New 3-Oxo-chol-4-en-24-oic Acids from the Marine Soft Coral *Eleutherobia* sp.

Sarah C. Lievens, Håkon Hope, and Tadeusz F. Molinski*

Department of Chemistry, One Shields Avenue, University of California, Davis, California 95616

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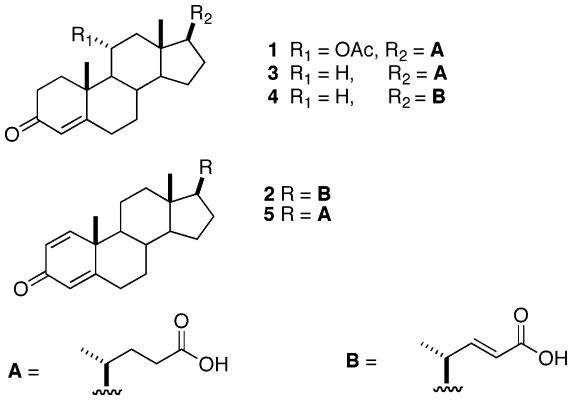
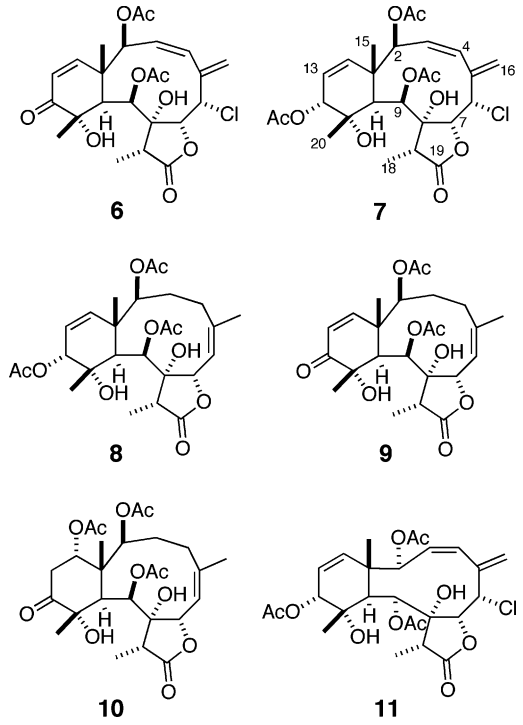
Two novel cholic acid derivatives and three known steroids were isolated from an octocoral *Eleutherobia* sp. from Pohnpei Micronesia along with the known briareins minabein-1 (**6**), minabein-4 (**7**), minabein-6 (**8**), minabein-8 (**9**), and minabein-9 (**10**). X-ray crystallographic analysis of the known compound minabein-4 allowed assignment of its correct relative and absolute stereochemistry. Consequently, a different structure proposed for the same compound (named independently as nui-inoalide-D) is incorrect.

In the last 25 years octocorals have yielded hundreds of novel diterpenes with nearly 300 compounds in the briarein family alone.[1] Other representative octocoral-derived compounds include the eunicellin-based eleuthosides from *Eleutherobia* and *Sarcodictyon* spp. (Alcyoniidae),[2] particularly the antimitotic eleutherobin,[3] and various cembranoids.[4,5] Not only diterpenes but also novel bioactive sterols, such as the cytotoxic steroidal glycosides riiseins A and B,[6] have been isolated from octocorals. During a search for new eleuthesides from *Eleutherobia* sp. we isolated two novel cholic steroids, 11-acetyl-3-oxo-chol-4-en-24-oic acid (**1**) and 3-oxo-chol-1,4,22-trien-24-oic acid (**2**), as well as three known cholic acids, 3-oxo-chol-4-en-24-oic acid (**3**),[7,8] 3-oxo-chol-4.22-dien-24-oic acid (**4**),[7,8] and 3-oxo-chol-1,4-dien-24-oic acid (**5**),[9] and the known briarein diterpenes minabein-1 (**6**), minabein-4 (**7**), minabein-6 (**8**), minabein-8 (**9**), and minabein-9 (**10**).[10] X-ray crystallographic structure determination was used to confirm the relative and absolute stereochemistry of **7**, thus solving an outstanding controversy over the correct structure. We also conclude that minabein-6 is identical to the compound reported subsequently as 11-hydroxyptilosarcenone.[11]



A MeOH/CH_2Cl_2 extract of the octocoral *Eleutherobia* sp. ("deadman's fingers"), collected from Pohnpei, Micronesia, was partitioned into fractions soluble in hexanes, $CHCl_3$, *n*-butanol, and water. The $CHCl_3$-soluble fraction was separated by size exclusion chromatography (Sephadex LH-20), and final purification of compounds was achieved using reversed-phase C_{18} HPLC to give compounds **1**−**10**. The known sterols 3-oxo-chol-4-en-24-oic acid (**3**),[7,8] 3-oxo-chol-4.22-dien-24-oic acid (**4**),[7,8] and 3-oxo-chol-1,4-dien-24-oic acid (**5**)[9] along with the briarein diterpenes minabein-1 (**6**), minabein-6 (**8**), minabein-8 (**9**), and minabein-9 (**10**)[10] were identified by comparison of spectroscopic data with literature values.



The structure of compound **1** was determined by comparison with the ^{1}H NMR data of compound **3**. HRFABMS of compound **1** gave a pseudomolecular ion $[M + H]^+$ at *m/z* 431.2793 (Δmmu = 0.5) corresponding to the formula $C_{26}H_{38}O_5$. The formula of **1**, together with an ESIMS/MS fragment ion at *m/z* 393.1 $[M + Na - CH_3COOH]^+$ attributed to elimination of acetic acid, suggested a new derivative of **3** formed by substitution of the steroid skeleton by an acetoxyl group. The ^{1}H NMR spectrum of **1** revealed new signals due to an *O*-acetyl group (δ 2.00, s, 3H) and a deshielded methine multiplet (δ 5.22, ddd, J = 10.6, 10.6, 5.2 Hz). The remaining ^{1}H NMR signals were essentially the same as those of compound **3**. Analysis of the J values for this signal showed it to be an axial proton on a chair-form cyclohexane ring coupled to two vicinal axial protons (each J = 10.6 Hz) and one equatorial proton (J = 5.2 Hz). Placement of an OAc group at either C-7 or C-11 would satisfy these requirements; however, NOE

* To whom correspondence should be addressed. Tel: +01 530 752-6358. Fax: +01 530 752-6358. E-mail: tfmolinski@ucdavis.edu.

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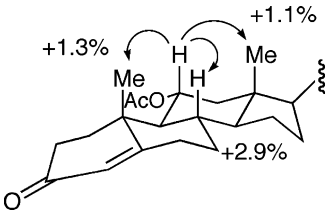

Figure 1. NOE correlations of **1**.

measurements (Figure 1) supported only the C-11 substitution and also secured the relative configuration of **1**. Irradiation of the signal at δ 5.22 ppm gave NOEs to both axial C-18 and C-19 methyl groups (δ 1.24, s, 3H; 0.77, s, 3H) and the axial H-8 proton (δ 2.25, m), under an overlapping complex of eight ^{1}H signals. The mutual NOEs allowed placement of the all three signals in *syn*-facial 1,3-diaxial relationships (Figure 1). Comparison of the ^{13}C NMR spectra for **1** and **3** confirmed the introduction of an acetate group at C-11. The C-11 CH_2 signal present in **3** (δ 21.0, t) was replaced by a methine ^{13}C NMR signal in **1** (δ 71.1, d). Other chemical shift changes at C-9 ($\Delta\delta = 1.1$) and C-12 ($\Delta\delta = 6.8$) are consistent with the constitution of **1** and the remaining NMR assignments made by comparison with literature data.[7,8] The absolute stereochemistry assigned to steroid **1** is based on the usual biosynthetic precedents.

The structure of compound **2** was determined from HRFABMS and comparison with the ^{1}H NMR data of compounds **4** and **5**. The HRFABMS gave a pseudomolecular ion $[M + H]^+$ at *m/z* 369.2423 corresponding to the formula $C_{24}H_{32}O_3$, which indicates the loss of H_2 from the formula of **5** and one additional degree of unsaturation. ^{1}H NMR of **2** gave three vinyl signals (δ 7.03 d, $J = 10.4$ Hz; δ 6.21, dd, $J = 10.4$, 2.0 Hz; δ 6.05, br s) indicative of the cross-conjugated 1,4-dien-3-one signals[9,12] found in compound **5** and two vinyl multiplets at δ 6.89 (dd, $J = 15.6$, 9.2 Hz) and 5.74 (d, $J = 15.6$ Hz) as in compound **4**. Thus, the additional double bond is located at C-22 and C-23 in conjugation with the carboxylic acid at C-24. NMR assignments are supported by analogy with literature values.[7−9]

Compound **6**, $[\alpha]_D$ −55° (*c* 0.15, $CHCl_3$), was isolated and identified as minabein-1, $[\alpha]_D$ −48 (*c*, 0.15, $CHCl_3$).[10] The same structure was reported contemporaneously as 11-hydroxyptilosarcenone, $[\alpha]_D$ −62.5° (*c* 0.74, CH_2Cl_2).[11]

The sample of *Eleutherobia* yielded a second diterpene **7** in the briarein family. A careful search of the literature uncovered two different structures and names—minabein-4 and nui-inoalide-D—assigned to one compound with spectral properties identical to those found in the present work. This was most apparent from analysis of the respective ^{13}C NMR chemical shifts of our sample with those reported for minabein-4 (Table 1), which revealed almost perfect matches. The structures proposed for minabein-4 (**7**)[10] and nui-inoalide-D (**11**)[13] have identical constitutions but are epimeric at C-2. Although NMR data for the compounds isolated in this study and the reported compounds were the same, their chiroptical properties showed inconsistencies. Minabein-4 is dextrorotatory ($[\alpha]_D$ −299° (*c* 0.88, $CHCl_3$),[10] which was almost double the value recorded for our recrystallized sample from CH_3CN, $[\alpha]_D$ −135° (*c* 0.12, $CHCl_3$)). Unfortunately, the optical activity of nui-inoalide-D was not reported. To resolve these anomalies, we undertook an X-ray crystallographic study of our sample.

Crystallization of **7** from acetonitrile gave thin, colorless plates (mp 229−231 °C), which were suitable for X-ray study. The structure is shown in Figure 2. Crystallographic data are summarized in the Supporting Information. Least-squares refinement was done with the program SHELXS-97.[14] Determination of the absolute configuration was based on the anomalous scattering from the Cl atom. The relative stereochemistry about C-2 and C-9 of the briarein ring system was unambiguously shown to be (1*S*,2*S*,3*Z*,6*S*,-7*R*,8*R*,9*R*,10*S*,11*S*,12*R*,13*Z*,17*R*) with both the C2 and C9 acetoxyl groups *syn*-facial. The ring junction absolute stereochemistry of **7** is the same as that proposed for briarein A.[15,16] Thus, the structure of our compound **7** is identical with that reported for minabein-4[10] and is depicted, here, with correct absolute configuration. Consequently, the structure of nui-inoalide-D is incorrect. Since the report of minabein-4 has precedence, we propose the use of this name for compound **7** and that the redundant name nui-inoalide-D be discarded.

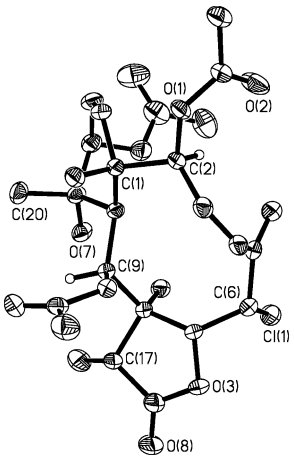

Figure 2. X-ray structure of minabein-4 (**7**) depicting the correct absolute configuration.

Table 1. ^{13}C NMR Chemical Shifts Reported for Briareins **7** and **11**

#	*a*	**7**[b]	**11**[b]	$\Delta\delta$[c]
1	45.7	45.8	45.8	0.1
2	77.5	77.5	77.5	0.0
3	129.1	129.1	129.2	0.1
4	129.0	129.1	129.0	0.1
5	137.1	137.2	137.1	0.1
6	61.9	61.8	62.0	0.2
7	78.8	79.0	78.8	0.2
8	83.0	83.0	83.1	0.1
9	69.1	69.1	69.1	0.0
10	39.1	39.1	39.2	0.1
11	75.5	75.1	75.5	0.4
12	72.7	72.7	72.8	0.1
13	121.2	121.2	121.2	0.0
14	142.4	142.4	142.4	0.0
15	14.8	14.8	14.8	0.0
16	116.8	116.7	116.7	0.1
17	45.5	45.5	45.5	0.0
18	6.9	6.9	7.0	0.1
19	175.0	175.1	174.9	0.2
20	23.5	23.4	23.5	0.1
AcO	172.1	171.9	172.0	0.2
	170.1	170.1	170.0	0.1
	169.8	169.8	169.7	0.1
AcO	20.9	20.9	20.9	0.0
	21.1	21.1	21.1	0.0
	22.0	22.0	22.0	0.0

[a] Sample from present work ($CDCl_3$ 100 MHz). [b] Values from ref 10 ($CDCl_3$). [c] Values from ref 12 ($CDCl_3$). [d] Maximum difference between δ's in each row.

Except for antifeedant properties described for **3**,[7] no extensive bioactivity has been reported for these compounds. Compounds **1**, **2**, **4**, **6**, **7**, **9**, and **10** were assayed for cytotoxicity against cultured HCT-116 cells at a con-

centration of 24 μg/mL, but no significant cytotoxicity was observed for any of the compounds.

The structures of two novel cholic acid sterols **1** and **2** were identified, and the absolute stereochemistry of the known compound **7** was confirmed by an X-ray structure determination. Additionally it was concluded that the structure of compound **6** is identical to the reported structure for both minabein-1 and 11-hydroxyptilosarcenone. Since the report of minabein-1 precedes that of 11-hydroxyptilosarcenone, we propose that further use of the latter redundant name be discarded.

Experimental Section

General Experimental Procedures. IR, UV, and optical rotations were measured using a Mattson Galaxy Series FTIR 3000, a Hewlett-Packard 8452A diode array spectrophotometer, and a Jasco DIP-370 digital polarimeter, respectively. IR spectra were recorded from thin films deposited on NaCl plates. ^{1}H NMR and ^{13}C NMR spectra were measured at 399.7673 and 100.5155 MHz, respectively, in $CDCl_3$ on a Varian Inova 400 spectrometer. Spectra were processed using the manufacturer's software and referenced to residual protiated solvent signal (δ_H 7.24, δ_C77.00 ppm). LRESIMS and MS/MS were obtained by direct infusion into a Thermo-Finnigan LCQ Deca ion trap. Reversed-phase HPLC was carried out using Varian Dynamax or Rainin Rabbit HP pumps and Dynamax or Microsorb C_{18} columns (C_{18}, 100 Å, 3 μm, 10 $\times$ 250 mm) with UV and refractive index detection. High-resolution mass measurements were provided by the UC Riverside MS facility.

Animal Material. *Eleutherobia* sp. (02-12-053) was collected in December 2002 using scuba from Mwant Pass, Pohnpei, Micronesia, and stored partially frozen for 5 days. The specimen consisted of small 0.5−1.0 cm by 1.0−3.0 cm fingers of orange, yellow, and red. The cones were very tough and durable with a knobby texture. A preserved type sample is archived at the University of California, Davis.

Extraction. The tissue (231.9 g wet) was refrozen and homogenized in the presence of 1:1 CH_2Cl_2/MeOH (1750 mL) at high speed in a Waring blender ($\times$3). The filtered extract was concentrated, and the remaining MeOH-soluble portion was diluted with an equal volume of water before sequential extraction with hexanes, $CHCl_3$, and finally, *n*-butanol. The $CHCl_3$-soluble portion was concentrated to give a red extract (1.25 g), which was further fractionated by size-exclusion chromatography (Sephadex LH-20, 1:1 hexane/CH_2Cl_2, then MeOH). Fractions were pooled on the basis of TLC and further purified by reversed-phase HPLC to give compounds **1** (1.17 mg), **2** (1.07 mg), **3** (6.0 mg), **4** (1.08 mg), **5** (10.9 mg), **6** (5.67 mg), **7** (5.7 mg), **9** (15.14 mg), and **10** (4.09 mg).

11-Acetoxy-3-oxo-chol-4-en-24-oic acid (1): clear glass. $[\alpha]^{20}_D$ +63.9° (*c* 0.072, EtOH); UV ($CHCl_3$) λ 258 nm (log ϵ 2.95), 304 (2.02); IR (NaCl) ν_{max} 3100, 2955, 2881, 1729, 1677, 1384, 1247, 1025 cm^{-1}; ^{1}H NMR δ 5.74 (1H, s, H-4), 5.22 (1H, dt, J = 10.6 Hz, 5.2 Hz, H-11), 2.43−1.03 (24H, c, 2 H-1, 2 H-2, 2 H-6, 2 H-7, H-8, H-9, 2 H-12, H-14, 2 H-15, 2 H-16, H-17, H-20, 2 H-22, 2H-23) 2.00 (3H, s, H-26),1.24 (3H, s, H-19), 0.89 (3H, d, J = 6.0 Hz, H-21), 0.77 (3H, s, H-18); ^{13}C NMR δ 199.5 (C, C-3), 170.14 (C, C-24, C-25, or C-5), 170.07 (C, C-24, C-25, or C-5), 170.04 (C, C-24, C-25, or C-5) 124.7 (CH, C-4), 71.1(CH, C-11), 55.7 (CH, C-14 or C-20), 55.5 (CH, C-14 or C-20), 54.8 (CH, C-9), 46.4 (CH_2, C-12), 42.7 (C, C-13), 39.7 (C, C-10), 36.6 (CH_2, C-1), 35.1 (CH, C-8 or C-17), 35.0 (CH, C-8 or C-17), 34.1 (CH_2, C-2), 33.5 (CH_2, C-6), 31.8 (CH_2 C-7), 30.5 (CH_2, C-22 or C-23), 30.4 (CH_2, C-22 or C-23), 28.0 (CH_2, C-16), 24.1 (CH_2, C-15), 22.0 (CH_3, C-26), 18.3 (CH_3, C-19 or C-21), 18.1 (CH_3, C-19 or C-21), 12.8 (CH_3, C-18); HRFABMS *m/z* 431.2793 [M + H]$^+$ (calcd for $C_{26}H_{39}O_5^+$, 431.2798).

3-Oxo-chol-1,4,22-trien-24-oic acid (2): clear glass, $[\alpha]^{20}_D$ −39.6° (*c* 0.045, EtOH); IR (NaCl) ν_{max} 3410, 3198, 2938, 2870, 1713, 1660, 1611, 1450, 1384, 1242, 887, 755 cm^{-1}; ^{1}H NMR δ 7.03 (1H, d, J = 10.4 Hz, H-1), 6.89 (1H, dd, J = 15.6, 9.2 Hz, H-22), 6.21 (1H, dd, J = 10.4, 2.0 Hz, H-2), 6.05 (1H, br s, H-4), 5.74 (1H, d, J = 15.6 Hz, H-23), 2.45−0.88 (18 H, c, 2 H-6, 2 H-7, H-8, H-9, 2 H-11, 2 H-12, H-14, 2 H-15, 2 H-16, H-17, H-20), 1.21 (3H, s, H-19), 1.08 (3H, d, J = 6.4 Hz, H-21), 0.76 (3H, s, H-18); ^{13}C NMR δ 188.5 (C, C-3), 173.2 (C, C-24), 169.3 (C, C-1), 156.9 (C, C-5), 155.9 (CH, C-22), 127.5 (CH, C-4), 123.8 (CH, C-2), 118.0 (CH, C-23), 55.2 (CH, C-14 or C-17), 54.7 (CH, C-14 or C-7), 52.3 (CH, C-9), 43.6 (C, C-13), 43.0 (C, C-10), 39.8 (CH, C-20) 39.3 (CH_2, C-12), 35.5 (CH, C-8), 33.6 (CH_2, C-6), 32.8 (CH_2, C-7), 30.6 (CH, C-8), 27.9 (CH_2, C-16), 24.4 (CH_2, C-15), 22.8 (CH_2, C-11), 19.0 (CH_3, C-19 or 21), 18.7 (CH_3, C-19 or 21), 12.3 (CH_3, C-18); HRFABMS *m/z* 369.2429 [M + H]$^+$ (calcd for $C_{24}H_{33}O_3^+$, 369.2423).

3-Oxo-chol-4-en-24-oic acid (3): white solid (6.0 mg) from methanol, $[\alpha]^{20}_D$ +46.7° (*c* 0.30, EtOH); UV, IR, ^{1}H and ^{13}C NMR, and HRFABMS data were identical to literature values.[7,8]

3-Oxo-chol-4.22-dien-24-oic acid (4): clear glass UV, IR, ^{1}H NMR, and HRFABMS data were identical to literature values.[7,8]

3-Oxo-chol-1,4-dien-24-oic acid (5): white solid, $[\alpha]^{20}_D$ +17° (*c* 0.55, EtOH); UV, IR, ^{1}H and ^{13}C NMR, and HRFABMS data were identical to literature values.[9,12]

Minabein-1 (6): white solid, $[\alpha]_D^{20}$ −55° (*c* 0.15, $CHCl_3$); UV, IR, ^{1}H NMR, ^{13}C NMR, and ESIMS data were identical to literature values.[10,11]

Minabein-4 (7): thin prismatic leaves, 5.7 mg (acetonitrile); mp 229−231 °C; $[\alpha]^{20}_D$ −135° (*c* 0.12, $CHCl_3$); UV, IR, and HRFABMS data were identical to literature values.[10,13] See Table 1 for ^{13}C NMR data.

Minabein-6 (8): white solid (mixed with minabein-4); ^{13}C NMR and ESIMS data were identical to literature values.[10]

Minabein-8 (9): white solid, $[\alpha]^{20}_D$ −24.4° (*c* 0.74, $CHCl_3$); UV, IR, ^{1}H and ^{13}C NMR, and ESIMS data were identical to literature values.[10]

Minabein-9 (10): white solid, $[\alpha]^{20}_D$ +9.5° (*c* 0.25, $CHCl_3$); UV, IR, ^{1}H and ^{13}C NMR, and ESIMS data were identical to literature values.[10]

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Supporting Information Available: Crystallographic data for compound **7** and ^{1}H and ^{13}C data for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk.

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