Tetrahedron Vol. 49, No. 2, pp. 319-328, 1993
Printed in Great Britain

0040-4020/93 $5.00+.00

STRUCTURES AND BIOACTIVITIES OF NEW ASBESTININ DITERPENOIDS FROM THE CARIBBEAN GORGONIAN OCTOCORAL *BRIAREUM ASBESTINUM*[1]

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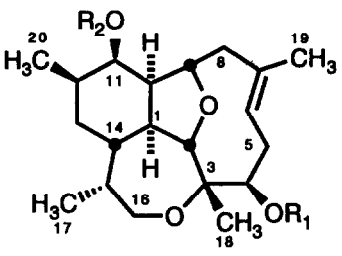
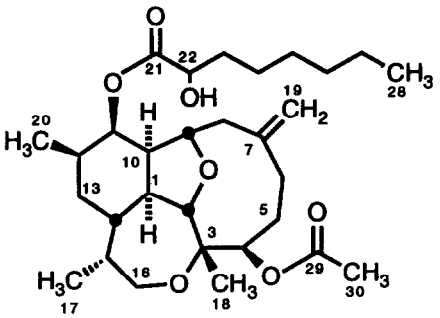
(*Received in USA* 17 *July* 1992; *accepted* 14 *October* 1992)

Abstract: Five new diterpenoids representatives from the rare skeletal class asbestinins have been isolated from the Caribbean gorgonian octocoral *Briareum asbestinum*. The structures of these secondary metabolites, named asbestinin-6 (**1**), asbestinin-7 (**4**), asbestinin-8 (**5**), asbestinin-9 (**10**) and asbestinin-10 (**11**), were defined by chemical and spectroscopic methods. The specimens of *B. asbestinum* collected in Puerto Rico yielded exclusively diterpenoids possessing the asbestinane carbon skeleton thus suggesting lack of biosynthetic versatility for this gorgonian. Diterpenoids **1**, **4**, **5**, **10** and **11** showed significant cytotoxicity when screened against a panel of five human tumor cell lines.

Gorgonian octocorals (Phylum Cnidaria, Order Gorgonacea) and soft corals (Alcyonacea, Phylum Cnidaria) are recognized as rich sources of biologically active and structurally interesting terpenoids.[2] In the course of our investigations on biologically active substances from Puerto Rican marine animals, we isolated from the Caribbean gorgonian octocoral *Briareum asbestinum* (Pallas) five new diterpenoids possessing the uncommon asbestinane carbon skeleton. Diterpene metabolites of this type are very rare and continue to intrigue natural products chemists because of the structural novelty and complexity and the interesting biological activity associated with these compounds. While more than 400 diterpenoids having the cembrane skeleton have been isolated from gorgonians (marine sea whips and sea fans) and soft corals, thus far only eleven asbestinin diterpenes have been isolated from these marine organisms.[3-5] The specimens of *B. asbestinum* collected from two distinct locations near Puerto Rico were frozen immediately after collection and subsequently freeze-dried. Conventional extraction procedures were used and the extracts were fractionated extensively using size exclusion chromatography and normal- and reversed-phase adsorbents to give the five new diterpenoids (see Experimental). Although a large number of diterpenes that have the briarein skeleton have been isolated from several gorgonians belonging to the *Briareum* species,[6] the specimens of *B. asbestinum* collected near Puerto Rico yielded exclusively diterpenes possessing the asbestinane carbon skeleton. Compounds **1**, **4**, and **5** were distinctively major diterpene metabolites (0.131% dry wt) and were isolated from specimens of *B. asbestinum* collected off the West coast of Puerto Rico (Mona Island) while compounds **10** and **11** were minor components (0.031% dry wt) isolated from specimens collected off the East coast (Palomino Island).



1 R_1 = $COCH_3$ R_2 = COC_7H_{15}
2 R_1 = $COCH_3$ R_2 = COC_3H_7
3 R_1 = H R_2 = H



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The new compounds reported here exhibited in vitro antitumor activity against the human colon (HCT 116), human breast (MCF-7), melanoma (SK5-MEL), T cell leukemia (CCRF-CEM) and kidney carcinoma (A 498) cell lines with IC_{50} values of 0.15-20 μg/mL. The complete structural assignments of diterpenes **1**, **4**, **5**, **10**, and **11** have been accomplished by chemical methods and from in-depth NMR spectral analyses in particular ^1H-^1H and ^1H-^{13}C chemical shift correlation NMR spectroscopy.

A molecular formula of $C_{30}H_{48}O_6$ was established for asbestinin-6 (**1**) from HREIMS (504.34430, calcd 504.34506), plus ^1H-NMR and ^{13}C-NMR data (see Tables 1 and 2). The ^1H-NMR spectrum contained signals for an acetate at δ 2.08, and four separate signals comprising the six methylenes and the methyl group of a caprylate ester. Ions corresponding to sequential losses of acetic acid and caprylic acid from the molecular ion were evident from the mass spectrum of **1**. Subtraction of the 10 carbons associated with the ester groups left 20 carbons, suggestive of a diterpene skeleton (5 unsaturations). The IR spectrum contained two ester bands at 1737 and 1731 cm^{-1} and indicated the absence of hydroxyl groups. Since the ^{13}C-NMR spectrum did not contain a signal for a tetrasubstituted carbon atom bearing carbon substituents, as found at the carbocyclic ring junction in the briarein series, nor did the mass spectrum indicate the presence of halogens, diterpene **1** was assigned a structure based on the asbestinane carbon skeleton. Chemical shifts of the protonated carbons were assigned by a 2D ^1H-^{13}C heteronuclear correlation experiment (HETCOR, J = 140-160 Hz) and on the basis of ^{13}C-APT, Selective Pulse INEPT NMR experiments,[7] conventional COSY spectra and on the basis of ^{13}C chemical shift arguments. From these combined data it became evident that compound **1** differed from asbestinin-1 (**2**), a known compound isolated from specimens of *B. asbestinum* collected in Belize[3] and Honduras,[4] only in the fatty acid with which the C-11 alcohol is esterified, caprylic versus butyric. To confirm this, we cleaved the acetate and caprylate side chains from **1** with $LiAlH_4$/ether at 25°C to afford the known crystalline diol **3** by comparison to authentic material with regard to melting point, ^1H- and ^{13}C-NMR, IR and MS spectra.[3,4] As was the case for asbestinin-1 (**2**), the three overlapping signals at δ 5.30 (H-4, H-6, and H-11) in asbestinin-6 (**1**)

could be resolved in C_6D_6 solution.[4] This allowed us now to establish unambiguously the locus of the caprylate versus acetate groups directly from ^1H-^1H COSY experiments and by comparison of the ^1H- and ^{13}C-NMR spectral data of **1** and **2** in C_6D_6 solution.

Although the ^{13}C-NMR spectrum of the less abundant metabolite asbestinin-7 (**4**) showed only 28 resonances (the ^{13}C-NMR signals at δ 38.30 and 34.67 are believed to be overlapped with another signal), the analysis of the spectral data established a molecular formula of $C_{30}H_{48}O_7$. Compound **4** possessed a mass spectral molecular ion which was only 16 amu's larger than that of asbestinin-6 (**1**). The ^1H-NMR and ^{13}C-NMR spectra indicated the presence of a α-hydroxy caprylate ester, an acetate ester and an exocyclic methylene group. The methylene proton signals at δ 5.31 (br s, 1H) and 5.14 (br s, 1H) combined with the absence of a 3H singlet near δ 1.90, indicated that the double bond in **4** had shifted to an exocyclic position. The infrared spectrum contained a broad hydroxyl band at 3330 cm^{-1} and two ester bands at 1737 and 1731 cm^{-1}. Ions corresponding to sequential losses of an acetic acid and a α-hydroxy caprylic acid moiety from the molecular ion were also evident. That the caprylate ester group had undergone α-hydroxylation in **4** was evident from the appearance of a new downfield signal at δ 87.01 (C-22) in the ^{13}C-NMR spectrum and a broad doublet at δ 4.53 (H-22) in the ^1H-NMR spectrum (see Tables 1 and 2). A ^1H-^1H COSY experiment showed that H-22 was not directly connected to any of the protons in the asbestinane skeleton. From the spectroscopic data accumulated, it appears evident that asbestinin-6 (**1**) and asbestinin-7 (**4**) differ only by a change from methylolefin to exomethylene, a rather common occurrence among natural products, and by α-hydroxylation of the caprylate side chain. The relative positions of the acetate and α-hydroxy caprylate groups in asbestinin-7 (**4**) were determined unambiguously through ^1H-^1H COSY and INEPT experiments. For instance, because in $CDCl_3$ solution H-4 and H-11 do not overlap in **4**, the ^{13}C-NMR signal assignments of carbons C-4, C-11, C-21, and C-29 were made from single frequency on-resonance decoupling experiments by irradiating the H-4 and H-9 signals individually. Thus, selective irradiation of the signal at δ 5.37 (H-11) caused enhancement of the ^{13}C-NMR signal at δ 171.21 (C-21) while irradiation of the signal at δ 4.15 (H-9) caused a similar enhancement of the ^{13}C-NMR signal at δ 73.52 (C-11). The relative stereochemistry for all the substituents on the complex tetracyclic array was determined to be identical to those of asbestinin-6 (**1**) and asbestinin-1 (**2**) by analysis of proton-proton coupling constants (see Table 1), NOE experiments and ^{13}C-NMR chemical shift comparisons.[4,5] Moreover, the relative stereochemistry of asbestinin-7 (**4**) was in line also with the identical stereochemistry of these groups in the 4-deoxyasbestinin series **6-9**.[5,8] Thus, the complete structure of asbestinin-7 with all relative stereochemistry (excepting that of C-22) is described by formula **4**. The relative stereochemistry of the hydroxyl group at position C-22 of the caprylate side chain remains as yet unassigned.

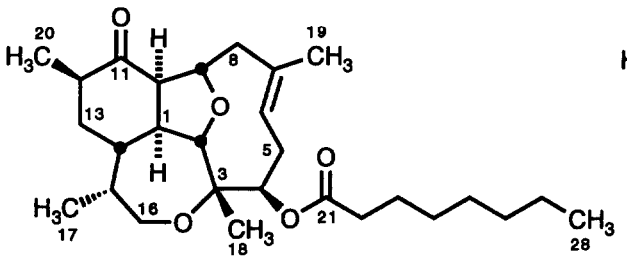
Table 1. ^{1}H-NMR (300-MHz) Spectral Data of the Asbestinins 6-10 in $CDCl_3$.

Assignments were aided by ^{1}H-^{1}H COSY, spin splitting patterns, selective decoupling experiments and comparison of J values. The δ values are in ppm and are referenced to the residual $CHCl_3$ signal (7.26 ppm).

H	Asbestinin-6	Asbestinin-7	Asbestinin-8	Asbestinin-9	Asbestinin-10
	δ, mult, J (Hz), intgrtn	δ, mult, J (Hz), intgrtn	δ, mult, J (Hz), intgrtn	δ, mult, J (Hz), intgrtn	δ, mult, J (Hz), intgrtn
H_1	2.23, m, 1H	2.71, m, 1H	2.88, m, 1H	2.39, m, 1H	2.40, m, 1H
H_2	4.00, d, 8 0, 1H	3.85, d, 9.3, 1H	3.93, d, 8.1, 1H	3.73, d, 9.3, 1H	3.74, d, 9.3, 1H
H_4	5.30, m, 1H	5.52, br t, 1H	5.34, m, 1H	-	-
H_5 (α)	2.69, m, 1H	2.49, m, 1H	2.68, m, 1H	2.79, dd, 15.0, 7.7, 1H	2.78, dd, 15.0, 8.1, 1H
(β)	2.07, m, 1H	2.34, m, 1H	2.09, m, 1H	2.45, m, 1H	2.46, m, 1H
H_6 (α)	5.30, m, 1H	1.55, m, 1H	5.34, m, 1H	2.24, m, 1H	2.27, m, 1H
H_6 (β)	-	1.55, m, 1H	-	1.69, m, 1H	1.75, m, 1H
H_8 (α)	1.99, d, 7.0, 1H	2.25, m, 1H	1.91, d, 7.1, 1H	2.11, d, 13.2, 1H	2.13, d, 13.2, 1H
(β)	2.44, dd, 13.5, 6.5, 1H	2.36, m, 1H	2.55, m, 1H	3.29, dd, 13.2, 6.5, 1H	3.33, dd, 13.1, 6.9, 1H
H_9	4.05, m, 1H	4.15, br t, 5.7, 1H	5.04, dd, 6.6, 1.5, 1H	3.98, m, 1H	4.01, dd, 6.5, 4.1, 1H
H_{10}	2.06, m, 1H	2.10, m, 1H	2.78, m, 1H	2.02, m, 1H	2.08, m, 1H
H_{11}	5.30, m, 1H	5.37, dd, 5 4, 2.1, 1H	-	5.26, m, 1H	5.25, m, 1H
H_{12}	1.95, m, 1H	2 02, m, 1H	2.50, m, 1H	2.05, m, 1H	2.03, m, 1H
H_{13} (α)	1.43, m, 1H	1.50, m, 1H	2.20, m, 1H	1.49, m, 1H	1.50, m, 1H
(β)	0.98, br d, 13.2, 1H	1.00, br d, 13.5, 1H	1.38, br d, 12.0, 1H	1.02, m, 1H	1.03, br d, 13.7, 1H
H_{14}	1.89, m, 1H	1.82, m, 1H	1.27, m, 1H	1.83, m, 1H	1.85, m, 1H
H_{15}	1.68, m, 1H	1.59, m, 1H	1.73, m, 1H	1.58, m, 1H	1.60, m, 1H
H_{16} (α)	3.32, dd,13.5, 5.1, 1H	3.40, dd, 12.9, 2.4, 1H	3.35, dd, 13.5, 4.2, 1H	3.45, dd, 12.9, 2.6, 1H	3.46, dd, 13.0, 2.9, 1H
(β)	3.80, dd, 13.5,1.8, 1H	3.66, d, 12.9, 1H	3.80, br d, 13.5, 1H	3.72, m, 1H	3.72, d, 13.0, 1H
Me_{17}	0.73, d, 6 9, 3H	0.87, d, 7.1, 3H	0.87, d, 7.0, 3H	0.89, d, 7.3, 3H	0.90, d, 6.9, 3H
Me_{18}	1.32, s, 3H	1.27, s, 3H	1.29, s, 3H	1.24, s, 3H	1.25, s, 3H
H_{19} (α)	1.84, s, 3H	5.31, br s, 1H	1.89, s, 3H	5.23, br s, 1H	5.25, br s, 1H
H_{19} (β)	-	5.14, br s, 1H	-	5.14, br s, 1H	5.15, br s, 1H
Me_{20}	0.88, d, 7.3, 3H	0.91, d, 7.1, 3H	1.11, d, 7.0, 3H	0.90, d, 7.1, 3H	0.90, d, 6.9, 3H
H_{22}	2.29, t, 7.5, 2H	4.53, br d, 3.0, 1H	2.31, t, 7.5, 2H	2.28, t, 7.6, 2H	2.06, s, 3H
H_{23}	1.57, m, 2H	2.07, m, 2H	1.63, m, 2H	1.60, m, 2H	-
H_{24}	1.25, m, 2H	1.25, m, 2H	1.27, m, 2H	0.92, t, 7.3, 3H	-
H_{25}	1.25, m, 2H	1.25, m, 2H	1.27, m, 2H	-	-
H_{26}	1.25, m, 2H	1 25, m, 2H	1.27, m, 2H	-	-
H_{27}	1.25, m, 2H	1.25, m, 2H	1.27, m, 2H	-	-
H_{28}	0.85, t, 7.8, 3H	0.87, t, 8 1, 3H	0.87, t, 8 0, 3H	-	-
Me_{30}	2.08, s, 3H	2.07, s, 3H	-	-	-

Table 2. ^{13}C-NMR (75-MHz) Spectral Data of the Asbestinins 6-10 in $CDCl_3$.[a]					
	1	4	5	10	11
C	Asbestinin-6	Asbestinin-7	Asbestinin-8	Asbestinin-9	Asbestinin-10
	δ, (mult)	δ, (mult)	δ, (mult)	δ, (mult)	δ, (mult)
1	38.20 (d)	38.79 (d)	43.05 (d)	40.12 (d)	40.07 (d)
2	94.53 (d)	93.46 (d)	92.26 (d)	93.15 (d)	93.18 (d)
3	78.97 (s)	77.20 (s)	79.00 (s)	77.25 (s)	77.23 (s)
4	72.28 (d)	69.27 (d)	72.64 (d)	206.67 (s)	206.40 (s)
5	28.95 (t)	34.67 (t)	29.09 (t)	37.49 (t)	37.55 (t)
6	124.95 (d)	25.20 (t)	125.18 (d)	35.97 (t)	35.76 (t)
7	129.28 (s)	144.38 (s)	129.05 (s)	146.74 (s)	146.59 (s)
8	44.19 (t)	38.30 (t)	43.68 (t)	41.57 (t)	41.51 (t)
9	80.79 (d)	83.39 (d)	75.72 (d)	79.88 (d)	79.88 (d)
10	48.27 (d)	47.05 (d)	55.45 (d)	47.83 (d)	47.85 (d)
11	73.71 (d)	73.52 (d)	212.12 (s)	72.71 (d)	73.05 (d)
12	30.92 (d)	31.24 (d)	40.80 (d)	31.11 (d)	31.07 (d)
13	31.28 (t)	31.46 (t)	34.67 (t)	31.62 (t)	31.55 (t)
14	37.59 (d)	38.30 (d)	41.36 (d)	37.42 (d)	37.32 (d)
15	36.66 (d)	36.64 (d)	36.93 (d)	36.68 (d)	36.64 (d)
16	67.51 (t)	67.81 (t)	66.67 (t)	68.18 (t)	68.07 (t)
17	11.33 (q)	10.86 (q)	11.48 (q)	10.98 (q)	10.95 (q)
18	19.64 (q)	17.44 (q)	19.24 (q)	24.10 (q)	23.97 (q)
19	18.79 (q)	117.40 (t)	18.82 (q)	114.05 (t)	114.07 (t)
20	18.07 (q)	17.29 (q)	15.99 (q)	18.06 (q)	17.97 (q)
21	173.43 (s)	171.21 (s)	173.49 (s)	173.64 (s)	170.99 (s)
22	34.59 (t)	87.01 (d)	34.67 (t)	36.53 (t)	21.12 (q)
23	25.07 (t)	34.67 (t)	25.15 (t)	18.44 (t)	-
24	29.04 (t)[b]	29.03 (t)[b]	29.02 (t)[b]	13.72 (q)	-
25	28.93 (t)[b]	28.91 (t)[b]	28.99 (t)[b]	-	-
26	31.64 (t)	31.74 (t)	31.71 (t)	-	-
27	22.55 (t)	22.64 (t)	22.62 (t)	-	-
28	14.01 (q)	14.08 (q)	14.07 (q)	-	-
29	171.37 (s)	173.17 (s)	-	-	-
30	21.23 (q)	21.35 (q)	-	-	-

[a]Assignments were made on the basis of ^1H-^{13}C COSY, ^1H-^1H COSY and proton attachments via APT. The δ values are in ppm and are referenced to the solvent peak (77.0 ppm). [b]Values with identical superscripts in each column may be interchanged.



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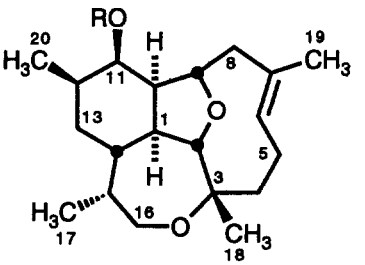
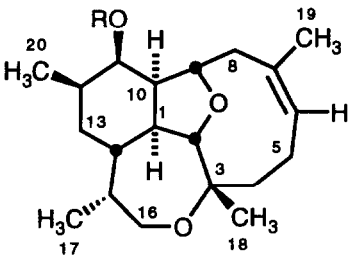


6 R = COC_3H_7
7 R = $COCH_3$

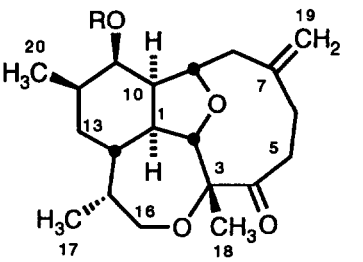
Asbestinin-8 (**5**) was isolated as a colorless oil and possessed a mass spectral molecular ion which was 44 amu's smaller than that of **1**.This difference is consistent with replacement of an acetoxy group with a ketone functionality; this was also suggested by a ^{13}C-NMR singlet at δ 212.12, the absence of a 3H singlet near δ 2.00 in the ^{1}H-NMR and the presence of a strong C=O stretching absorption at 1720 cm^{-1} in the IR spectrum. A caprylate ester moiety in asbestinin-8 was evident from the ^{1}H- and ^{13}C-NMR spectra and by the presence of a major ion at *m/z* 316.20372 for $C_{20}H_{28}O_3$ which reflects a fragmentation of M^+-$C_8H_{16}O_2$ (caprylic acid) from the molecular composition of $C_{28}H_{44}O_5$. That the ketone functionality in **5** must be placed at C-11 stems from the overall downfield shift experienced by most of the protons in the cyclohexane ring and by H-9 in the tetrahydrofuran ring moiety. Examination of molecular models clearly indicates that if we place the C=O group at C-11, the ensuing cyclohexanone ring adopts a boat conformation causing H-9 and Me-20 to lie within the deshielding zone of the carbonyl group. These protons should therefore experience a significant downfield shift. Indeed, H-9 and Me-20 appear now at δ 5.04 (vs. 4.05 in **1**) and 1.11 (vs. 0.88 in **1**), respectively. During an INEPT experiment, irradiation of the signal at δ 5.34 (H-4) caused enhancement of the ^{13}C-NMR signals at δ 173.49 (C-21), 29.09 (C-5) and 79.00 (C-3). Therefore, the caprylate ester group must be located now on the 10-membered carbocyclic ring at C-4. The relative stereochemistry of the substituents around the asbestinane carbon skeleton and the *E* geometry of the Δ^6 trisubstituted double bond were established spectroscopically as before[5] and by chemical correlation to known asbestinin diol **3** upon reduction with $LiAlH_4$/ether at 25°C. As predicted from the inspection of molecular models, the reduction of the cyclohexanone moiety in asbestinin-8 takes place from the less hindered side of the ring to produce the desired stereoisomer with very high stereoselectivity.

Along with known diterpenoids **6-9** (0.71% dry wt) which belong to the recently discovered 4-deoxyasbestinin series,[5] two previously undescribed minor metabolites, named asbestinin-9 (**10**) and asbestinin-10 (**11**), were isolated from a specimen of *B. asbestinum* collected off the East coast of Puerto Rico near Palomino Island. The new

asbestinin metabolites **10** and **11** have many characteristic signals found in the other asbestinins. The ^{1}H-NMR spectrum of the first of these, asbestinin-9 (**10**), contained signals for a butyrate ester group (δ 2.28, 2H, t, C-22; 1.60, 2H, m, C-23; 0.92, 3H, t, C-24) and an exocyclic methylene group (δ 5.23, br s, 1H; 5.14, br s, 1H). The presence of the butyrate moiety in asbestinin-9 was established also from the ^{13}C-NMR spectra and by the presence of a strong ion at *m/z* 316.20474 for $C_{20}H_{28}O_3$ due to loss of a $C_4H_8O_2$ fragment (butyric acid) from the actual molecular composition of $C_{24}H_{36}O_5$. No hydroxyl absorption was observed in the IR spectrum; instead a strong ketone stretching absorption at 1729 cm^{-1} and that of an ester band at 1736 cm^{-1} were observed. A ^{1}H-^{1}H COSY experiment placed the butyrate ester group at C-11 while the new ketone functionality (δ 206.67) was established at C-4 as suggested by the noticeable downfield shift experienced by the H-5αβ and H-6αβ protons. The placement of the keto group at C-4 leaves the proton sequence H-5 to H-6 as a separate spin system, a fact consistent with the observed ^{1}H-NMR data. The ^{1}H-NMR spectrum of asbestinin-10 (**11**) ($C_{22}H_{32}O_5$ by HRFABMS analysis) was quite similar to that of compound **10**. The signals due to H-5, H-6 and H-11 were almost identical to those of **10**, suggesting that these two compounds possess the same type of substituted asbestinane ring. The mass spectral molecular ion of **11** was 28 amu's smaller than that of **10**; this difference is consistent with replacement of a butyrate ester group in **10** with an acetoxy group. This was confirmed by the presence of an intense ion at *m/z* 317 for $C_{20}H_{29}O_3$ in the low resolution FAB mass spectrum due to loss of a $C_2H_4O_2$ fragment (acetic acid) from the molecular ion.



8 R = COC_3H_7
9 R = $COCH_3$



10 R = COC_3H_7
11 R = $COCH_3$

BIOLOGICAL ACTIVITY: The new asbestinin diterpenes reported here were not active against *Pseudomonas aeruginosa*, *Escherichia coli* or *Staphylococcus aureus* at doses of 10, 5, and 1μg of test compound per disc. However, all the asbestinins displayed significant antitumor activity. The cytotoxic activities of the new compounds against several human tumor cell lines were as follows: asbestinin-6 (**1**) [MCF-7 (IC_{50} = 1.5μg/mL); CCRF-CEM (IC_{50} = 0.5μg/mL); HCT 116 (IC_{50} = 5μg/mL)], asbestinin-7 (**4**) [MCF-7 (IC_{50} = 9μg/mL); CCRF-CEM (IC_{50} = 0.15μg/mL); HCT 116 (IC_{50} = 5μg/mL)],

asbestinin-8 (**5**) [MCF-7 (IC_{50} = > 50μg/mL); CCRF-CEM (IC_{50} = 2.5μg/mL); HCT 116 (IC_{50} = 10μg/mL)], asbestinin-9 (**10**) [SK5-MEL (IC_{50} = > 50μg/mL); A 498 (IC_{50} = > 50μg/mL); HCT 116 (IC_{50} = 20μg/mL)], asbestinin-10 (**11**) [SK5-MEL (IC_{50} = > 50μg/mL); A 498 (IC_{50} = 15μg/mL); HCT 116 (IC_{50} = > 50μg/mL)].

EXPERIMENTAL

General Experimental Procedures.- Infrared spectra were recorded on a Nicolet 600 FT-IR spectrophotometer. Proton and carbon-13 NMR spectra were recorded on a General Electric Multinuclear QE-300; proton-NMR chemical shifts are recorded with respect to the residual $CHCl_3$ signal (7.26 ppm). Carbon-13 chemical shifts are reported in ppm relative to $CDCl_3$ (77.0 ppm). Optical rotations were determined on a Perkin-Elmer Polarimeter Model 243B. Low resolution mass spectra were recorded on a Hewlett-Packard 5995A spectrometer. Column chromatography was performed on Analtech Si gel (35-75 mesh) and TLC analyses were carried out using Analtech glass packed precoated Si gel plates. All solvents used were either spectral grade or were distilled from glass prior to use.

Collection and Extraction. Minced and freeze-dried specimens of *B. asbestinum* (668.7g) collected at Mona Island were extracted exhaustively with $CHCl_3$-MeOH (1:1) (6 x 1L) and after filtration the crude extract was evaporated under vacuum to yield a residue (45.82g) that was partitioned between hexane and H_2O (3 x 500mL). The hexane extract was subsequently filtered, and the filtrate was concentrated *in vacuo* to yield 20.2g of a dark green oily residue. A portion of the residue (11.0g) was dissolved in a small volume of toluene and the resulting concentrate was fractionated by size exclusion chromatography on a Bio-Beads SX-2 column with toluene. The combined diterpene-containing fractions (TLC guided) were concentrated to obtain a yellow-orange oil (6.83g) that was chromatographed over a Si gel column (240g) with 20% EtOAc in hexane. The less polar portion of the lipids was fractionated roughly into fractions A through H on the basis of TLC analysis. Repeated column chromatography of fraction F (*ca.* 483mg) by HPLC [Ultrasphere-ODS Si gel with MeOH-H_2O (9:1)] gave pure asbestinin-6 (**1**) (*ca.* 400mg). Fraction E (75.8mg), which consisted by TLC analyses of a mixture of **1**, **4** and **5**, was dissolved in MeOH and purified through reversed-phase HPLC [Ultrasphere-ODS Si gel with MeOH-H_2O (93:7)] to give another *ca.* 30mg of pure **1** (combined weight *ca.* 430mg; 0.12% dry wt) and 15mg (0.004% dry wt) and 25mg (0.006% dry wt) of analytically pure asbestinin-7 (**4**) and asbestinin-8 (**5**), respectively. Specimens of *B. asbestinum* collected at Palomino Island (424g, dry weight) were extracted as described before.[5] Purification of a portion of the hexane solubles (7.1g) by successive size exclusion [Bio-Beads SX-2, toluene], adsorption [Si gel, 20% EtOAc in hexane] and reversed-phase HPLC chromatography [Ultrasphere-ODS Si

gel with MeOH-H_2O (7:3)] gave, after elution of the known 4-deoxyasbestinins **6-9** (0.735g; 0.34% dry wt),[5] analytically pure asbestinin-9 (**10**) (29mg; 0.013% dry wt) and asbestinin-10 (**11**) (40mg; 0.018% dry wt).

Asbestinin-6 (**1**)- Colorless oil: $[\alpha]_D^{25}$ -75.9° (c = 4.08, $CHCl_3$); IR (neat) 2960, 2930, 2873, 1737, 1731, 1454, 1367, 1232, 1176, 1106, 1066, 1018, 802 cm^{-1}; HREIMS *m/z* $[M]^+$ 504.34430 (1.5%) ($C_{30}H_{48}O_6$ requires 504.34506), 444 (1.4), 360 (10), 300 (10), 279 (8), 219 (26), 174 (30), 127 (17), 93 (26), 57 (100); ^1H- and ^{13}C-NMR in $CDCl_3$ (see Tables 1 and 2); ^1H-NMR (C_6D_6, 300-MHz) d 5.64 (1H, dd, J=6.3, 11.0 Hz, H-4), 5.49 (1H, br t, J=8.4 Hz, H-6), 5.28 (1H, dd, J=3.0, 4.8 Hz, H-11); ^{13}C-NMR (C_6D_6, 75-MHz) d 172.76(s), 170.63(s), 129.17(s), 125.67(d), 94.85(d), 81.05(d), 79.51(s), 73.64(d), 72.69(d), 67.57(t), 48.75(d), 44.49(t), 38.55(d), 38.45(d), 37.59(d), 34.95(t), 32.08(t), 31.57(t), 31.20(d), 29.86(t), 29.39(t), 29.36(t), 25.58(t), 22.96(t), 20.71(q), 19.83(q), 18.81(q), 18.34(q), 14.25(q), 11.68(q).

Asbestinin-7 (**4**)- Colorless oil: $[\alpha]_D^{25}$ +5.0° (c = 3.2, $CHCl_3$); IR (neat) 3330, 2959, 2930, 2875, 1737, 1731, 1461, 1374, 1260, 1236, 1173, 1097, 1018, 930, 804, 757 cm^{-1}; HREIMS *m/z* $[M]^+$ 520.33599 (6%) ($C_{30}H_{48}O_7$ requires 520.33997), 506 (6), 502 (4), 460 (4), 458 (5), 446 (2), 376 (4), 333 (3), 316 (7), 219 (25), 174 (27), 122 (46), 57 (100); ^1H- and-^{13}C-NMR (see Tables 1 and 2).

Asbestinin-8 (**5**)- Colorless oil: $[\alpha]_D^{25}$ -49.0° (c = 3.9, $CHCl_3$); IR (neat) 2959, 2928, 2871, 1731, 1720, 1455, 1373, 1260, 1170, 1102, 1066, 1015, 865, 800 cm^{-1}; HREIMS *m/z* $[M]^+$ 460.31745 (4%) ($C_{28}H_{44}O_5$ requires 460.31885), 316 (28), 235 (14), 191 (15), 127 (28), 81 (27), 57 (100); ^1H- and ^{13}C-NMR (see Tables 1 and 2).

Asbestinin-9 (**10**)- Colorless oil: $[\alpha]_D^{27}$ -78.0° (c = 2.0, $CHCl_3$); IR (neat) 2963, 2936, 2876, 1736, 1729, 1687, 1377, 1248, 1183, 1074, 1012, 754 cm^{-1}; HREIMS *m/z* $[M]^+$ 404.25391 (70%) ($C_{24}H_{36}O_5$ requires 404.25625), 316 (12), 258 (10), 124 (100), 71 (32); ^1H- and ^{13}C-NMR (see Tables 1 and 2).

Asbestinin-10 (**11**)- Colorless oil: $[\alpha]_D^{25}$ -81.5° (c = 0.76, $CHCl_3$); IR (neat) 2968, 2926, 2872, 1731, 1726, 1687, 1638, 1440, 1385, 1235, 1126, 918, 758 cm^{-1}; HRFABMS *m/z* $[M+1]^+$ 377.2328 (100%) ($C_{22}H_{33}O_5$ requires 377.2328), 317 (85); ^1H- and ^{13}C-NMR (see Tables 1 and 2).

Reduction of Asbestinin-6 (1) with Lithium Aluminum Hydride. A solution of asbestinin-6 (30mg, 0.06 mmol) in dry ether (5mL) was added to a suspension of $LiAlH_4$ (5mg) in dry ether (5mL), and the mixture was stirred for 30 min at 25°C. Excess reagent was destroyed by addition of EtOAc (1mL) followed by dropwise

addition of 0.1N HCl. The ether layer was separated and dried over Na_2SO_4, and the solvent was removed under vacuum to give an oily residue (20mg). Recrystallization from 1:1 ether/hexane gave white needles of the diol **3**: mp 154-156°C; ^1H- and ^{13}C-NMR, IR and MS spectra were identical with the authentic material.[3] The same general procedure was followed for the reduction of asbestinin-8 (**5**) (10mg, 0.02 mmol) with $LiAlH_4$/ether at 25°C to give **3**, identical in all respects with authentic material.

ACKNOWLEDGMENTS

The assistance of José J. Morales, Miriam Ortiz, Luis A. Ríos, Deborath Parrilla, Javier J. Soto and Dr. José E. García in specimen collection is gratefully acknowledged. The efforts of Marta Romero and Arlín Toro in obtaining valuable antimicrobial measurements and of Dr. Louis R. Barrows from the University of Utah Center for Drug Discovery for cytotoxicity assays on human tumor cells are also acknowledged. We extend our sincere appreciation to the crew of the R/V Isla Magueyes for their assistance during a trip to Mona Island and to Miss Mariliz Ortiz for the initial extraction of some specimens of *B. asbestinum*. HREIMS, HRFABMS and LRFABMS spectral determinations were performed by the Midwest Center for Mass Spectrometry, a National Science Foundation Regional Facility (Grant No. CHE8211164). This study was supported in part by the National Science Foundation EPSCoR Program (Grant No. R118610677) and the NSF-MRCE Program (Grant No. R11-8802961). O. M. Cóbar thanks the Agency for International Development (AID) for a pre-doctoral fellowship.

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