Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 47 (2006) 2197–2200
Tetrahedron Letters

Coelodiol and coeloic acid, *ent*-isocopalane diterpenes from the Indonesian sponge *Coelocarteria* cfr. *singaporensis*

Ernesto Fattorusso,[a] Adriana Romano,[a] Orazio Taglialatela-Scafati,[a,*] Giorgio Bavestrello,[b] Patrizia Bonelli[c] and Barbara Calcinai[d]

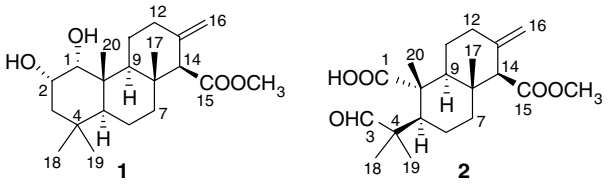
[a]*Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli 'Federico II', Via D. Montesano 49, 80131 Napoli, Italy*
[b]*Dipartimento di Scienze del Mare, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy*
[c]*Istituto Nazionale Tumori-Fondazione Pascale, Via M. Semmola, 80131 Napoli, Italy*
[d]*Dipartimento per lo Studio del Territorio e delle sue Risorse, Università di Genova, Corso Europa 26, 16132 Genova, Italy*

Received 10 January 2006; accepted 20 January 2006
Available online 10 February 2006

Abstract—Two novel *ent*-isocopalane diterpenes, coelodiol (**1**) and coeloic acid (**2**), the latter characterized by an unique oxidative degradation of ring A, have been isolated from the Indonesian sponge *Coelocarteria* cfr. *singaporensis*. The stereostructure of these metabolites has been established through interpretation of NMR data and application of the exciton chirality CD method. Coelodiol (**1**) and coeloic acids (**2**) were found to inhibit the growth of MKN-45 cell line (human gastric adenocarcinoma).
© 2006 Elsevier Ltd. All rights reserved.

Marine sponges of the family Isodictyidae (order Poecilosclerida, suborder Mycalina) have been scarcely investigated for their chemical composition. Among the few genera of this family, sponges belonging to the genus *Isodictya* have been shown to elaborate purine and tryptophan derivatives;[1,2] on the contrary, secondary metabolites produced by other genera, and, among them, by the genus *Coelocarteria* are hitherto unknown.

As part of our ongoing screening for bioactive secondary metabolites from Indonesian marine invertebrates, we have recently analyzed the organic extract of the sponge *Coelocarteria* cfr. *singaporensis* (Isodictyidae) collected in 2003 in the Bunaken Marine Park (North Sulawesi, Indonesia). This is a massive sponge, hard but friable, characterized by a subhemispherical body, partially buried into the sediment. It has short and composed, branched inhalant fistules and an exhalant cribrous area organized in one large fistule. Commonly, the sponge is covered by numerous epibionts and only the fistules are visible. In the area of the Bunaken Park it lives in shallow waters in crevices, up to 40 m depth. From the organic extract of this organism, we have isolated two new *ent*-isocopalane diterpenes, named coelodiol (**1**) and coeloic acid (**2**), the latter characterized by a unique oxidative degradation of ring A, whose stereostructure elucidation is herein detailed.



A specimen of *C.* cfr. *singaporensis* was extracted sequentially with fresh MeOH at room temperature. The combined MeOH extracts were concentrated to a brown gum, which was then partitioned between water and EtOAc. The EtOAc phase (7.5 g) was chromatographed over a silica column using *n*-hexane, *n*-hexane/EtOAc, and EtOAc/MeOH mixtures of increasing polarity. A fraction eluted with *n*-hexane/EtOAc 7:3 was further purified by normal-phase HPLC (eluent: *n*-hexane/EtOAc 65:35) to give pure samples of coelodiol (**1**, 5.2 mg) and coeloic acid (**2**, 1.8 mg).

ESI-MS of coelodiol (**1**), $[\alpha]_D$ +4.0 (*c* 0.2, $CHCl_3$), showed pseudomolecular ion peak at *m*/*z* 373 $[M+Na]^+$, while the peak at *m*/*z* 350.2466 in the

Keywords: *Coelocarteria* cfr. *singaporensis*; Indonesia; *ent*-Isocopalane; Diterpenes; Structure elucidation; NMR; Exciton chirality.
* Corresponding author. Tel.: +39 081 678509; fax: +39 081 678552; e-mail: scatagli@unina.it

0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2006.01.109

HR-EIMS indicated the molecular formula $C_{21}H_{34}O_4$ (calcd *m*/*z* 350.2457) for **1**. The ^1H and ^{13}C NMR spectra of **1** (Table 1, C_6D_6) were analyzed with the help of the 2D NMR HMQC spectrum, disclosing the presence of the following moieties: (i) five methyl groups, all resonating as singlets in the ^1H NMR spectrum (δ_H 0.87, 0.97, 1.06, 1.30, 3.38), the last ascribable to a methoxy group (δ_C 51.5); (ii) a geminally disubstituted double bond (δ_H 5.10, br s, and 4.90, br s, δ_C 109.5; δ_C 145.0); (iii) an ester group (δ_C 171.8), also indicated by the IR ($CHCl_3$) absorption at ν_{max} 1742 cm^{-1}. Thus, taking into account the five unsaturation degrees implied by molecular formula, coelodiol (**1**) has to possess a tricyclic structure. Inspection of the 2D NMR COSY spectrum of **1** allowed the arrangement of the remaining proton signals into the three spin systems (A–C) highlighted in bold in Figure 1. In particular, spin system A contains, in addition to a methylene, two consecutive oxygenated methines (δ_H 3.20, δ_C 76.3; δ_H 3.60, δ_C 74.4), accounting for the two remaining oxygen atoms of the molecular formula. In addition, it should be noted that spin system C comprises also the protons of the sp^2 methylene (H_2-16), as well as H-14, on the basis of

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR data of coelodiol (**1**) and coeloic acid (**2**) in C_6D_6

Pos.	1		2	
	δ_H, mult., *J* in Hz	δ_C, mult.	δ_H, mult., *J* in Hz	δ_C, mult.
1	3.20, d, 3.5	76.3, d		182.0, s
2	3.60, ddd, 9.5, 3.5, 3.0	74.4, d		
3a	1.57, dd, 12.5, 3.0	33.0, t	9.26, s	204.3, d
3b	1.42, dd, 12.5, 9.5			
4		42.5, s		58.4, s
5	1.16, dd, 13.0, 2.8	48.4, d	1.13, dd, 12.5, 2.5	49.2, d
6a	1.39[a]	20.5, t	1.36[a]	21.0, t
6b	1.36[a]		1.31[a]	
7a	1.26[a]	39.6, t	1.30[a]	38.9, t
7b	1.25[a]		1.28[a]	
8		40.2, s		39.4, s
9	1.67, dd, 12.0, 3.0	50.7, d	1.64, dd, 12.0, 3.0	53.1, d
10		42.0, s		51.0, s
11a	1.47, m	24.5, t	1.43, m	24.4, t
11b	1.38[a]		1.37[a]	
12a	2.31, ddd, 12.5, 3.8, 2.0	36.0, t	2.23, ddd, 12.0, 3.8, 2.0	36.9, t
12b	2.08, ddd, 12.5, 12.5, 3.5		2.10, ddd, 12.0, 12.0, 3.5	
13		145.0, s		143.7, s
14	2.98, s	64.3, d	2.73, s	63.7, d
15		171.8, s		172.0, s
16a	5.10, br s	109.5, t	5.01, br s	109.9, t
16b	4.90, br s		4.81, br s	
17	1.30, s	16.3, q	1.06, s	16.1, q
18	0.97, s	26.0, q	0.87, s	23.2, q
19	0.87, s	32.1, q	0.92, s	33.0, q
20	1.06, s	17.8, q	1.03, s	19.5, q
OCH_3	3.38, s	51.5, q	3.34, s	51.5, q

[a] Overlapped with other signals.

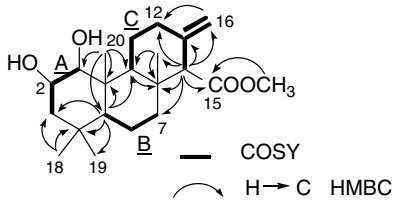


Figure 1. COSY and $^{2,3}J$ H→C HMBC correlations of coelodiol (**1**).

long-range allylic coupling of H_2-16 both with the multiplets at δ_H 2.08 and 2.31 (H_2-12) and the singlet at δ_H 2.98 (H-14).

Information arising from the 2D NMR HMBC spectrum allowed us to join all the substructures resulting from the above analysis, thus assembling the gross structure of coelodiol (**1**). Particularly informative were the $^{2,3}J_{C,H}$ correlations of H-5 (δ_H 1.16) with C-4, C-18, C-19, C-1, C-9, C-10, and C-20 and those of the downfield resonating methine H-14 (δ_H 2.98) with C-15, C-12, C-13, C-16, C-7, C-8, C-9, and C-17. The network of significant HMBC cross-peaks is depicted in Figure 1.

The relative configuration of **1** has been established on the basis of the proton–proton coupling constants (see Table 1) and the dipolar couplings (evidenced through a ROESY spectrum) shown in Figure 2. In particular, the *trans*-AB ring junction has been deduced by the ROESY cross-peaks H_3-20/H-2 and H-5/H-3b(ax); analogously, the dipolar couplings H-9/H-5 and H_3-17/H_3-20 implied a *trans*-BC ring junction. Taking into account the axial orientation of H-2, the relatively small coupling constant $J_{H1–H-2}$ (3.5 Hz) is indicative of an equatorial H-1; thus, H-1 and H-2 are cis (eq–ax) oriented. Finally, the ROESY correlation H-9/H-14 indicated the cis relationship of these protons and, consequently, the relative configuration at C-14.

Coelodiol (**1**) belongs to the diterpenoid class of isocopalanes, which is very rare in nature and apparently restricted to the marine environment. Interestingly, this skeleton has been found in two completely enantiomeric forms: the 'isocopalane' family, identifiable by the α-orientation of methyls at C-8 and C-10, has been isolated from some nudibranches[3,4] and, very recently, also from the sponge *Mycale* aff. *graveley*.[5] The enantiomeric '*ent*-isocopalane' family is typical of marine sponges.[6,7] Consequently, in order to assign **1** to one of these two classes, an unambiguous determination of its absolute configuration was needed. Taking advantage of the pres-

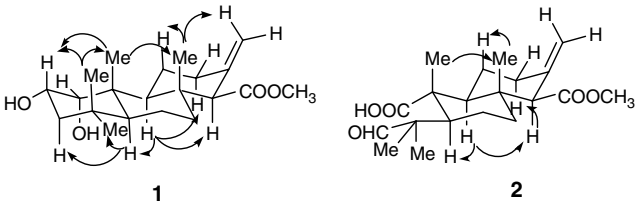


Figure 2. Diagnostic ROESY correlations detected for coelodiol (**1**) and coeloic acid (**2**).

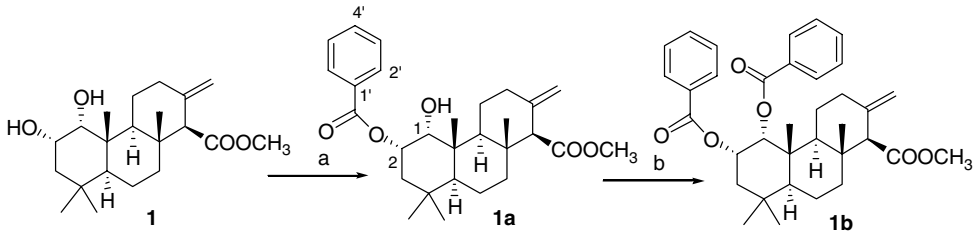


Figure 3. Benzoylation of coelodiol (**1**): (a) PhCOCl/Py/DMAP and (b) BzOTf/CH_2Cl_2.

ence of a 1,2-diol system in **1**, we decided to face this problem through the use of the non-empirical exciton chirality CD method that links the sign of the long wavelength Cotton effect with the chirality of the benzoate-bearing carbinol C–O bonds.[8] Attempts to obtain the 1,2-dibenzoate derivative of **1** by using benzoyl chloride in pyridine invariably produced only the 2-monobenzoate **1a**[9] (Fig. 3). Benzoylation of the axial hydroxy group at position 1 was successfully accomplished by treating **1a** with benzoyl trifluoromethanesulfonate (BzOTf) in CH_2Cl_2 at −78 °C,[10] obtaining the 1,2-dibenzoate **1b** in 35% yield.[11] The CD spectrum of **1b** in MeOH/dioxane 9:1 showed typical exciton-split Cotton effects with opposite signs centered upon the UV absorption of the benzoate chromophore: $\Delta\varepsilon_{222}$ +6.7 and $\Delta\varepsilon_{236}$ −15.2. The negative sign of the Cotton effect at the longer wavelength indicates the negative chirality between the two dipoles of the benzoate chromophores ('dibenzoate chirality rule') (Fig. 4),[8] thus assigning coelodiol to the *ent*-isocopalane class, as reported in **1**.

A second diterpene derivative, coeloic acid (**2**),[12] $[\alpha]_D$ −2.0 (*c* 0.15, $CHCl_3$), showed ESI-MS pseudomolecular ion peaks at *m*/*z* 373 $[M+Na]^+$ and 395 $[M-H+2Na]^+$, while HR measurement of the EIMS peak at *m*/*z* 350 revealed the molecular formula $C_{20}H_{30}O_5$ (meas. *m*/*z* 350.2099, calcd *m*/*z* 350.2093) for **2**. With the help of the 2D HMQC NMR spectrum, ^1H and ^{13}C NMR spectral data (Table 1, C_6D_6) of coeloic acid (**2**) were compared with the corresponding data of coelodiol (**1**), and several resemblances, but also some differences appeared evident. In particular, four methyl singlets, a methyl ester group, and an *exo*-methylene group were still present in **2**; on the other hand, the 1,2-diol system was missing, while an aldehyde group [NMR: δ_H 9.26, δ_C 204.3; IR ($CHCl_3$): ν_{max} 1735 cm^{-1}] and a carboxylic acid [NMR: δ_C 182.0; IR ($CHCl_3$): ν_{max} 1710 cm^{-1}] resulted to be part of structure **2**. Consequently, since coeloic acid (**2**) contains three carbonyl groups and a double bond, only two rings are needed to account for the six unsaturation degrees implied by molecular formula.

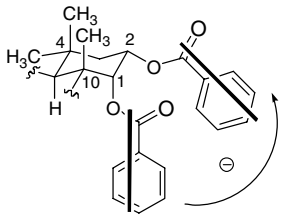


Figure 4. The negative chirality between the two benzoate dipoles of coelodiol-1,2-dibenzoate (**1b**).

Combined analysis of 2D NMR COSY, HMQC, and HMBC spectra of coeloic acid (**2**), assisted by comparison with parallel spectra of coelodiol (**1**), revealed that all the structural differences between **2** and **1** are confined to ring A. Indeed, the ^1H and ^{13}C NMR resonances assigned to nuclei from positions 5 to 20 of **2** proved to be quite similar to those of the corresponding atoms in coelodiol (**1**) (as can be seen in Table 1), and, furthermore, all the diagnostic COSY and HMBC correlations detected for protons and carbons belonging to BC rings of coelodiol (see Fig. 1) were identically detected also for coeloic acid (**2**). Additional correlations evident in the HMBC spectrum of **2** allowed us to draw its planar structure. In particular, $^3J_{H/C}$ cross-peaks of H_3-20 with C-9, C-5, and with the carbon resonating at δ_C 182.0 allowed the placement of the carboxylic group at C-10. Analogously, the $^3J_{H/C}$ cross-peaks of both H_3-18 and H_3-19 with C-5 and with the carbon resonating at δ_C 204.3 were in agreement with the linkage at C-4 of the two methyls and the aldehyde groups.

The pattern of proton–proton coupling constants and the series of ROESY cross-peaks (Fig. 2) were used to establish the relative configuration of coeloic acid (**2**) that, eventually, resulted to be identical to that of coelodiol (**1**). Due to the limited amount of material available, determination of absolute configuration of coeloic acid (**2**) was not accomplished; anyway, in structure **2** it has been reported with the same *ent*-isocopalane configuration of coelodiol (**1**).

The carbon skeleton of coeloic acid (**2**) is unique and, to our knowledge, it represents the first example in nature of a *seco*-norisocopalane diterpene. Coeloic acid (**2**) can derive from coelodiol (**1**) (or from a related *ent*-isocopalane) through an extensive oxidative degradation of ring A, resulting in the formation of a carboxylic acid at position 1, of an aldehyde group at position 3, and in the complete loss of carbon 2. This kind of degradation is not only unique among isocopalanes, but it is very rare also within the entire class of terpenoids. One of the few examples of related molecules is represented by oryzalides, antibacterial C_{19}-kaurane diterpenes isolated from rice plant.[13] Interestingly, in this case, the aldehyde group is located at position 1 and the carboxylic acid at position 3.

Coelodiol (**1**) and coeloic acids (**2**) were found to inhibit the in vitro growth of MKN-45 cell line (human gastric adenocarcinoma)[14] at 20 and 40 μg/mL, respectively. Further biological studies, aimed at establishing the mechanism of this activity, will be reported elsewhere in the future.

Acknowledgement

Financial support was provided by M.I.U.R., PRIN 2003 (Progetto Sostanze Naturali ed Analoghi Sintetici ad Attività Antitumorale).

References and notes

1. Moon, B.; Baker, B. J.; McClintock, J. B. *J. Nat. Prod.* **1998**, *61*, 116–118.
2. Moon, B.; Park, Y. C.; McClintock, J. B.; Baker, B. J. *Tetrahedron* **2000**, *56*, 9057–9062.
3. Gavagnin, M.; Ungur, N.; Castelluccio, F.; Muniain, C.; Cimino, G. *J. Nat. Prod.* **1999**, *62*, 269–274.
4. Gavagnin, M.; De Napoli, A.; Castelluccio, F.; Cimino, G. *Tetrahedron Lett.* **1999**, *40*, 8471–8475.
5. Rudi, A.; Benayahu, Y.; Kashman, Y. *J. Nat. Prod.* **2005**, *68*, 280–281.
6. de Miranda, D. S.; Brendolan, G.; Imamura, P. M.; Sierra, M. G.; Marsaioli, A. J.; Ruveda, E. A. *J. Org. Chem.* **1981**, *46*, 4851–4856.
7. Zubia, E.; Gavagnin, M.; Scognamiglio, G.; Cimino, G. *J. Nat. Prod.* **1994**, *57*, 725–731.
8. (a) Nakanishi, K.; Harada, N. *J. Am. Chem. Soc.* **1969**, *91*, 3989–3991; (b) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy—Exciton Coupling in Organic Stereochemistry*; Univ. Sci. Books: Mill Valley, USA, 1983.
9. Coelodiol-1-benzoate (**1a**): amorphous solid; $[\alpha]_D^{25}$ +2 (*c* 0.1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 8.09 (2H, d, $J = 7.0$ Hz, H-2′), 7.59 (2H, t, $J = 7.0$ Hz, H-3′), 7.45 (1H, t, $J = 7.0$ Hz, H-4′), 5.19 (1H, ddd, $J = 9.5$, 3.5, 3.0 Hz, H-2), 4.79 (1H, br s, H-15a), 4.65 (1H, br s, H-15b), 3.73 (1H, d, $J = 3.5$ Hz, H-1), 3.69 (3H, s, OCH_3), 2.93 (1H, br s, H-14), 2.35 (1H, ddd, $J = 12.5$, 3.8, 2.0 Hz, H-12a), 2.12 (1H, dt, $J = 12.5$, 3.5 Hz, H-12b), 1.89 (1H, dd, $J = 12.5$, 3.0 Hz, H-3a), 1.77 (1H, overlapped, H-3b), 1.77 (1H, overlapped, H-6a), 1.75 (1H, overlapped, H-6b), 1.66 (1H, m, H-11a), 1.60 (1H, overlapped, H-11b), 1.60 (1H, overlapped, H-7a), 1.59 (1H, overlapped, H-9), 1.58 (1H, overlapped, H-7b), 1.23 (1H, dd, $J = 13.0$, 2.8 Hz, H-5), 1.15 (3H, s, H-17), 1.08 (3H, s, H-20), 0.97 (3H, s, H-19), 0.91 (3H, s, H-18). ESI-MS: *m*/*z* 477 $[M+Na]^+$.
10. Brown, L.; Koreeda, M. *J. Org. Chem.* **1984**, *49*, 3875–3880.
11. Coelodiol-1,2-dibenzoate (**1b**): amorphous solid; $[\alpha]_D^{25}$ n.d.; ^1H NMR (500 MHz, $CDCl_3$): δ 8.09 (4H, d, $J = 7.0$ Hz, H-2′), 7.59 (4H, t, $J = 7.0$ Hz, H-3′), 7.45 (2H, t, $J = 7.0$ Hz, H-4′), 5.36 (1H, ddd, $J = 9.5$, 3.2, 3.0 Hz, H-2), 5.28 (1H, d, $J = 3.2$ Hz, H-1), 4.77 (1H, br s, H-15a), 4.68 (1H, br s, H-15b), 3.70 (3H, s, OCH_3), 2.78 (1H, br s, H-14), 2.37 (1H, ddd, $J = 12.5$, 3.8, 2.0 Hz, H-12a), 2.12 (1H, dt, $J = 12.5$, 3.5 Hz, H-12b), 1.92 (1H, dd, $J = 12.5$, 3.0 Hz, H-3a), 1.80 (1H, overlapped, H-3b), 1.78 (1H, overlapped, H-6a), 1.77 (1H, overlapped, H-6b), 1.66 (1H, m, H-11a), 1.65 (1H, overlapped, H-11b), 1.60 (1H, overlapped, H-9), 1.59 (1H, overlapped, H-7a), 1.58 (1H, overlapped, H-7b), 1.25 (1H, dd, $J = 13.0$, 2.8 Hz, H-5), 1.20 (3H, s, H-17), 1.07 (3H, s, H-20), 0.98 (3H, s, H-19), 0.91 (3H, s, H-18). ESI-MS: *m*/*z* 581 $[M+Na]^+$.
12. We preferred to number coeloic acid (**2**) by using the same numbering system of the isocopalane skeleton. Although, in this case, position 2 must be skipped, there is the advantage of an easy comparison of ^1H and ^{13}C NMR resonances of coeloic acid (**2**) with those of the corresponding nuclei in the *ent*-isocopalane coelodiol (**1**).
13. (a) Kono, Y.; Uzawa, J.; Kobayashi, K.; Suzuki, Y.; Uramoto, M.; Sakurai, A.; Watanabe, M.; Teraoka, T.; Hosokawa, D. *Agric. Biol. Chem.* **1991**, *55*, 803–811; (b) Kono, Y.; Kojima, A.; Nagai, R.; Watanabe, M.; Kawashima, T.; Onizawa, T.; Teraoka, T.; Watanabe, M.; Koshino, H.; Uzawa, J.; Suzuki, Y.; Sakurai, A. *Phytochemistry* **2004**, *65*, 1291–1298.
14. MKN-45 (human gastric adenocarcinoma) cell line was grown in RPMI 1640 supplemented with 20% foetal bovine serum (BioWhittaker) and 2 mM L-glutamine (MP Biomedicals). Samples of 10×10^3 cells/well were plated in 96-well culture plates and allowed to adhere for 24 h at 37 °C in a humidified atmosphere with 5% CO_2. The cells were incubated in medium containing different concentrations of coelodiol (**1**) and coeloic acid (**2**). After 6 days, the proliferation rate was assessed by counting the cells after staining with trypan blue solution (MP Biomedicals) through phase contrast microscopy.