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J. Nat. Prod., **1993**, 56 (7), 1051-1056• DOI:
10.1021/np50097a007 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 7, pp. 1051-1056, July 1993

DITERPENES FROM THE GORGONIAN CORAL *ERYTHROPODIUM CARIBAEORUM* FROM THE SOUTHERN CARIBBEAN

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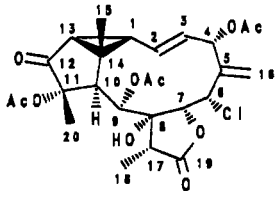
ABSTRACT.—*Erythropodium caribaeorum* obtained off the coast of Tobago has yielded the known diterpenes erythrolide A [**1**], erythrolide B [**2**], and erythrolide E [**3**] as well as a new diterpene designated erythrolide J [**4**]. The structure of compound **4** was determined by high resolution nmr studies.

Well over one hundred diterpenes possessing the briarane skeleton have been isolated from marine coelenterates mainly from the sub-class Octocorallia (1). Recent reports of seven new compounds of this type from *Erythropodium caribaeorum* Duchassaing & Michelotti (Gorgonidae) and nine from a *Briareum* sp. as well as twenty-five from the encrusting gorgonian *Solenopodium stechei* collected on the Great Barrier Reef off Australia have added significantly to this group of diterpenes (2,3).

E. caribaeorum is an encrusting gorgonian that is not readily distinguished from *Briareum asbestinum*. Previous investigations of this organism have yielded erythrolides A–I (2,4). Erythrolides A [**1**] and B [**2**] have not been reported thus far from any other organism and may well be chemotaxonomic markers for *E. caribaeorum*. We report here our investigation of *E. caribaeorum* collected off the coast of Tobago. In addition to compounds **1** and **2**, we also isolated erythrolide E [**3**] (2) and a new diterpene which we have named erythrolide J [**4**].

Erythrolides A [**1**], B [**2**], and E [**3**] were identified by comparison of their respective ^{1}H-nmr spectral data with those reported in the literature (2,4). The complete ^{1}H and



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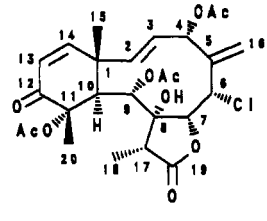

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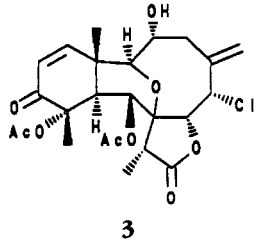

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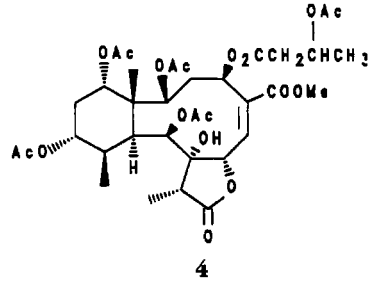

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TABLE 1. ^{1}H-nmr Data for Compounds **1–4** in $CDCl_3$.[a]

Proton	Compound			
	1	**2**	**3**	**4**[b]
H-1	2.30 (8.0,7.0)	—	—	—
H-2	6.45 (17.0,7.0,2.0)	6.20 (16.0)	4.18 (1.7)	4.88 (8.5)
H-3	5.84 (17.0,2.0)	5.51 (16.0)	3.96 (bs)	2.91 (m)
H-3′	—	—	—	2.09 (m)
H-4	5.99 (s)	5.53 (bs)	2.73 (13.8,3.0)	5.82 (12.4,5.8)
H-4′	—	—	2.75 (13.8,4.1)	—
H-6	4.54 (9.8)	5.03 (2.0)	4.78 (1.0)	6.87 (10.0,1.1)
H-7	5.13 (9.8)	5.64 (2.0)	4.36 (1.0)	5.60 (10.0)
H-9	5.42 (2.0)	5.47 (bs)	5.72 (2.5)	5.25 (bs)
H-10	2.94 (bs)	3.78 (bs)	3.20 (2.5)	2.94 (4.0)
H-11	—	—	—	1.99 (m)
H-12	—	—	—	4.81 (2.0)
H-13	1.92 (8.0)	6.06 (10.0)	6.00 (10.1)	1.99[c] (m)
H-14	—	6.60 (10.0)	6.63 (10.1)	4.74 (3.1)
H-15	1.56 (s)	1.40 (s)	1.62 (s)	1.14 (s)
H-16	5.59 (s)	5.55 (s)	5.67 (s)	—
H-16′	5.47 (s)	5.54 (s)	5.39 (s)	—
H-17	3.14 (8.0)	2.74 (7.6)	2.68 (7.5)	2.56 (7.1)
H-18	1.19 (8.0)	1.24 (7.6)	1.03 (7.5)	1.24 (7.1)
H-20	1.37 (s)	1.40 (s)	1.45 (s)	1.13 (7.6)
MeCO	2.21 (s)	2.15 (s)	2.24 (s)	2.24 (9-Ac)
	2.12 (s)	2.10 (s)	2.10 (s)	2.04 (12-Ac)
	2.01 (s)	1.96 (s)	—	2.00 (14-Ac)
	—	—	—	1.95 (2-Ac)
OMe	—	—	—	3.84 (s)

[a]Chemical shifts were measured at 400 MHz. Coupling constants (in Hz) are in parentheses.

[b]See Figure 1, partial structure **D** for the assignment of the 3-acetoxybutanoyl moiety in erythrolide J [**4**].

[c]Average value for an incompletely resolved CH_2 group.

^{13}C assignments for all three compounds were achieved by the use of COSY, HETCOR, and FLOCK (5) experiments. Our assignments were in complete agreement with those reported for erythrolide E [**3**]. However, for erythrolides A [**1**] and B [**2**], our assignments (Tables 1 and 2), in a few instances, are at variance with those reported in the literature (2,4).

An inspection of the HETCOR spectrum of **1** revealed that C-6 was directly attached to a proton at δ 4.54. This proton was coupled (J=9.8 Hz) to a proton at δ 5.13, whose directly attached carbon resonated at δ 80.7, indicating that H-6 and H-7 should be interchanged. The HETCOR spectrum also revealed that H-1 (δ 2.30) was directly attached to a carbon at δ 37.3, while H-13 (δ 1.92) was attached to a carbon at δ 39.8. This observation indicated that the assignments for C-1 and C-13 should be interchanged. In the FLOCK spectrum of **1**, the C-14 carbon had long-range connectivity with methyl protons at δ 1.37, which were directly attached to a carbon at δ 21.7. In a similar fashion the C-11 carbon at δ 83.1 showed long-range correlations to methyl protons at δ 1.56. Again, from the HETCOR experiment, these methyl protons were directly attached to a carbon at δ 22.7. These observations indicated that the H-15/C-15 and H-20/C-20 assignments should be interchanged. The HETCOR spectrum along with the COSY experiment also indicated that C-7 and C-9 should be interchanged. A detailed analysis of COSY, HETCOR, and FLOCK spectra of **2** revealed that H-6 and H-7 should be interchanged, as well as C-10 and C-17, and C-15 and C-20.

TABLE 2. ^{13}C-nmr Data for Compounds **1–4** in $CDCl_3$.[a]

Carbon	Compound			
	1	**2**	**3**	**4**[b]
C-1	37.3	42.0	36.3	45.3
C-2	126.7	144.9	86.2	73.7
C-3	133.0	131.5	69.9	36.8
C-4	75.7	73.6	41.3	67.9
C-5	138.5	141.7	138.3	137.5
C-6	59.4	65.7	59.3	138.4
C-7	80.7	79.2	85.7	76.9
C-8	87.3	81.1	83.1	82.6
C-9	80.5	77.8	68.8	75.6
C-10	43.7	48.8	41.7	33.4
C-11	83.1	80.9	80.5	42.9
C-12	205.0	195.5	194.0	72.7
C-13	39.8	126.3	124.5	24.0
C-14	29.7	154.5	152.3	74.4
C-15	21.7	20.9	21.2	14.9
C-16	127.9	115.8	123.0	167.2
C-17	43.9	44.8	48.9	43.6
C-18	9.5	9.3	6.8	6.4
C-19	175.1	176.2	174.0	175.5
C-20	22.7	22.8	22.3	15.1
Acetates	171.9	169.5	169.9	170.4 (12-Ac)
	20.9	21.0	21.3	21.4
	169.9	169.4	169.9	170.4 (14-Ac)
	21.2	21.2	21.1	21.1
	167.9	168.9	—	170.1 (2-Ac)
	21.7	21.2	—	20.9
	—	—	—	169.2 (9-Ac)
	—	—	—	21.6
OMe	—	—	—	52.9

[a]Chemical shifts were measured at 100.6 MHz.

[b]See Figure 1, part structure **D** for the assignments for the 3-acetoxybutanoyl moiety in erythrolide J [**4**].

TABLE 3. NOe Data for Erythrolide J [**4**].[a]

Irradiated	Observed	% Enhancement
δ 1.13 (H-20)	δ 5.25 (H-9)	3.0
	δ 4.81 (H-12)	4.0
	δ 2.24 (9-Ac)	0.8
δ 1.14 (H-15)	δ 4.74 (H-14)	5.0
	δ 2.24 (9-Ac)	1.0
	δ 1.95 (2-Ac)	0.6
δ 1.24 (H-18)	δ 5.25 (H-9)	1.0
	δ 2.56 (H-17)	7.0
δ 4.88 (H-2)	δ 5.82 (H-10)	3.0
	δ 2.94 (H-4)	3.0
δ 5.82 (H-4)	δ 4.88 (H-2)	4.0

[a]NOe data obtained at 400 MHz using nOe difference spectroscopy.

Erythrolide J [**4**], [α]D +17.3°, was isolated as an amorphous solid and had the molecular formula $C_{35}H_{48}O_{17}$ on the basis of hrfabms (positive). ^{1}H- and ^{13}C-nmr data along with ir spectroscopy indicated the following functional groups: (a) six *O*-acyl groups of which five were -OAc, (b) one -CO_2Me, (c) one γ- lactone (ir 1778 cm^{-1}), (d) one hydroxyl (ir 3430 cm^{-1}), and (e) a trisubstituted double bond. These accounted for all seventeen oxygen atoms in the molecule and for ten double bond equivalents, which suggested that compound **4** was bicarbocyclic.

A combination of ^{1}H nmr, COSY, HETCOR, and the FLOCK pulse sequence (5) led to the partial structures shown in Figure 1. Partial structure **A** was supported by a uv absorption at λ max (MeOH) 225 nm (ε 3200), and the proton on the β-carbon (δ 6.87) of the conjugated system was shown from COSY data to be adjacent to a proton (δ 5.60) on an oxygen-bearing sp^3 hybridized carbon atom.

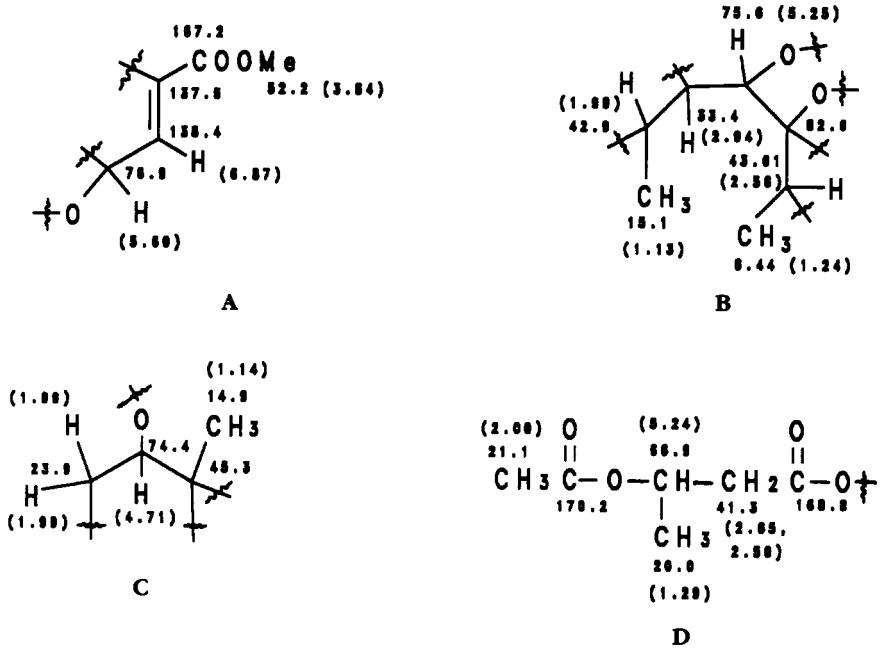

FIGURE 1. Partial structures and nmr assignments for compound **4**.

Partial structure **B** showed two secondary methyl groups and a tertiary C-O moiety adjacent to a secondary C-O. The fragment **C** related the only quaternary carbon in the molecule with a methyl singlet and an adjacent secondary oxygenated carbon. Finally, an acetoxy-bearing four-carbon unit which must be a side chain on the main skeleton was revealed as the fragment **D**. In addition, from COSY data two three-carbon units, each showing a 1,3 relationship of two secondary oxygenated carbons separated in each case by a methylene carbon, were revealed.

The partial structures **A–D** and the latter two 3-carbon fragments could be assembled as shown in Figure 2, in which all the direct connectivities were revealed by COSY and FLOCK data except C-1/C-10 and C-4/C-5. The 3-acetoxybutanoyl moiety as well as the acetate groups were assigned on the basis of a series of selective INEPT experiments. Erythrolide J was thus shown to have structure **4** excluding the stereochemistry.

The stereochemistry of erythrolide J was determined from a series of nOe difference experiments as follows. In the cyclohexane ring Me-15 and H-14 are on the same face of the molecule since there is an nOe relationship between the protons concerned.

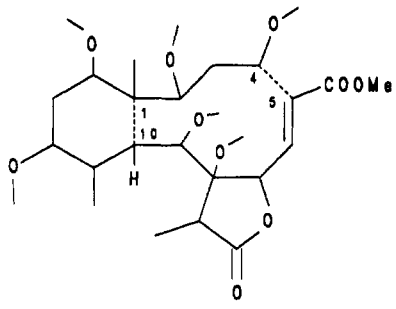

FIGURE 2. Assembly of partial structures for compound **4** based on COSY and FLOCK data.

Similarly there is an nOe relationship between Me-20 and H-12; their cis relationship was supported by the lack of observable coupling between them. The nOe enhancement of H-10 when H-2 was irradiated confirmed that H-2 was trans to the methyl attached to C-1 and on the same side of the molecule as H-10; this also supported the attachment of C-1 to C-10.

The large coupling between H-6 and H-7 (J=10.0 Hz) revealed their anti-parallel relationship. The acetate attached to C-9 had a β orientation since there was an nOe interaction between its methyl group (δ 2.24) and both Me-15 and Me-20. The stereochemistry at C-7 and C-8 was assigned by analogy with related briaranes (6).

The nOe interaction between Me-18 and H-9 was consistent with an α orientation of the methyl group interacting with a proton at C-9 in an α-pseudoequatorial disposition. The 3-acetoxybutanoyl moiety at C-4 had a β orientation since irradiation of the C-4 proton caused an enhancement of the proton at C-2, while irradiation of H-2 resulted in an nOe interaction with H-4.

Erythrolide J [**4**] is only the second naturally occurring briarane diterpene so far reported with the Me-16 group oxygenated up to the level of a carboxylate moiety (6). While a large variety of *O*-acyl groups other than the common acetate group have been observed in these compounds, (2, 7–11) this is the first report of a briarane diterpene bearing a 3-acetoxybutanoyl substituent.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were taken on a Kofler hot stage apparatus and are uncorrected. Uv spectra were obtained on a Cary 14UV spectrophotometer in MeOH solutions. Ir spectra were obtained on a Nicolet 3DX FTIR spectrometer. The nmr spectra were recorded on a Varian XL-400 spectrometer in $CDCl_3$ solutions with TMS as an internal standard. Nuclear Overhauser enhancements were determined from nOe difference spectra produced by on-resonance and off-resonance irradiation of the peak of interest. To obtain sufficient signal/noise ratio for accurate nOe measurements, 128 transients were collected per spectrum, with the acquisitions alternated in blocks of 16 to minimize effect of spectrometer instabilities. Exponential line broadening of 1 Hz was applied to each spectrum prior to generating difference spectra. A VG 70-250S mass spectrometer operating at 70 eV was used to obtain ms.

EXTRACTION AND ISOLATION.—A sample of *E. caribaeorum* was collected at La Guira Reef (−7 m) in southwest Tobago and was immediately stored in Me_2CO. The organism was identified by Mr. Richard Laydoo of the Institute of Marine Affairs, Trinidad and Tobago, where a voucher specimen has been deposited. The sample was subsequently macerated in the solvent and exhaustively extracted with Me_2CO. The extract was concentrated to a small volume, diluted with H_2O, and extracted with EtOAc to give a brown oil (4.67 g). The crude extract was suspended in 95% aqueous MeOH and extracted with petroleum ether, further diluted with H_2O, and re-extracted with EtOAc. The EtOAc fraction was washed with 5% Na_2CO_3, dried over Na_2SO_4, and the solvent evaporated to give a dark brown viscous oil (2.98 g, 1.58% based on the dried wt of the extracted organism).

The EtOAc extract (1.40 g) was chromatographed on Si gel using CH_2Cl_2 with increasing concentrations of EtOAc as eluent. Fractions eluting wth 15% EtOAc were combined and separated by preparative tlc in petroleum ether-CH_2Cl_2-Me_2CO (4:1:1) to give erythrolides A [**1**] (18 mg) and B [**2**] (160 mg). A

further portion (0.60 g) of the EtOAc extract was separated by preparative tlc in petroleum ether-CH_2Cl_2-Me_2CO (3:1:1) followed by rechromatography on a chromatotron [petroleum ether-CH_2Cl_2-Me_2CO (4:1:1)] and subsequent preparative tlc in CH_2Cl_2-MeOH (50:1) to give erythrolide E [**3**].

Erythrolide A [**1**].—Mp 177–179° ($CHCl_3$/MeOH) (14 mg); ir (Nujol) 3420, 1765, 1745 cm^{-1}; uv (MeOH) 215 nm (ε 8600); eims *m/z* $[M]^+$ 538 (2%), 503 (6), 478 (12), 436 (61), 418 (27), 401 (26), 376 (100), 341 (60), 323 (28).

Erythrolide B [**2**].—Colorless glass (150 mg): ir (neat) 3520, 1785, 1740, 1690 cm^{-1}; uv (MeOH) 224 nm (ε 9100); eims *m/z* $[M]^+$ 538 (2), 503, (7), 478 (3), 436 (65), 401 (6), 376 (9), 186 (12).

Erythrolide E [**3**].—Compound **3** was obtained as a white amorphous solid (7 mg): cims (isobutane) *m/z* $[M+H]^+$ 497 (47%), 437 (100), 377 (57), 135 (73); hreims 496.1484, calcd for $C_{24}H_{29}O_9Cl$, 496.1500. A sample of *E. caribaeorum* was collected at Milford Bay (−15 m), Tobago, steeped in Me_2CO, and extracted as before. The final EtOAc extract gave an orange-brown viscous solid (1.23 g, 1.98% of the dried wt of the organism).

Chromatography of the extract (0.95 g) was done with C_6H_6 containing increasing concentrations of EtOAc. The fraction (38.9 mg) eluting with 40% EtOAc was subjected to preparative tlc on Si gel in petroleum ether-Me_2CO-EtOAc (5:1:1) to give compound **4** as an amorphous solid (14.3 mg).

Erythrolide J [**4**].—$[\alpha]_D$ +17.3° (*c*=0.33, $CHCl_3$); ir ($CHCl_3$) 3430, 1778, 1739, 1728 cm^{-1}; uv (MeOH) 225 nm (ε 3200); fabms (positive) *m/z* $[M+H]^+$ 741 (0.5%), 681 (1), 621 (1), 595 (7), 534 (22), 492 (35) 372 (23), 69 (100); hrfabms (positive) 741.3032, calcd for $C_{35}H_{49}O_{17}$ $[M+H]^+$ 741.2970.

ACKNOWLEDGMENTS

Research in the Toronto laboratory was supported by grants from the Natural Sciences and Engineering Research Council of Canada. One of us (WFT) gratefully acknowledges receipt of a CIDA/NSERCC research associateship.

LITERATURE CITED

1. D.J. Faulkner, *Nat. Prod. Rep.*, **5**, 541 (1988), and references cited therein.
2. E.O. Pordesimo, F.J. Schmitz, L.S. Ciereszko, M.B. Hossain, and D. van der Helm, *J. Org. Chem.*, **56**, 2344 (1991).
3. S.J. Bloor, F.J. Schmitz, M.B. Hossain, and D. van der Helm, *J. Org. Chem.*, **57**, 1205 (1992).
4. S.A. Look, W. Fenical, D Van Engen, and J. Clardy, *J. Am. Chem. Soc.*, **106**, 5026 (1984).
5. W.F. Reynolds, S. McLean, M. Perpick-Dumont, and R.G. Enriquez, *Magn. Reson. Chem.*, **27**, 162 (1989).
6. S.J. Wratten and D.J. Faulkner, *Tetrahedron*, **35**, 1907 (1979).
7. B.F. Bowden, J.C. Coll, I.M. Vasilescu, and P.N. Alderslade, *Aust. J. Chem.*, **42**, 1727 (1989).
8. A. Clastres, P. Laboute, A. Ahond, C. Poupat, and P. Potier, *J. Nat. Prod.*, **47**, 162 (1984).
9. A. Clastres, A. Ahond, C. Poupat, P. Potier, and S.K. Kan, *J. Nat. Prod.*, **47**, 155 (1984).
10. S. Isaacs, S. Carmely, and Y. Kashman, *J. Nat. Prod.*, **53**, 596 (1990).
11. A. Groweiss, S.A. Look, and W. Fenical, *J. Org. Chem.*, **53**, 2401 (1988).

Received 19 October 1992