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Antiproliferative Compounds from *Ocotea macrocarpa* from the Madagascar Dry Forest[1]

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Abstract

Bioassay-directed fractionation of an antiproliferative ethanol extract of the roots of *Ocotea macrocarpa* (Lauraceae) afforded the new butanolide macrocarpolide A (**1**), and the two new secobutanolides macrocarpolides B (**2**) and C (**3**), together with the known butanolides linderanolide B (**4**) and isolinderanolide (**5**). The structure elucidation of all compounds was carried out based on NMR and mass spectroscopic data analyses. The absolute configurations of all compounds isolated were determined by comparison of their optical rotation values with those found in literature. Compounds **1**–**5** showed good antiproliferative activities against the A2780 ovarian cell line, with IC_{50} values of 2.57 ± 0.12 (**1**), 1.98 ± 0.23 (**2**), 1.67 ± 0.05 (**3**), 2.43 ± 0.41 (**4**), and 1.65 ± 0.44 μM (**5**), respectively.

Keywords

Antiproliferative activity; *Ocotea macrocarpa*; Butanolide; Lauraceae

As a part of the Madagascar International Cooperative Biodiversity Group (ICBG) program,[2ab] an ethanol extract of the roots of *Ocotea macrocarpa* was found to have

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Supplementary data

Supplementary data associated with this article, consisting of experimental procedures and ^{1}H NMR spectra for compounds **1**–**5**, can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.XXXX.YY.ZZZ

moderate activity against the A2780 ovarian cancer cell line (IC_{50} 3.9 μg/ml). This extract was thus selected for further evaluation for the presence of novel anticancer agents. The plant genus *Ocotea*, the largest member of the Lauraceae family, comprises approximately 350 species that are distributed throughout tropical and subtropical climates. Most species are found in the Americas from Mexico to Argentina, seven species are found in Africa, one specie is found in the Canary Islands, and about 34 recognized species are found in Madagascar.[3ab] Some species are used in traditional medicine, including for treatment of fever and malaria.[4] Chemical investigations on various *Ocotea* species have led to the isolation of a wide range of secondary metabolites including alkaloids, flavonoids, lignans, and terpenoids, many of which exhibited interesting antiproliferative, antifungal, antiherpetic, antiinflammatory, and antimicrobial activities.[5abcdefg]

Bioassay-guided isolation of an extract of the roots of *Ocotea macrocarpa* produced five bioactive compounds: one new butanolide (**1**), two new secobutanolides (**2** and **3**), and two known butanolides, linderanolide B (**4**)[6] and isolinderanolide (**5**).[7] The structures of the known compounds were determined by a comparison of their ^{1}H NMR and mass spectra data with literature data, together with a comparison of their optical rotation values with the literature values.

Compound **1** was isolated as a colorless oil. The molecular formula was determined to be $C_{20}H_{34}O_3$ by HRESIMS ($[M + H]^+$, *m/z* 323.2586, cal. for $C_{20}H_{35}O_3^+$ 323.2581). The IR exhibited the characteristic absorption bands at 3450 cm^{-1} for a hydroxyl group, and 1760 and 1700 cm^{-1} for an α,β-unsaturated-γ-lactone.[8] The UV spectrum of **1** had an absorption maximum at 226 nm. The IR, UV and ^{1}H NMR spectroscopic data of **1** were comparable to those of **4** and **5**, suggesting that **1** had the same β-hydroxy-γ-methylene-α,β-unsaturated γ-lactone skeleton. The proton signal at δ_H 7.10 (dt, *J* = 7.8, 2.0 Hz, 1H, H-1′) in **1** differed significantly from the corresponding signals in **4** and **5** at δ_H 6.68 (td, *J*= 7.8, 2.0 Hz, 1H, H-1′), suggesting the *E* configuration for $\Delta^{3(1')}$ in **1**.[89] The ^{1}H NMR spectrum of **1** also displayed resonances assignable to two exomethylene protons appearing at δ_H 4.96 and δ_H 4.72 (dd, *J* = 2.8, 1.4 Hz, each 1H, H_2-6), one oxymethine at δ_H 5.26 (brs, 1H, H-4), and two deshielded methylene protons at δ_H 2.50 and δ_H 2.43 (dt, *J* = 14.8, 7.2 Hz, each 1H, H_2-2′). The positions of these protons were assigned from HMBC experimentation (Fig. 2). The exocyclic olefinic signals at δ_H 4.96 and δ_H 4.72 (H_2-6) were correlated with both a quaternary carbon at δ_C 157.8 (C-5) and a methine carbon at δ_C 66.7 (C-4). Carbon 5 also correlated with the oxymethine signal at δ_H 5.26 (H-4). Furthermore, clear long range correlations between both the oxymethine proton at δ_H 7.10 (H-1′) to the carbonyl carbon at δ_C 166.1 (C-2) were observed in the HMBC spectrum.

In addition, a broad peak at δ_H 1.25–1.31 (28H, H-3′–14′) and a triplet at δ_H 0.88 (*J* = 7.0 Hz, H-15′) were attributed to the methylene protons in a long alkyl chain and the terminal methyl group in **1**, respectively. Compound **1** showed an $[\alpha]^{21}_D$ value of −11.11 (*c* 0.27, MeOH), indicating the *S* configuration at C-4 as described for previously reported butanolides.[910ab] The complete assignments of all protons and carbons of **1** (Table 1) were accomplished by further interpretation of its HMBC and HSQC spectra. Thus, the structure of **1** was elucidated as (3*E*,4*S*)-4-hydroxy-5-methylene-3-pentadecylidene-dihydro-furan-2-one, and named macrocarpolide A.

Compound **2**, a colorless oil, had a molecular formula of $C_{23}H_{42}O_4$, as deduced from its HRESIMS spectrum (*m/z* 383.3157 $[M+H]^+$, calcd. for $C_{23}H_{43}O_4^+$, 383.3156). The IR spectrum of **2** showed absorption bands characteristic of hydroxyl (3458 cm^{-1}), ester (1734 cm^{-1}), and ketone (1715 cm^{-1}) groups. The UV absorption at 222 nm together with its IR and ^1H NMR spectroscopic data indicated a secobutanolide skeleton.[910b] Comparison of the ^1H NMR spectroscopic data of **2** with those of **1** revealed that the ^1H NMR of **2** exhibited additional signals at δ_H 3.73 (s, 3H, 1-OMe) and δ_H 2.15 (s, 3H, H-3′), but lacked the signals at δ_H 4.96 and δ_H 4.72 in **1**. This fact confirmed the presence of a methoxy and an acetyl group, and the absence of the α,β-unsaturated-γ-lactone ring in **2**. In the HMBC spectrum, protons of the acetyl group at δ_H 2.15 (H-3′) showed correlations to an oxymethine group at δ_C 73.5 (C-1′). The methoxy protons at δ_H 3.73 (1-OMe) correlated with a carbonyl carbon at δ_C 166.7 (C-1), and the olefinic proton at δ_H 7.08 (t, *J*= 7.7 Hz, H-3) exhibited cross peaks with both the oxymethine carbon (δ_C 73.5, C-1′) and the carbonyl carbon (δ_C 166.7, C-1). Those correlations confirmed the assignment of a secobutanolide skeleton to **2**. By the same analysis used to characterize compound **1**, the deshielded methylene group of **2** was assigned at C-4 by the HMBC correlation between δ_H 2.35 (q, *J* = 7.6 Hz, 2H, H-4) and the quaternary olefinic carbon at δ_C 129.9 (C-2). Furthermore, the presence of an *E* trisubstituted double bond was evident from the characteristic chemical shift of the olefinic proton at δ_H 7.08 (H-3), compared to that of known compounds with a *Z* conformation (δ_H 6.69).[910b]

The positive optical activity (+2.23, *c* 2.24, MeOH) of **2** indicated that C-1′ possessed the *S* configuration.[11abc] Similarly to **1**, the complete assignments of all protons and carbons of **2** (Table 1) were accomplished by further interpretation of its HMBC and HSQC spectra. From the above data, compound **2** was assigned as (2*E*)-2-[(1*S*)-1-hydroxy-2-oxo-propyl]-nonadec-2-enoic acid methyl ester, and named macrocarpolide B.

The molecular formula of compound **3** ($C_{21}H_{38}O_4$, HRESIMS *m/z*: 355.2856 $[M+H]^+$, calcd. for $C_{21}H_{39}O_4^+$, 355.2843) differed from that of **2** by C_2H_4, suggesting a two-carbon deletion in the side chain. Analysis of the UV, IR and ^1H NMR spectra revealed **3** to be a similar secobutanolide to **2**, with the same *E* geometry of the trisubstituted double bond [δ_H 7.08 (t, *J* = 7.0 Hz, 1H, H-3)], but with two carbons less in the alkyl chain. Similarly to **2**, the *S* configuration at C-1′ was deduced by the positive optical rotation value of +2.27 (*c* 0.88, MeOH).[11abc] The complete assignments of all protons and carbons of **3** (Table 2) were accomplished by interpretation of its HMBC and HSQC spectra. Therefore, compound **3** was assigned as (2*E*)-2-[(1*S*)-1-hydroxy-2-oxo-propyl]-heptadec-2-enoic acid methyl ester, and named macrocarpolide C.

Compounds **1**–**5** showed good antiproliferative activities against the drug-sensitive A2780 ovarian cell line[12] as previously described[13] using paclitaxel (IC_{50} 0.073 ± 0.015 μM) as the positive control. Their IC_{50} values were 2.57 ± 0.12 (**1**), 1.98 ± 0.23 (**2**), 1.67 ± 0.05 (**3**), 2.43 ± 0.41 (**4**), and 1.65 ± 0.44 μM (**5**). The similar IC_{50} values for the five compounds suggests that they have a similar mechanism of action, possibly as Michael acceptors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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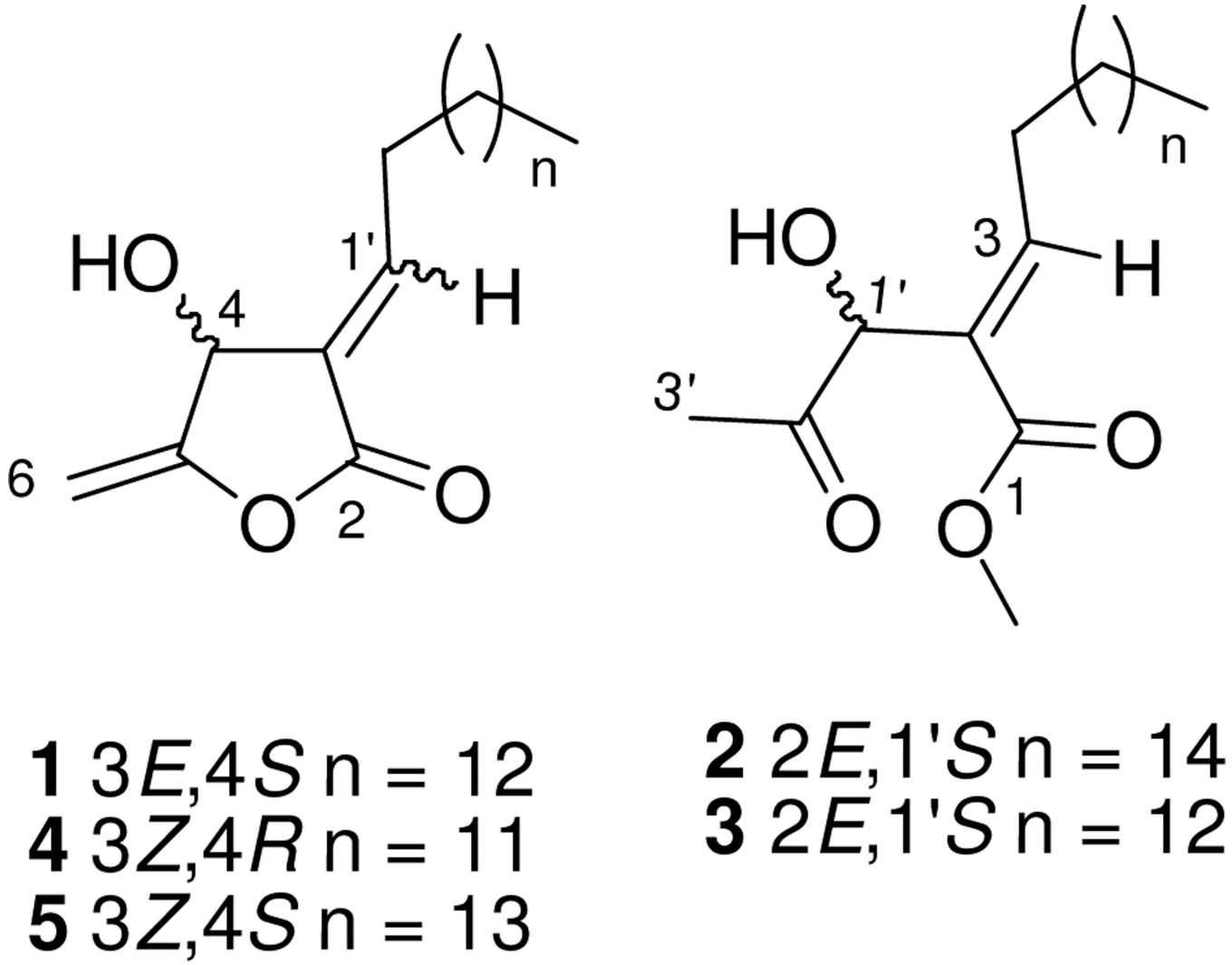

1 3*E*,4*S* n = 12
4 3*Z*,4*R* n = 11
5 3*Z*,4*S* n = 13

2 2*E*,1'*S* n = 14
3 2*E*,1'*S* n = 12

Figure 1.
Structures of compounds **1**–**5**.

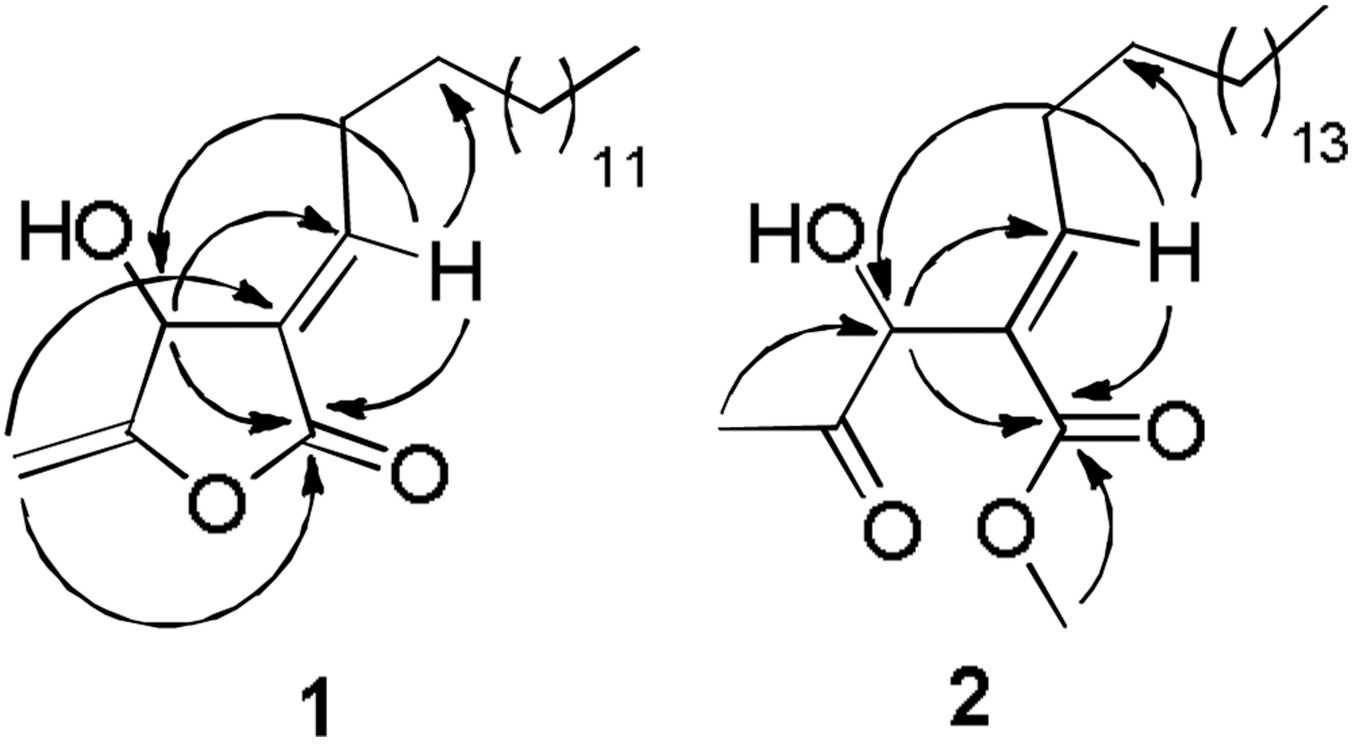

Figure 2.
Key HMBC correlations of **1** and **2**.

Table 1

^{1}H and ^{13}C NMR data for compound **1**.[a]

Posn	δ_H[b]	δ_c[c]
2		166.1 (C)
3		127.4 (C)
4	5.26 brs	66.7 (CH)
5		157.8 (C)
6	4.96 dd (2.8, 1.4) 4.72 dd (2.8, 1.4)	91.5 (CH_2)
1′	7.10 dt (7.8, 2.0)	150.3 (CH)
2′	2.50 dt (14.6, 7.7) 2.43 dt (14.6, 7.7)	29.8 (CH_2)
3′	1.25–1.31	28.3 (CH_2)
4′	1.25–1.31	29.8–29.5 (CH_2)
5′	1.25–1.31	29.8–29.5 (CH_2)
6′	1.25–1.31	29.8–29.5 (CH_2)
7′	1.25–1.31	29.8–29.5 (CH_2)
8′	1.25–1.31	29.8–29.5 (CH_2)
9′	1.25–1.31	29.8–29.5 (CH_2)
10′	1.25–1.31	29.8–29.5 (CH_2)
11’	1.25–1.31	29.8–29.5 (CH_2)
12′	1.25–1.31	29.8–29.5 (CH_2)
13′	1.25–1.31	32.1 (CH_2)
14′	1.25–1.31	22.8 (CH_2)
15′	0.88 t (7.0)	14.3 (CH_3)

[a] Assignments based on analysis of 2D NMR spectra.

[b] Data (δ) measured at 500 MHz; brs = broad singlet, dd= doublet of doublets, dt = doublet of triplets. *J* values are in Hz and are omitted if the signals overlapped as multiplets. The overlapped signals were assigned from HSQC and HMBC spectra without designating multiplicity.

[c] Data (δ) measured at 125 MHz; CH_3, CH_2, CH, and C multiplicities were determined by HSQC experiment.

Table 2

^{1}H and ^{13}C NMR data for compounds **2** and **3**.[a]

	2		3	
Posn	**δ_H[b]**	**δ_c[c]**	**δ_H[b]**	**δ_c[c]**
1		166.7 (C)		166.7 (C)
2		129.9 (C)		129.9 (C)
3	7.08 t (7.7)	149.3 (CH)	7.08 t (7.0)	149.3 (CH)
4	2.35 q (7.6)	28.9 (CH_2)	2.35 q (7.6)	28.9 (CH_2)
5	1.25–1.31	28.4 (CH_2)	1.26 br s	28.4 (CH_2)
6	1.25–1.31	29.8–29.5 (CH_2)	1.26 br s	29.8–29.5 (CH_2)
7	1.25–1.31	29.8–29.5 (CH_2)	1.26 br s	29.8–29.5 (CH_2)
8	1.25–1.31	29.8–29.5 (CH_2)	1.26 br s	29.8–29.5 (CH_2)
9	1.25–1.31	29.8–29.5 (CH_2)	1.26 br s	29.8–29.5 (CH_2)
10	1.25–1.31	29.8–29.5 (CH_2)	1.26 br s	29.8–29.5 (CH_2)
11	1.25–1.31	29.8–29.5 (CH_2)	1.26 br s	29.8–29.5 (CH_2)
12	1.25–1.31	29.8–29.5 (CH_2)	1.26 br s	29.8–29.5 (CH_2)
13	1.25–1.31	29.8–29.5 (CH_2)	1.26 br s	29.8–29.5 (CH_2)
14	1.25–1.31	29.8–29.5 (CH_2)	1.26 br s	29.8–29.5 (CH_2)
15	1.25–1.31	29.8–29.5 (CH_2)	1.26 br s	32.1 (CH_2)
16	1.25–1.31	29.8–29.5 (CH_2)	1.26 br s	29.8–29.5 (CH_2)
17	1.25–1.31	32.1 (CH_2)	0.88 t (7.0)	14.3 (CH_3)
18	1.25–1.31	22.8 (CH_2)		
19	0.88 t (7.0)	14.3 (CH_3)		
1′	4.90 brd (4.9)	73.5 (CH)	4.90 brs	73.5 (CH)
2′		206.2 (C)		206.2 (C)
3′	2.15 s	25.0 (CH_3)	2.15 s	25.0 (CH_3)
1-OMe	3.73 s	52.2 (CH_3)	3.73 s	52.2 (CH_3)

[a] Assignments based on analysis of 2D NMR spectra.

[b] Data (δ) measured at 500 MHz; brs = broad singlet, dd= doublet of doublets, dt = doublet of triplets. *J* values are in Hz and are omitted if the signals overlapped as multiplets. The overlapped signals were assigned from HSQC and HMBC spectra without designating multiplicity.

[c] Data (δ) measured at 125 MHz; CH_3, CH_2, CH, and C multiplicities were determined by HSQC experiment.