported from the species *Excoecaria agallocha* L. of Okinarvan origin. These include some 2,3-seco and 3,4-seco derivatives also. The Indian species has been found to be prolific in elaborating equally large number of new diterpenoids which included pimaranes and isopimaranes also in addition to the above three classes.

Full details of HMBC correlations noticed in the compounds (**1–3**) are given in the supplementary material (Table 3).

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 $CHCl_3$. ^{1}H 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 Rudolph 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 *Excoecaria agallocha* L. were collected from Corangi Mangrove forest near Bhiravapalem in the Godavari Estuary (Latitude 16° 15′ N and Longitude 82° 15′ E) in March 1998 and were identified by Professor B. Kondala Rao, Department of Marine Living Sources, Andhra University, Visakhapatnam. A voucher specimen (Code: AU1/160) was deposited at the Marine Museums of the School of Chemistry, Andhra University and the National Institute of Oceanography, Goa.

3.3. Extraction and isolation

The details of extraction of the plant material, isolation of the compounds and their structural elucidation were presented earlier (Anjaneyulu and Lakshmana Rao, 2000; Anjaneyulu et al., 2002). Further purification of the column fractions 162–170 (*n*-hexane:EtOAc; 6.5:3.5) furnished agallochin M (0.003%, **1**) and agallochin N (0.0025%, **2**). The residue from the column fractions 175–180 (*n*-hexane:EtOAc; 6.0:4.0) furnished agallochin O (0.0035%, **3**).

3.3.1. *Agallochin M (**1**)*

Colorless oil, $[\alpha]_D^{25}-46.4°$ (*c* 0.2, $CHCl_3$). IR (Nujol) ν_{max} 3352, 2949, 1736, 1637, 1439, 1390, 1195, 1089, 914, 862 and 756 cm^{-1}. Found. C 69.40%, H 9.56%, $C_{21}H_{36}O_5$ requires C 69.80%, H 9.78%. (+) ve FABMS *m/z* (relative intensity): 351 $[M+H-H_2O]^+$(20), 333(55), 315(70), 301(10), 275(15), 209(40), 169(100), (154(60), 121(35). ^1H NMR (300 MHz, $CDCl_3$): see Table 1; and ^{13}C NMR (75 MHz, $CDCl_3$,): see Table 2.

3.3.2. *Agallochin N (**2**)*

Colorless needles from MeOH, mp 160–162 °C, $[\alpha]_D^{25}-54.2°$ (*c* 0.1, $CHCl_3$). IR (Nujol) ν_{max} 3441, 2928, 1765, 1718, 1460, 1396, 1253, 1143, 1076, and 758 cm^{-1}. Found C 69.02%, H 8.52%, $C_{21}H_{32}O_5$ requires C 69.23%, H 8.79%. (+) ve FABMS *m/z* (relative intensity): 365 $[M+H]^+$(95), 347(20), 307(95), 289(70), 242(25), 209(15), 154(100), 120(45). ^1H NMR (300 MHz, $CDCl_3$): see Table 1; and ^{13}C NMR (75 MHz, $CDCl_3$,): see Table 2.

3.3.3. *Agallochin O (**3**)*

Colorless oil, $[\alpha]_D^{25}-28.0°$ (*c* 0.1, $CHCl_3$). IR (Nujol) ν_{max} 3422, 2928, 1738, 1637, 1442, 1381, 1120, 1020, 895, and 752 cm^{-1}. Found C 75.48%, H 9.40%, $C_{21}H_{32}O_3$ requires C 75.90%, H 9.63%. (+) ve FABMS *m/z* (relative intensity): 315 $[M+H-H_2O]^+$(100), 301(10), 259(10), 227(10), 199(15), 171(15), 136(100), 106(40). ^1H NMR (300 MHz, $CDCl_3$): see Table 1; and ^{13}C NMR (75 MHz, $CDCl_3$,): see Table 2.

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