Agallochaols A and B, Two New Diterpenes from the Chinese Mangrove *Excoecaria agallocha* L.

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Two new *ent*-isopimarane diterpenoids, agallochaols A (**1**) and B (**2**), were isolated from the dried stems and leaves of the mangrove *Excoecaria agallocha* L. Their structures were established on the basis of spectroscopic data and chemical evidence.

Introduction. – *Excoecaria agallocha* L. (Eupharbiaceae) is a shrub widely distributed on seashores and edge mangroves throughout tropical Africa, Asia, and northwest Australia. The bark and wood of *E. agallocha* have been used in traditional medicines for the treatment of flatulence in Thailand [1][2], and its leaves and latex have been used as fish poisons in India [3].

The piscicidal constituent of the twigs and bark of *E. agallocha*, native to Okinawa, has been characterized as the daphnane diterpene ester excoecariatoxin that was known as skin irritant and tumor promoter [4][5]. A novel phorbol ester, an anti-HIV principle, has also been isolated from the leaves and stems of *E. agallocha* collected in Australia [1][2]. The isolation and structural elucidations of a large number of new diterpenoids with different skeletons such as manoyl oxides, labdanes, beyeranes, isopimaranes, and kauranes, along with some known derivatives, have been reported very recently [6–10].

In the course of our research on biologically active substances from Chinese marine organisms [11–13], a sample of mangrove *E. agallocha* collected from Guangxi Province, China, was investigated. Two new *ent*-isopimarane diterpenes, named agallochaol A (**1**) and agallochaol B (**2**), respectively, were isolated from this plant. In the present paper, we report the isolation and structural elucidation by spectroscopic methods of these new compounds.

Results and Discussion. – The chipped stems and leaves of *E. agallocha* were extracted exhaustively with MeOH. The MeOH extract was partitioned consecutively between H_2O and petroleum ether, H_2O and AcOEt, and H_2O and BuOH. The AcOEt fraction was subjected to chromatography (silica gel and *Sephadex LH-20*) to give agallochaols A (**1**, 13 mg) and B (**2**, 25 mg).

Agallochaol A (**1**) was obtained as a UV-absorbing colorless oil. Its ESI-MS (positive ion) displayed two pseudo-molecular ions at *m/z* 319 ($[M+H]^+$) and 341 ($[M+Na]^+$). The HR-ESI-MS experiment established the molecular formula $C_{20}H_{30}O_3$ (*m/z* 319.2303 ($[M+H]^+$) and 341.2079 ($[M+Na]^+$), indicating six degrees

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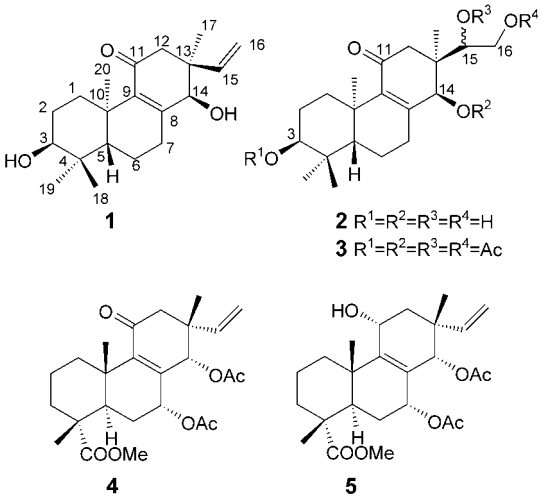

of unsaturation. The structure of **1** was elucidated on the basis of extensive spectroscopic analysis and comparison with model compound **4**, an oxidated derivative of the isopimarane diterpenoid methyl (7α,11α,14α)-7,14-bis(acetyloxy)-11-hydroxy-isopimara-8,15-dien-18-oate[1]) (**5**) [14]. The relative configuration of **1** was deduced from a NOESY experiment, and the absolute configuration was determined as (3*S*,5*S*,10*R*,13*S*,14*R*), inverted to that of **4** [14], by analysis of the CD profile ($\Delta\varepsilon_{252}$ – 2.53) of **1**, which is opposite of that of **4**.

Analysis of the ^{13}C-NMR and DEPT spectra (*Table*) of **1** assigned three of six unsaturation degrees to one exocyclic C=C bond and one endocyclic C=C bond, resonating at δ 140.8 (*d*) and 116.3 (*t*), and δ 153.8 (*s*) and 142.6 (*s*), respectively, and one carbonyl resonance appearing at δ 197.1 (*s*). Consequently, the remaining unsaturations were due to three rings. In addition, the ^{13}C-NMR spectrum (*Table*) showed the signals of two O-bearing C-atoms at δ 75.4 (*d*) and 76.6 (*d*). The remaining signals observed between δ 47.3 and 17.4 were attributed to thirteen sp^3 C-atoms (four Me, five CH$_2$, one CH, and three C). The signals at δ(C) 197.1, 142.6, and 153.8, suggested the presence of a tetrasubstituted enone chromophore. The UV spectrum showed a λ_{max} at 246 nm (log ε 3.32) consistent with such an enone system. The ^{1}H-NMR spectrum (*Table*) showed five downfield signals between δ 5.88 and 3.45, assigned to olefinic and CH–O protons, and four tertiary Me signals (δ 1.18, 1.11, 0.98, 0.89; each 3 H, *s*). The *m* integrating for eleven protons between δ 2.68 and 1.38 were due to the five CH$_2$ and one CH groups as established by the HMQC experiment. The proton signals at δ 5.88 (*dd*, *J* = 17.6 Hz, 10.8 Hz, 1 H), 5.23 (*d*, *J* = 10.8 Hz, 1 H), and 5.10 (*d*, *J* = 17.6 Hz, 1 H) indicated the presence of a terminal CH=CH$_2$ moiety.

An isopimarane skeleton for **1** was suggested after detailed analysis of the 2D-NMR spectra (^{1}H,^{1}H COSY, HMQC, and HMBC) and comparison with the data of **4** [14]. The ^{1}H,^{1}H COSY experiment allowed us to establish separate spin systems of **1** revealing the connectivities H–C(3)/CH$_2$(2) and H–C(15)/H–C(16a)/H–C(16b)). The suggested molecular framework of **1** was confirmed by the HMBC spectrum (*Fig. 1*) showing the long-range correlations H–C(3)/C(1), Me(18) (Me(19))/C(3), C(4) and C(5), H–C(14)/C(17), C(7), C(13), C(12), C(15), C(9), and C(8), H–C(15)/C(12), C(13), and C(14), and Me(17)/C(12), C(13), C(14), and C(15). The similarity of the data of **1** with those of model compound **4**, except for the positions of C(3), C(7), C(14), and C(18), further confirmed the proposed molecular framework.

The NOE correlations (*Fig. 2*) H–C(14)/Me(17), H$_\alpha$–C(7), and H$_\alpha$–C(12), H$_\alpha$–C(6)/Me(20), and H$_\alpha$–C(2)/Me(20), H$_\alpha$–C(1), H$_\alpha$–C(3), and Me(19) suggested the α-orientation of these protons. On the other

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[1]) Isopimara-8,15-diene = (4a*S*,7*S*,10a*S*)-7-ethenyl-1,2,3,4,4a,5,6,7,8,9,10,10a-dodecahydro-1,1,4a,7-tetramethylphenanthrene; for systematic names, see *Exper. Part*.

Table. *^{1}H- and ^{13}C-NMR Data*[a]) *of Agallochaols A* (**1**)[b]) *and B* (**2**)[c])

	1		**2**	
	δ(H)	δ(C)[d])	δ(H)	δ(C)[d])
H_α–C(1)	2.52–2.57 (*m*)	28.6 (*t*)	2.43 (*dt*, *J* = 13.3, 3.6)	30.3 (*t*)
H_β–C(1)	1.35–1.43 (*m*)		1.41–1.50 (*m*)	
H_α–C(2)	1.96–2.05 (*m*)	25.8 (*t*)	1.93–2.02 (*m*)	27.2 (*t*)
H_β–C(2)	1.56–1.63 (*m*)		1.54–1.60 (*m*)	
H_α–C(3)	3.45 (br. *t*, *J* = 2.8)	75.4 (*d*)	3.37 (br. *t*, *J* = 2.8)	76.4 (*d*)
C(4)		37.6 (*s*)		39.1 (*s*)
H_β–C(5)	1.50 (br. *d*, *J* = 12.7)	45.0 (*d*)	1.54–1.60 (*m*)	46.6 (*d*)
H_α–C(6)	1.42–1.47 (*m*)	17.4 (*t*)	1.51–1.54 (*m*)	19.0 (*t*)
H_β–C(6)	1.68–1.71 (*m*)		1.72 (*dd*, *J* = 11.9, 7.4)	
H_α–C(7)	2.56–2.63 (*m*)	31.4 (*t*)	2.62–2.70 (*m*)	33.8 (*t*)
H_β–C(7)	2.28 (*dd*, *J* = 20.2, 5.5)		2.27 (*dd*, *J* = 20.4, 5.6)	
C(8)		153.8 (*s*)		156.7 (*s*)
C(9)		142.6 (*s*)		143.6 (*s*)
C(10)		37.4 (*s*)		38.8 (*s*)
C(11)		197.1 (*s*)		201.0 (*s*)
H_α–C(12)	2.15 (*d*, *J* = 15.6)	47.2 (*t*)	1.97 (*d*, *J* = 14.4)	46.9 (*t*)
H_β–C(12)	2.69 (*d*, *J* = 15.6)		2.73 (*d*, *J* = 14.4)	
C(13)		43.5 (*s*)		43.9 (*s*)
H_α–C(14)	3.88 (*s*)	76.6 (*d*)	3.90 (*s*)	76.6 (*d*)
H–C(15)	5.88 (*dd*, *J* = 17.6, 10.8)	140.8 (*d*)	3.81 (*dd*, *J* = 8.0, 3.3)	76.5 (*d*)
H_a–C(16)	5.23 (*d*, *J* = 10.8)	116.3 (*t*)	3.64 (*dd*, *J* = 11.3, 3.4)	64.1 (*t*)
H_b–C(16)	5.10 (*d*, *J* = 17.6)		3.50 (*dd*, *J* = 11.2, 7.9)	
Me(17)	1.11 (*s*)	23.5 (*q*)	0.89 (*s*)	18.2 (*q*)
Me(18)	0.98 (*s*)	28.2 (*q*)	0.97 (*s*)	29.5 (*q*)
Me(19)	0.89 (*s*)	22.3 (*q*)	0.89 (*s*)	23.3 (*q*)
Me(20)	1.18 (*s*)	19.4 (*q*)	1.18 (*s*)	20.6 (*q*)

[a]) *Bruker DRX*-400-MHz spectrometers, assignments made by ^{1}H,^{1}H COSY, HMQC, HMBC, and NOESY experiments. [b]) In $CDCl_3$, chemical shifts [ppm] referred to $CHCl_3$ (δ(H) 7.26) and $CDCl_3$ (δ(C) 77.0). [c]) In CD_3OD, chemical shifts [ppm] referred to MeOH (δ(H) 3.30) and CD_3OD (δ(C) 49.0). [d]) By DEPT sequence.

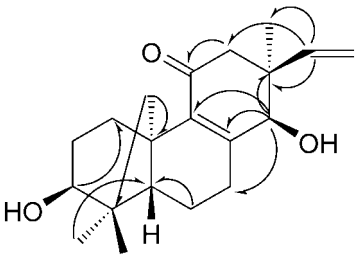

Fig. 1. *Key HMBC correlations of* **1**

hand, the NOEs H–C(5)/H_β–C(7) and Me(18), H_β–C(6)/H_β–C(7), and Me(18), H_β–C(1)/H_β–C(2) indicated that all these protons were on the other side of the rings and were assigned as the β protons.

Agallochaol B (**2**) was shown to be the 15,16-dihydroxy derivative of **1**. Its molecular formula, deduced from HR-ESI-MS (*m*/*z* 353.2313 ($[M+H]^+$), corresponds to $C_{20}H_{32}O_5$, of 34 mass units more than that of **1**. Comparison of the spectral data of **2** with those of **1** clearly indicated that it differs from **1** only in the exocyclic C(15)=C(16)

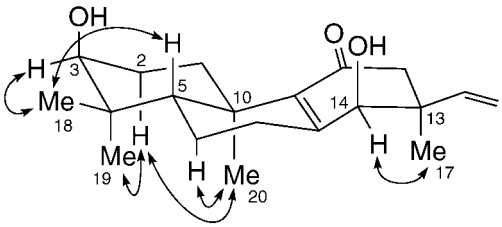

Fig. 2. *Key NOE correlations of* **1**

bond, where the three olefinic protons are replaced by a CH–O and an *AB*-type CH_2–O group. To confirm this structure, **2** was treated with Ac_2O-pyridine yielding the expected tetraacetoxy derivative **3**. The remaining of structure **2** is the same as in compound **1**. Thus **2** was determined as (3β,14β)-3,14,15,16-tetrahydroxy-*ent*-isopimar-8-en-11-one. The configuration at C(15) remains unknown.

The splitting pattern (*ABX*) and coupling constants of H–C(15) (δ 3.50 (*dd*, J = 11.2, 7.9 Hz)) and CH_2(16) (δ 3.81 (*dd*, J = 8.0, 3.3 Hz, H_a–C(16)) and 3.64 (*dd*, J = 11.3, 3.4 Hz, H_b–C(16)) [15] of **2** supported the assignment (*Table*). In the ^{1}H-NMR spectrum of **3**, the expected 4 *s* of 4 Ac groups appeared and at δ 2.01, 2.03, 2.05, and 2.10 (each 3 H, *s*), consistent with a 168 mass-unit enhancement for the molecular ion (ESI-MS: 543 ($[M+Na]^+$)) as compared to **2**.

The crude MeOH extract of *E. agallocha* exhibited weak antitumor activity against A-549 and HL-60, but agallochaols A and B proved inactive. Bioassays for antifungal and antibiotic of **1** and **2** are currently ongoing.

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Experimental Part

General. Column chromatography (CC): commercial silica gel (*Qing Dao Hai Yang Chemical Group Co.*; 100–200 and 200–300 mesh). TLC: precoated silica gel plates (*Yan Tai Zi Fu Chemical Group Co.*; *G60 F-254*) were used for anal. TLC. Optical rotation: *Perkin-Elmer 341* polarimeter. UV Spectra: *Varian Cary-300-Bio* spectrophotometer; λ_{max} (log ε) in nm. CD: λ ($\Delta\varepsilon$) in nm. IR Spectra: *Nicolet Magna-FT-IR-750* spectrometer; $\tilde{\nu}_{max}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker DRX-400* (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer; chemical shifts δ in ppm, with residual $CHCl_3$ (δ(H) 7.26, δ(C) 77.0) or CD_3OD (δ(H) 3.30, δ(C) 49.0) as internal standard, coupling constant J in Hz, assignments supported by ^{1}H,^{1}H COSY, HMQC, and HMBC experiments. ESI-MS and HR-ESI-MS: *Q-TOF Micro* LC-MS-MS spectrometer in *m*/*z*.

Plant Material. Plant material was collected in Guangxi Province, China, in 1999, and identified as *E. agallocha* L. by Prof. *Jin-Gui Shen* of the Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. A voucher specimen is available for inspection at the Institute of Materia Medica, SIBS-CAS.

Extraction and Isolation. Dried ground stems and leaves (4.0 kg) of *E. agallocha* were extracted with MeOH (3 × 5 l). The MeOH extract was evaporated to give a residue (410 g), which was dissolved in H_2O (1000 ml). This soln. was partitioned consecutively between H_2O and petroleum ether, H_2O and AcOEt, and H_2O and BuOH. The AcOEt extract was evaporated to give a residue (100 g), which was separated by CC (silica gel (100–200 mesh, 1.5 kg), petroleum ether/AcOEt 90:10, 80:20, 70:30, 60:40, 50:50, and Me_2CO): *Fractions 1–16*. *Fr. 7* and *14* were further purified by CC (silica gel, $CHCl_3$/MeOH; then *Sephadex LH-20*, 100% MeOH); pure **1** (13 mg) and **2** (25 mg).

(3β,5β,10α,13β,14β)-3,14-Dihydroxyisopimara-8,15-dien-11-one (= *(1*R,2S,*4b*R,7S,*8a*S)*-2-Ethenyl-2,3,4b,5,6,7,8,8a,9,10-dodecahydro-1,7-dihydroxy-2,4b,8,8-tetramethylphenanthren-4(1*H)*-one*; **1**): Colorless thick oil. $[\alpha]_D^{20} = -12$ ($c = 0.65$, $CHCl_3$). UV (MeOH): 246 (3.32). CD ($c = 0.120$, MeOH): 331 (+0.96), 276 (0), 252 (−2.53), 234 (0), 219 (+1.96), 199 (+1.69). IR (KBr): 3425 (OH), 2926, 1716, 1649, 1592, 1458, 1415, 1377, 1261, 1034, 802, 756, 665. ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz): *Table*. ESI-MS: 319 ($[M+H]^+$), 341 ($[M+Na]^+$). HR-ESI-MS: 319.2303 ($[M+H]^+$ $C_{20}H_{31}O_3^+$; calc. 319.2273), 341.2079 ($C_{20}H_{30}O_3Na^+$; calc. 341.2093).

(3β,5β,10α,13β,14β)-3,14,15,16-Tetrahydroxyisopimar-8-en-11-one (= *(1*S,2R,*4b*R,7S,*8a*S)*-2-(1,2-Dihydroxyethyl)-2,3,4b,5,6,7,8,8a,9,10-decahydro-1,7-dihydroxy-2,4b,8,8-tetramethylphenanthren-4(1*H)*-one*; **2**): Colorless thick oil. $[\alpha]_D^{20} = -30$ ($c = 1.00$, MeOH). UV (MeOH): 248 (3.56). IR (KBr): 3382 (OH), 2958, 1653, 1456, 1417, 1386, 1290, 1028, 677. ^{1}H-NMR (CD_3OD, 400 MHz) and ^{13}C-NMR (CD_3OD, 100 MHz): *Table*. ESI-MS: 353 ($[M+H]^+$). HR-ESI-MS: 353.2313 ($C_{20}H_{33}O_5^+$; calc. 353.2328).

(3β,5β,10α,13β,14β)-3,14,15,16-Tetrakis(acetyloxy)isopimar-8-en-11-one (**3**). Treatment of **2** (15 mg) with Ac_2O/pyridine 1:1 (3 ml) at r.t. for 48 h yielded **3** (14 mg). Colorless oil. ^{1}H-NMR ($CDCl_3$, 400 MHz): 1.36–1.27 (*m*, H_β−C(1)); 2.54 (*dt*, *J* = 13.5, 3.4, H_α−C(1)); 1.62–1.68 (*m*, H_β−C(2)); 1.88–1.97 (*m*, H_α−C(2)); 4.67 (br. *t*, *J* = 2.6, H_α−C(3)); 1.41–1.48 (*m*, H_β−C(5)); 1.62–1.68 (*m*, H_β−C(6)); 1.41–1.48 (*m*, H_α−C(6)); 2.29–2.24 (*m*, CH_2(7)); 2.79 (*d*, *J* = 14.6, H_β−C(12)); 2.18 (*dd*, *J* = 14.4, 1.51, H_α−C(12)); 5.19 (*d*, *J* = 1.2, H_α−C(14)); 5.21 (*dd*, *J* = 8.3, 3.0, H−C(15)); 4.30 (*dd*, *J* = 11.8, 3.0, H_a−C(16)); 3.95 (*dd*, *J* = 11.8, 8.3, H_b−C(16)); 0.92 (*s*, Me(17)); 1.06 (*s*, Me(18)); 0.87 (*s*, Me(19)); 1.18 (*s*, Me(20)); 2.10, 2.05, 2.03, 2.01 (4*s*, 4 Ac). ESI-MS: 543 ($[M+Na]^+$).

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