Pergamon

Tetrahedron Letters 41 (2000) 1401–1403

TETRAHEDRON LETTERS

Brassicolene, a novel cytotoxic diterpenoid from the Formosan soft coral *Nephthea brassica*

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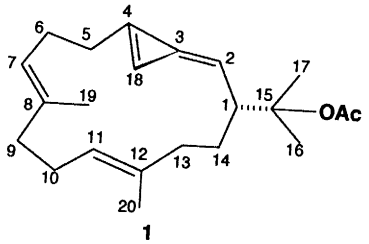
Received 19 October 1999; revised 29 November 1999; accepted 3 December 1999

Abstract

A novel cytotoxic diterpenoid, brassicolene (**1**), has been isolated from the soft coral *Nephthea brassica.* The structure of **1** (novel carbon skeleton) was determined by 1D and 2D spectral analysis.

Keywords: soft coral; *Nephthea brassica*; cytotoxic; diterpenoid; brassicolene.

In previous papers,[1,2] we reported a tetraterpene and a norditerpene with novel skeletons from Formosan soft corals *Sinularia flexibilis* and *S. inelegans*, respectively. In a continuing search for bioactive substances from marine organisms, the Formosan soft coral *Nephthea brassica* Kükenthal (Family Nephtheidae) was selected for study since its CH_2Cl_2 extracts showed significant cytotoxicity in several tumor cell lines as determined by standard procedures.[3,4] Bioassay-guided fractionation resulted in the isolation of a novel cytotoxic diterpenoid, brassicolene (**1**), which was obtained as a colorless oil, $[\alpha]^{25}_{D}$ +16.2 (*c* 0.06, $CHCl_3$). Its UV absorption at λ_{max} (log ε) 226 nm (4.16) exhibited the presence of a conjugated diene. Analysis of HREIMS revealed a molecular formula of $C_{22}H_{32}O_2$ [M^+ *m/z* 328.2390 (Δ −1.2 mmu)], which indicated seven degrees of unsaturation. Its IR spectrum (KBr) suggested the presence of an ester carbonyl group (1726 cm^{-1}).



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PII: S0040-4039(99)02302-3

Table 1
^{1}H and ^{13}C NMR data of brassicolene (**1**) (400 and 100.6 MHz, respectively, in $CDCl_3$). The chemical shifts are given in ppm relative to TMS, and coupling constants (*J*) in Hz

pos.	δ_H; mult.; *J*	δ_C;mult.	HMBC	COSY	NOESY
1	3.25; ddd; 13, 3, 0.6	46.4; d	3, 14, 15		18, 20
2	7.09; d; 0.6	137.5; d	3, 4, 18	18	5
3		153.5; s			
4		124.2; s			
5	2.57; m	23.1; t	4, 6		2, 18
6α	2.28; m	26.2; t	4, 5, 7, 8	5, 7	
6β	2.41; m				18
7	4.94; m	125.7; d	19	6	5, 6, 9, 10
8		134.4; s			
9	2.09; m	39.0; t	10		7, 9, 10
10	2.01; m	23.8; t		11	9, 11
11	4.42; m	125.5; d	10, 13, 20	10, 20	10, 13, 18
12		132.9; s			
13	1.88; m	37.4; t	11, 14	14	11
14α	1.67; m	23.9; t	13	1, 13	18
14β	1.83; m				1
15		84.1; s			
16	1.40; s	23.7; q	1, 15		1
17	1.42; s	24.8; q	1, 15		1
18	5.74; s	111.1; d	2, 3, 4	2	1, 5, 6β, 7, 14α, 19
19	1.57; s	15.4; q	7, 8, 9	7	18
20	1.50; s	14.7; q	11, 12, 13	11	1
OAc	1.97; s	22.5; q			
		170.4; s			

^{1}H and ^{13}C NMR spectral data (Table 1) showed the structure of **1** contained an acetoxyisopropyl side chain (δ_C 24.8 q, 23.7 q, 84.1 s, 22.5 q, 170.4 s; δ_H 1.40, 1.42, 1.97, 3H each, s each), two isolated methyl-bearing trisubstituted double bonds (δ_H 4.94 m, 4.42 m, 1.57 bs, 1.50 bs, 3H each; δ_C 125.7 d, 134.4 s, 125.5 d, 132.9 s), a trisubstituted cyclopropene double bond (δ_H 5.74, 1H, s; δ_C 111.1 d, 124.2 s),[5] a trisubstituted double bond (δ_H 7.09, 1H, d, *J*=0.6 Hz; δ_C 137.5 d, 153.5 s), one methine carbon (δ_C 46.4 d) and six methylene carbons (δ_C 23.1, 26.2, 39.0, 23.8, 37.4, 23.9). These data suggested that **1** may possess a bicyclo [12.1.0] pentadecane skeleton with functionalities of an acetoxyisopropyl, two isolated methyl-bearing trisubstituted double bonds, a cyclopropene, and a trisubstituted double bond conjugated with the cyclopropene double bond. The acetoxyisopropyl at C-1 was confirmed by HMBC correlations between H-1 to C-3, C-14 and C-15, H-16 to C-1 and C-15, and H-17 to C-1 and C-15. The vinyl methyl group at C-8 was confirmed by HMBC correlations between H-19 to C-7, C-8 and C-9 and H-7 to C-19. The other vinyl methyl group at C-12 was revealed by the HMBC correlations between H-11 to C-10, C-13 and C-20, and H-20 to C-11, C-12 and C-13. The acetoxyisopropyl and the methyl-bearing trisubstituted olefins were further connected by HMBC correlations as shown in Table 1. The position of the cyclopropene and its conjugated olefin was confirmed by HMBC correlations between H-1 to C-3, C-14 and C-15, H-2 to C-3, C-4 and C-18, and H-18 to C-2, C-3 and C-4. The α configuration of the acetoxyisopropyl at C-1 was determined by NOESY experiment, which showed correlations between H-1 and H-14β, 18 and 20. Brassicolene (**1**) exhibited cytotoxicity against A-549 and P-388 cell culture system with ED_{50} of 3.62 and 0.86 μg/ml, respectively.

Acknowledgements

We wish to thank Professor Chang-Feng Dai of the Institute of Oceanography, Taiwan National University, for identification of the soft coral sample. This work was supported by grants from the National Science Council of Taiwan.

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