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BRIARANE AND ASBESTINANE DITERPENES FROM *BRIAREUM ASBESTINUM*

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Abstract: Six new diterpenes, (**1-3** and **5-7**), were isolated from *Briareum asbestinum* collected off the coast of Tobago, West Indies. Their structures were determined by a combination of 2D NMR experiments.

Gorgonian octocorals of the genus *Briareum* (Order, Gorgonacea) have been the subject of several chemical investigations.[1-11] *Briareum asbestinum* (Pallas) is the most widely investigated of these organisms, and some collections have afforded briarane diterpenes,[1,2] while others have yielded asbestinanes.[7-11] We have investigated extracts of *B. asbestinum* collected off the coast of Tobago, West Indies, and report here the isolation and characterisation of six new diterpenes. Five of these belong to the briarane class, while one is an asbestinane.

Compound (**1**) is the first briarane diterpene containing a C-19 methyl ester. Its structure was solved by single-crystal X-ray structure analysis; a preliminary report on this compound was recently published.[13] Compound (**2**) was isolated as colourless crystals and had the molecular formula, $C_{27}H_{40}O_8$, on the basis of high resolution mass spectrometry. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) were similar to those of (**1**) and showed that (**2**) had one butyrate less. The COSY, HETCOR, and FLOCK spectra of (**2**) revealed that C-12 [δ_H 3.86 (bs)] had a free hydroxyl group and that the butyrate and acetate groups were at C-2 [δ_H 4.92 (dd, J = 10.1, 1.5 Hz)] and C-14 [δ_H 5.00 (dd, J = 3.0, 3.0 Hz)], respectively. In the FLOCK spectrum, the oxymethine carbon at δ 70.6

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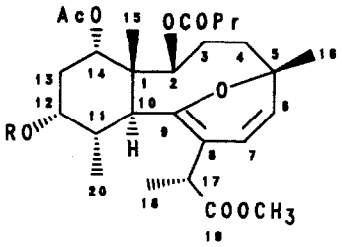
Table 1: ^{1}H NMR Assignments for Compounds (1) - (6).

Position	(1)[a,b]	(2)[b]	(3)[c]	(4)[c]	(5)[c]	(6)[b]
2	4.94	4.92	4.78	4.96	5.37	4.94
3	2.06	2.07	2.07	2.07	2.39	2.01
	1.39	1.39	1.39	1.33	2.22	1.78
4	2.20	2.21	2.23	2.30	5.02	3.88
	1.32	1.33	1.32	1.33	- -	1.95
6	5.58	5.58	5.59	5.56	5.16	5.16
7	5.93	5.94	5.95	5.91	- -	- -
9	- -	- -	- -	- -	2.90	3.22
	- -	- -	- -	- -	2.32	2.49
10	3.64	3.51	3.58	4.39	3.37	3.86
11	2.21	2.08	- -	- -	2.33	- -
12	5.11	3.86	3.02	4.31	- -	2.90
13	2.08	2.08	2.26	2.08	5.98	2.35
	2.08	2.08	2.07	2.08	- -	2.15
14	4.89	5.00	4.92	4.99	6.52	4.67
15	1.37	1.37	1.33	1.23	1.06	0.91
16	1.33	1.34	1.36	1.36	1.80	1.73
17	3.71	3.74	3.69	3.46	- -	- -
18	1.32	1.32	1.38	1.29	1.88	1.84
20	0.83	0.96	1.33	5.12	1.14	1.30
	- -	- -	- -	4.93	- -	- -
C-2 ester	2.15	2.17	2.17	2.19	2.08	2.06
	1.55	1.56	1.57	1.58	- -	- -
	0.91	0.91	0.92	0.93	- -	- -
C-14 ester	1.92	2.00	1.92	2.03	- -	2.01
OCH_3	3.83	3.81	3.80	3.79	- -	- -

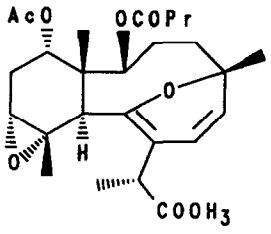
[a]δ_H for C-12 PrCO: 2.26 (H_2-2), 1.66 (H_2-3), 0.95 (H_3-4).

[b]Taken at 400 MHz with assignments based on HETCOR and FLOCK spectra.

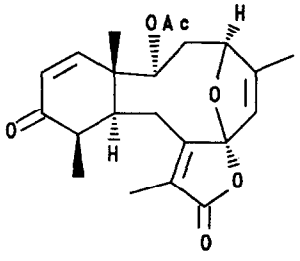
[c]Taken at 500 MHz with assignments based on HMQC and HMBC spectra.



(1) R = PrCO
(2) R = H
(4) R = H, $\Delta^{11(20)}$



(3)



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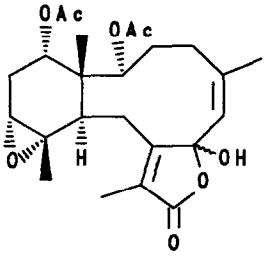


(6)

(C-12) showed three-bond connectivity to the C-20 methyl at δ 0.96. The relative stereochemistry of (2) was determined by comparison with (1) and from a series of nOe difference spectra. Compound (2) is therefore the 12-desbutoxy-12-hydroxy derivative of methyl briareolate (1).

Compound (3) was isolated as an amorphous powder and had similar ^1H and ^{13}C NMR spectra to (2) with resonances due to butyroxy, acetoxy, and epoxide groups. These were assigned to C-2 [δ_H 4.78 (bd, J = 9.4 Hz)], C-14 [δ_H 4.92 (bd, J = 6.6 Hz)], and C-12 [δ_H 3.02 (bd, J = 4.3 Hz)], respectively, on the basis of DQF-COSY, HMQC, and HMBC experiments (Tables 1 and 2). The presence of the epoxide was confirmed by the observation of ^{13}C resonances at δ 58.5 (C-11) and δ 57.0 (C-12). However, while the sample was still under NMR investigation, it underwent a complete transformation to compound (4)- presumably by the traces of HCl normally present in $CDCl_3$- precluding the complete characterisation of this compound. The methyl at δ 1.33 in (3) was replaced by methylene protons at δ 5.12 and 4.93 in (4), while the oxymethine proton was shifted downfield to δ 4.31 (dd, J = 3.9, 3.7 Hz). The complete assignment of the ^1H and ^{13}C NMR spectra (Tables 1 and 2) were achieved by COSY, HMQC, HMBC, and nOe difference experiments. When the oxymethine proton at δ 4.31 (H-12) was irradiated, enhancements were observed for the

Table 2: ^{13}C NMR Assignments for Compounds (1) - (6).[a]

C#	(1)[b]	(2)	(3)	(4)	(5)	(6)
1	45.7	45.9	44.4	46.9	45.4	43.2
2	78.0	78.0	77.4	78.0	79.8	79.0
3	28.7	28.7	28.2	28.5	31.2	30.1
4	34.9	34.6	34.6	33.9	86.6	27.3
5	75.1	75.1	75.1	75.1	147.4	144.3
6	129.8	129.8	130.2	130.0	119.9	123.7
7	120.2	120.4	120.1	120.8	118.2	107.4
8	117.3	117.3	118.5	117.0	158.0	160.4
9	151.0	151.4	149.4	150.5	26.4	25.2
10	37.4	36.4	43.2	40.6	42.9	35.9
11	31.8	33.5	58.5	146.4	44.6	59.4
12	72.1	70.6	57.0	71.7	200.0	59.1
13	31.2	33.6	28.6	33.6	129.0	26.8
14	74.0	75.4	72.3	74.9	155.5	73.5
15	15.1	14.9	14.0	14.7	17.2	16.4
16	23.8	23.6	23.7	23.8	12.9	23.4
17	37.7	37.6	37.5	37.7	124.6	125.0
18	14.5	14.3	14.7	14.7	9.9	9.7
19	174.3	174.6	174.3	174.8	171.0	171.8
20	15.2	15.3	20.8	113.3	12.3	23.2
C-2 ester	172.3	172.6	172.6	172.6	169.9	169.6
	36.5	36.4	36.4	36.3	20.8	21.4
	18.4	18.2	18.4	18.2	- -	- -
	13.9	13.7	13.7	13.7	- -	- -
C-14 Ac	169.8	170.0	170.4	170.2	- -	170.6
	21.1	21.2	20.4	21.3	- -	21.2
OCH_3	52.0	51.9	51.8	52.0		

[a]Taken at 100 MHz. [b] δ_C for C-12 PrCO: 172.3 (C-1), 36.8 (C-2), 18.6 (C-3), 14.0 (C-3).

exomethylene proton at δ 5.12 and another proton at δ 2.08 (H-13). On the other hand, irradiation of the C-14 proton at δ 4.99, caused enhancement of the angular methyl resonance at δ 1.23, suggesting that they were both ß-oriented.

Compound (5), $C_{22}H_{26}O_6$, was isolated as an amorphous powder. The ir spectrum had absorptions due to α,ß-unsaturated-γ-lactone (1753 cm^{-1}), ester (1733 cm^{-1}), and conjugated ketone (1681 cm^{-1}). The ^1H and ^{13}C NMR spectra (Tables 1 and 2) revealed that compound (5) was a briarane diterpene which possessed an α,ß unsaturated ketone, disubstituted, trisubstituted and tetrasubstituted double bonds, and one acetate. In addition, a quaternary carbon at δ 118.2 was assigned to a spiroketal-lactone that was also part of a dihydrofuran system. This carbon showed long-range correlations with the olefinic proton at δ 5.16 (C-6) as well as the C-9 methylene protons at δ 2.90 and δ 2.32, on the basis of an HMBC experiment. The HMBC spectrum in combination with an HMQC experiment, also revealed that an oxymethine proton at δ 5.02 (C-4) was part of the dihydrofuran system, and that the acetate was at C-2. Irradiation of the C-15 methyl at δ 1.06 resulted in nOe enhancements of H-2 (δ 5.37) and H-3ß (δ 2.39), indicating that they all had a ß-orientation, while irradiation of the C-20 methyl at δ 1.14 caused enhancement of resonances at δ 1.88 (H-18), and 2.33 (H-11) showing that it was ß-oriented. The C-4 proton at δ 5.02 was determined to have an α-orientation since a small enhancement was observed for H-3α when this proton was irradiated. The spiroketal-lactone-dihydrofuran system is unprecedented among the briarane diterpenes.

Compound (6), $C_{24}H_{32}O_8$, was isolated as a colourless gum and had ir absorbances at 3332 (OH), 1745 (α,ß-unsaturated-γ-lactone), and 1731 (ester) cm^{-1}. The ^1H NMR spectrum had resonances due to two acetates (δ 2.06 and 2.01), two olefinic methyls (δ 1.84 and 1.73), and two quaternary methyls (δ 1.30 and 0.91). The ^{13}C NMR spectrum revealed the presence of tetrasubstituted (δ 125.0 and 160.4) and trisubstituted (δ 123.7 and 144.3) double bonds, along with an epoxide (δ 59.1 and 59.4) and a hemiketal carbon (δ 107.4). A HETCOR spectrum indicated that the epoxide carbon at δ 59.1 was directly attached to a proton at δ 2.90, while a FLOCK spectra showed that both epoxide carbons had long-range correlations with the methyl singlet at δ 1.30 (20-H_3). Irradiation of the C-20 methyl resulted in a 7% nOe enhancement of H-12 (δ 2.90), showing that they were on the same face. The position of C-11 (δ 59.4) and C-12 (δ 59.1) indicate that the epoxide ring as α. In briaranes containing a ß 11,12-epoxide, these carbons resonate at significantly higher field.[2]

Irradiation of the C-15 methyl at δ 0.91 gave enhancement of H-14 (4%) and H-2 (2.5%).

Table 3: NMR Data for Compound (7).*

C#	δ_C	δ_H	C#	δ_C	δ_H
1	37.5	2.50	12	31.6	2.07
2	92.3	3.79	13	31.4	1.44, 1.02
3	77.8	- -	14	38.5	1.90
4	34.4	1.84, 1.42	15	36.7	1.61
5	78.9	4.51	16	68.0	3.82, 3.46
6	30.2	1.84, 1.50	17	11.1	0.90
7	76.4	- -	18	22.3	1.29
8	47.4	1.78, 1.67	19	22.4	1.12
9	74.0	4.52	20	17.4	0.89
10	48.8	1.76	21	171.4	- -
11	73.2	5.33	22	21.6	2.08

*δ_C at 100 MHz, δ_H at 400 MHz.

There was no nOe between H-2 and H-10. In addition, H-2 was a double doublet of small vicinal coupling (J = 4.2, 2.1 Hz). This is consistent with an α-orientation of both the C-2 and C-14 -OAc substituents and a pseudoequatorial disposition of H-2. Compounds (5) and (6) are related to briareolides A-I, recently isolated from a *Briareum* sp.,[2] while (6) is only the second briarane diterpene possessing the hemiketal lactone group.[14]

Compound (7) was isolated as a colourless gum and had the molecular formula $C_{22}H_{34}O_5$ (HREIMS: 378.2395). The ^1H NMR spectrum had signals due to two secondary methyl groups (δ 0.89, δ 0.90) and two quaternary methyls at lower field (δ 1.12, δ 1.29), and one acetate (δ 2.08). The ^{13}C NMR spectrum indicated the presence of seven carbons bearing single-bonded oxygen. Extensive analysis of a series of 2D NMR spectra (Table 3) indicated that (7) was an asbestinane diterpene containing one acetate at C-11 and an oxetane involving C-5 and C-7. The latter was clearly established by the presence of C-4, C-6 and C-8 as methylene carbons.

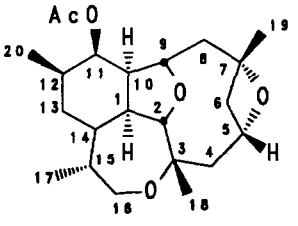
The relative stereochemistry involving the cyclohexane, oxepane and tetrahydrofuran rings was based on the similarity of the J-values of the protons H-1, H-2, H-10, H-11, H-13α, H-13ß, H-14 and H-15 with those of 11-acetoxy-4-deoxyasbestinin B[9] (Table 5).

The stereochemical relationships at C-3, C-5 and C-7 was based on nOe data. Thus, irradiation of the C-19 methyl (δ 1.12) caused an nOe enhancement of the oxetane proton at δ 4.51

Table 4: ^{1}H-^{1}H COSY Correlations for Compound (7).[a]

Proton	COSY	Proton	COSY
1	H-2,H-10,H14	9	H-8_α,H-8_β,H-10
2	H-1	10	H-1,H-9,H-11
4α	H-5	11	H-10,H-12
4ß	H-5	12	H-11,H-13,H-20
5	H-4_α,H-4_β,H-6_α,H-6_β	13α	H-12,H-14
6α	H-5	13ß	H-12,H-14
6ß	H-5	14	H-1,H-13_α,H-13_β,H-15
8α	H-9	15	H-14
8ß	H-9		

[a]Taken at 400 MHz.



(7)

Table 5: Comparison of ^{1}H-NMR Data of Compound (7) and 4-Deoxyasbestinin B.

Proton	Compound (7)	4-Deoxyasbestinin B[a]
1	2.50 (ddd, 9.0,9.0,9.0)	2.17 (ddd, 10.7,10.7,8.5)
2	3.79 (d, 9.0)	3.99 (d, 8.5)
10	1.76 (m)	2.05 (m)
11	5.33 (d, 5.0)	5.31 (dd, 5.2,2.7)
13α	1.44 (m)	1.47 (ddd, 13.7,13.7,9.7)
13ß	1.02 (dd, 13.5,3.0)	0.99 (ddd, 13.4,3.0,1.5)
14	1.90 (m)	1.87 (dddd)
15	1.61 (m)	1.64 (m)

[a]Taken from reference (9).

(7%), while irradiation of the C-18 methyl caused a weaker (2.5%) enhancement of this same proton. Compound (7) belongs to a small but growing number of 4-deoxyasbestinin diterpenes that have recently been isolated from Puerto Rican collections of *B. asbestinum*.[9-11]

EXPERIMENTAL

All samples of *Briareum asbestinum* were collected off the South-West coast of Tobago and identified by Mr Richard S. Laydoo, of the Institute of Marine Affairs , Trinidad and Tobago, where voucher specimens have been deposited.

Melting points were determined on a micro hot stage. IR spectra were obtained on a Nicolet 5DX FTIR spectrometer. UV spectra were recorded on a Cary 14UV instrument, while NMR spectra were recorded on a Varian XL-400 or Varian UNITY 500 spectrometer in $CDCl_3$ solutions with TMS as an internal standard. A VG 70-250S mass spectrometer (70 ev) was used to obtain electron impact spectra. In the FAB mode this same instrument was used to obtain FAB spectra in an ethylene glycol matrix.

General Extraction Procedure:

Four samples of *B. asbestinum* were collected at varying times in different locations off South-West Tobago, yielding the metabolites as indicated. The ground organism was extracted exhaustively with acetone and the solvent concentrated to yield an aqueous suspension. Partitioning with EtOAc afforded a crude EtOAc soluble fraction which was suspended in a H_2O/MeOH mixture and washed with petroleum ether (60-80°), and then with EtOAc to yield an EtOAc soluble fraction. This fraction was then subjected to extensive chromatography using vacuum liquid chromatography (VLC) and preparative TLC on silica gel to yield the various compounds. Percentage yields are based on dry weight of ground organism after extraction.

Methyl briareolate (1): A sample of *Briareum asbestinum* collected at Grouper Ground, Milford Bay, in July 1988 (-12 metres) yielded methyl briareolate (1) after final preparative TLC using petroleum ether/CH_2Cl_2/acetone (5:1:1 x2) as eluent - 17 mg (0.004%); colourless crystals, mp 165-167 °C; $[\alpha]_D$ +130.9° (*c* 0.11, $CHCl_3$); IR($CHCl_3$) 1730 cm^{-1}; UV(MeOH) 210 nm (ϵ 2700), 283 nm (ϵ 3700); EIMS 562 (M^+, 7%), 503(6), 474(100), 431(14), 403(32), 386(10), 287(27), 207(29), *Exact mass*: 562.3112, calcd. for $C_{31}H_{46}O_9$ 562.3138.

12-desbutoxy-12-hydroxy methyl briareolate (2): Compound (2) (along with compound (1)) was obtained from a sample collected in September 1990 (-15 metres) after final preparative TLC using $CHCl_3$/EtOAc (4:1) as eluent - 20.7 mg (0.02%); colourless crystals, mp 173-176 °C; $[\alpha]_D$ +154.7° (*c* 0.21, $CHCl_3$); IR ($CHCl_3$) 3430, 1731, 1640 cm^{-1}; UV(MeOH) 222 nm (ϵ 3200), 288 nm (ϵ 3800); EIMS 492 (M^+, 8%), 433(6), 404(100), 361(19), 345(18), 287(47), 221(37), 207(51), *Exact mass*: 492.2696 calcd. for $C_{27}H_{40}O_8$ 492.2723.

Compounds (3) and (5): Compounds (3) and (5) were the only metabolites isolated from a sample collected in September 1990 from Bucco Reef (-15 metres). Vacuum liquid chromatography gave two major fractions. The less polar fraction was separated by preparative TLC using petroleum ether/acetone (6:1 x2) to give compound (3) as a white amorphous powder (0.03%). On standing in the NMR tube in $CDCl_3$ this was transformed into compound (4) - a colourless gum ; IR ($CHCl_3$) 3499, 1728 cm^{-1}; EIMS 490 (M^+,43%), 431(27), 419(27), 402(100), 359(53), 343(23), 207(44), 194(53), *Exact mass*: 490.2545 calcd. for $C_{27}H_{38}O_8$ 490.2567.

Compound (5): Compound (5) was obtained from the more polar fraction after preparative TLC using CH_2Cl_2/acetone (40:1 x3) as an amorphous powder (10.4 mg, 0.03%); IR ($CHCl_3$) 1753, 1733, 1681 cm^{-1}; UV(MeOH) 235 nm (ϵ 5000); EIMS 386 (M^+, 14%), 344 (12), 326(68), 300(34), 222(85), 204(45), 178(64), 149(100), *Exact mass*: 386.1713 calcd. for $C_{22}H_{26}O_6$ 386.1729.

Compounds (6) and (7): A sample of *B. asbestinum* collected in March 1990 at Flying Reef (-10 metres) yielded compounds (6) and (7) as the only diterpenes. Compound (6) was isolated fron the less polar fraction by preparative TLC using petroleum ether/CH_2Cl_2/acetone (3:2:1 x2) as a white amorphous powder (15.2 mg, 0.002%); $[\alpha]_D$ -31.4° (*c* 0.54, $CHCl_3$); IR ($CHCl_3$) 3332, 1745, 1731 cm^{-1}; UV (MeOH) 234 nm (ϵ 5700); CIMS (Isobutane) 449 ($M+H^+$, 8%), 431(78), 389(45), 371(25), 329(50), 311(67), 61(100), *Exact mass*: 449.2181 calcd. for $C_{24}H_{33}O_8$ 449.2175.

Compound (7) was isolated from the more polar fraction by preparative TLC using CH_2Cl_2/MeOH (10:1) as a colourless gum (0.002%); $[\alpha]_D$ -5.8° (*c* 0.1, $CHCl_3$); IR($CHCl_3$) 1731 cm^{-1}; EIMS 378 (M^+, 100%), 339(32), 318(40), 281(76), 241(50), 221(71), 174(72), 105(90), *Exact mass*: 378.2395 calcd. for $C_{22}H_{34}O_5$ 378.2406.

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