New Diterpenes from the Common Caribbean Gorgonian *Briareum asbestinum* (Pallus)

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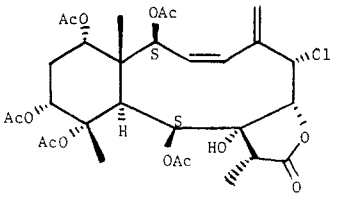
The major diterpenes in the toxic extracts of *Briareum asbestinum* (Pallus) from three Honduras Bay Island collections are reported. The structures and chemistry of two new asbestinins, **2** and **3**, and four known ones, **4**, **5**, **9**, and **10**, are discussed. Structures for **2** and **3** were derived from spectroscopic arguments and were confirmed by chemical interconversions. NMR experiments including ^{13}C at 90 MHz, a plot of J_R/J_{CH} with δ_H, and ^1H spin decouplings at 360 MHz enabled assignment of all 26 C's and 40 H's in **4**. These data served as a standard to completely assign the ^1H and ^{13}C NMR for natural products **2**, **3**, **5**, **9**, and **10** and for synthetic products **6** and **7**.

The possibility that marine natural products may protect exposed, sessile, coral reef invertebrates against grazing predators has been raised quite often recently.[1] Extracts from sponges and coelenterates in this group that show toxicity to fish quite often also contain structurally unusual compounds.[2] This, in part, has stimulated our systematic chemical study of abundant, toxic, soft-bodied invertebrates from the western Caribbean.

In April 1978 we collected the gorgonian *Briareum asbestinum* (Pallus) from Roatan Island, Honduras. Using the Bakus assay procedure,[1c] we found that a concentration of 320 μg/mL of a crude *Briareum* extract was highly toxic to goldfish (death in 10 min).

Briareum has been the subject of previous chemical study. Several years ago chlorine-containing diterpenes such as briarein A (**1**)[3,4] and the novel steroid gorgosterol[5] were observed from Jamaican collections. More recently, five compounds containing the new asbestinin diterpene skeleton were isolated from *B. asbestinum* collected from the Belize barrier reef.[6] Our strucutral work upon the Honduras biotoxic *Briareum* extracts has revealed two new asbestinins: asbestinin epoxide (**2**) and asbestinin-5 acetate **3**, which were accompanied by four other known[6] asbestinins **4**, **5**, **9**, and **10**. Structures and chemical and spectroscopic properties of the new asbestinins are described below *along with previously unreported spectroscopic details for the other known ones.*



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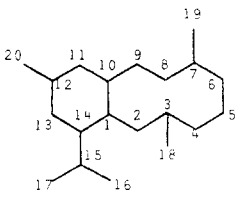
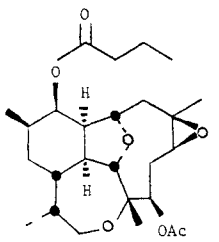


Asbestinane Skeleton



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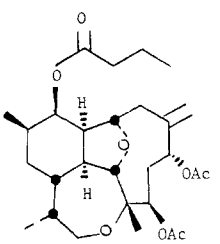

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Results and Discussion

Our first collection of *B. asbestinum* from Anthony's Key (Roatan Island, 1978) was a small one. It yielded a crude extract and chromatographic fractions which gave complex ^1H NMR spectra (360 MHz), showing that chlorinated briarein diterpenes were not major components.[7] Methanol extracts of larger collections of *B. asbestinum* 1 year later from three locations, including Anthony's Key and Lost Paradise (Roatan Island) and Pelican Point (Hog Island), gave crude oil yields which varied somewhat (0.67%, 5.0% and 1.8%, respectively, based upon dry weight) as did the major components. The Hog Island extract seemed less complex, and flash chromatography[8] followed by preparative high-performance LC yielded pure samples of two isomeric olefins, **4** and **5** (M^+, m/e 448,

(1) (a) Randall, J. E.; Hartman, W. D. *Mar. Biol. (Berlin)* **1968**, *1*, 216. (b) Kittredge, J. S.; Takashashi, F. T.; Lindsey, J.; Lasker, R. *Fish. Bull.* **1974**, *72*, 1. (c) Bakus, G. J.; Green, G. *Science* **1974**, *185*, 951. (d) Ne'eman, I.; Fishelson, L.; Kashman, Y. *Toxicon* **1974**, *12*, 593. (e) Green, G., *Mar. Biol. (Berlin)* **1977**, *40*, 207. (f) Bakus, G. J.; Thun, M. A. *Colloq. Int. CNRS* **1978**, *No. 291*, 417. (g) Berquist, P. R. "Sponges"; University of California Press: Berkeley, CA, 1978, Chapters 6 and 7.

(2) For some recent examples see: (a) Braekman, J. C.; Daloze, D.; Hulot, G.; Tursch, B.; Declercq, J. P.; Germain, G.; van Meerssche, M. *Bull. Soc. Chim. Belg.* **1978**, *87*, 917; (b) Tursch, B.; Braekman, J. C.; Daloze, D.; Dedeurwaerder, H.; Karlsson, R. *Ibid.* **1978**, *87*, 75; (c) Bowden, B. F.; Coll, J. C.; Mitchell, S. J.; Stokie, G. J. *Aust. J. Chem.* **1979**, *32*, 653.

(3) Birks, J. E.; Van der Helm, D.; Chang, C. Y.; Ciereszko, L. S., *Acta Crystallogr., Sect. B* **1977**, *B33*, 704.

(4) Slight errors have appeared in drawings of the briarein A structure in two recent reviews [Scheuer, P. J., Ed. "Marine Natural Products"; Academic Press: New York, 1978; Vol. II. Fenical, W. *Ibid.*, Chapter 3, p 209. Tursch, B.; Braekman, J. C.; Daloze, D.; Kaiser, M. *Ibid.*, Chapter 4, p 273] including in the former a missing OAc at C_7 (numbering of ref 3) and in the latter an *R* designation at C_7 which should be *S* as in ref 3.

(5) (a) Hale, R. L.; Declercq, J.; Tursch, B.; Djerassi, C.; Gross, R. A., Jr.; Weinheimer, A. J.; Gupta, K.; Scheuer, P. J. *J. Am. Chem. Soc.* **1970**, *92*, 2179. (b) Ciereszko, L. S.; Johnson, M. A.; Schmidt, R. W.; Koons, C. B. *Comp. Biochem. Physiol.* **1968**, *24*, 899.

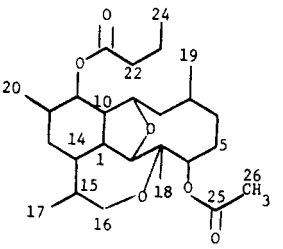
(6) Stierle, D. B.; Carte, B.; Faulkner, D. J.; Tagle, B.; Clardy, J. *J. Am. Chem. Soc.* **1980**, *102*, 5088.

(7) Briarein A and briarein B were isolated as extremely minor components (experimental section). See ^1H NMR data in: Wratten, S. J.; Fenical, W.; Faulkner, D. J.; Wekell, J. C. *Tetrahedron Lett.* **1977**, 1559.

(8) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

0022-3263/81/1946-0964$01.00/0

Table I. ^{1}H NMR Data[a] (Benzene-d_6, 360 MHz)

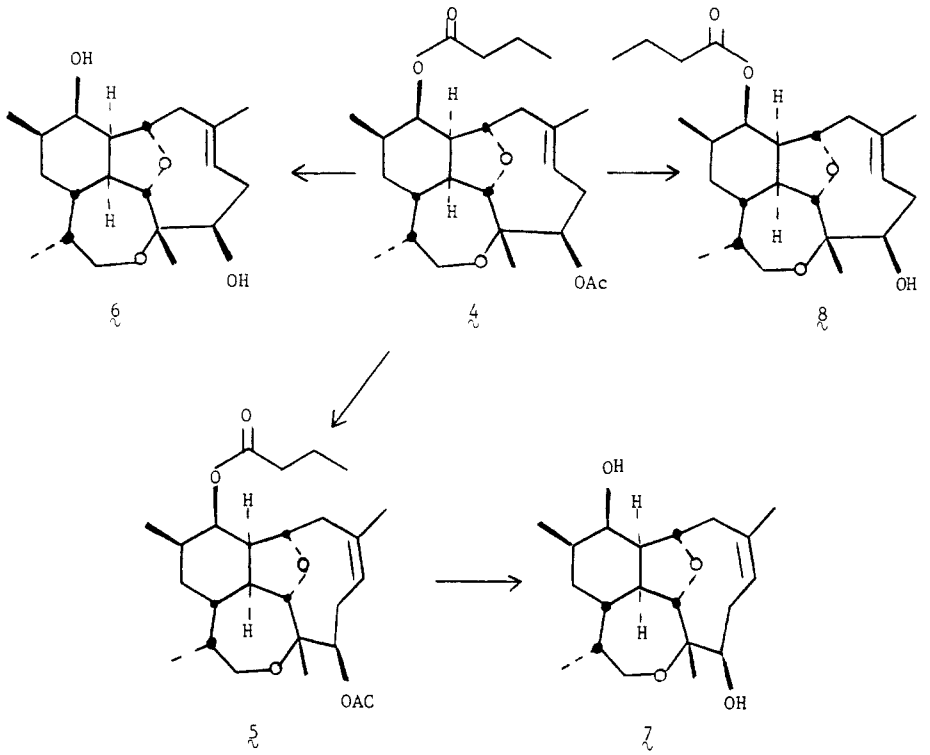


	4			6			5			7		
	δ	mult type	*J*, Hz[b]	δ	mult type	*J*, Hz	δ	mult type	*J*, Hz	δ	mult type	*J*, Hz
H_1	2.32	ddd	8, 9, 9	2.17	ddd	8, 9, 9	2.38	ddd	9, 9, 10	2.22	ddd	9, 11, 11
H_2	4.20	d	8	4.22	d	8	4.08	d	9	4.11	d	9
H_4	5.61	dd	7, 11	4.08	dd	6, 11	5.34	dd	7, 9	4.02	d	7
H_5	2.85 (α)	ddd	7, 9, 13	3.12	ddd	6, 9, 13	3.50	ddd	7, 10, 13	3.39	ddd	7, 10, 13.6
	2.22 (β)	ddd	9, 11, 13	2.38	ddd	8, 11, 13	1.90	m		2.27	m	
H_6	5.47	dd	9, 9	5.63	dd	8, 9	6.03	dd	7, 10	5.71	dd	7, 10
H_8	2.40 (α)	dd	7, 13	2.52	dd	7, 12	2.66	d	14	2.72	d	14.5
	1.76 (β)	d	13	1.79	d	12	1.54	dd	5, 14	1.69	dd	4.2, 14.5
H_9	4.16	dd	3, 7	4.16	dd	2, 7	4.25	dd	3, 5	4.31	dd	3.3, 6.7
H_{10}	1.80	m		1.61	ddd	2, 3, 9	1.80	m		1.64	ddd	3, 3, 9
H_{11}	5.34	dd	3, 4	3.15	dd	3, 5	5.36	dd	3, 5	3.12	dd	3, 3.5
H_{12}	1.58	m		1.31	m		1.55	m		1.42	m	
H_{13}	0.77 (α)	ddd	2, 3, 13	{0.80	ddd	3, 4, 13	0.72	ddd	2, 3, 13	{0.77	ddd	2, 3, 13
	1.21 (β)	ddd	10, 13, 13	{1.20	ddd	10, 13, 13	1.21	ddd	9, 13, 13	{1.25	ddd	9, 13, 13
H_{14}	1.91	dddd	3, 5, 10, 10	2.05	dddd	4, 4, 9, 10	1.90	m		2.04	dddd	4, 4, 11, 14
H_{15}	1.42	m		1.18	m		1.28	m		1.25	m	
H_{16}	3.79 (α)	dd	2, 13	3.68	d	13	3.75	d	13	3.63	d	13
	3.37 (β)	dd	4, 13	3.23	dd	2, 13	3.46	dd	3, 13	3.30	dd	3.3, 13
Me_{17}	0.85	d	7 (3 H)	0.72	d	7	0.85	d	7	0.81	d	7.1
Me_{18}	1.50	s	(3 H)	1.42	s		1.66	s		1.54	s	
Me_{19}	1.81	s	(3 H)	1.67	s		1.62	s		1.66	s	
Me_{20}	0.89	d	7 (3 H)	0.93	d	7	0.87	d	7	0.91	d	7.2
$(CH_2)_{22}$	2.16	t	(2 H)				2.13	t	7			
$(CH_2)_{23}$	1.58	m	(2 H)				1.23	m				
Me_{24}	0.82	t	(3 H)				0.84	t	7			
Me_{26}	1.83	s	(3 H)				1.76	s				

	2			3			10			9		
	δ	mult type	*J*, Hz	δ	mult type	*J*, Hz	δ	mult type	*J*, Hz	δ	mult type	*J*, Hz
H_1	2.45	ddd	10, 10, 10	2.77	ddd	9, 9, 9	2.50	ddd	9, 9, 10	2.52	ddd	9, 9, 9
H_2	4.01	d	10	3.89	d	9	3.87	d	9	3.85	d	9
H_4	5.50	d	6	5.82*	br s		5.72*	br s		5.99	dd	3, 12
H_5	2.75 (α)	ddd	6, 10, 14				3.07	br d		2.98	dd	12, 14
	2.05 (β)	dd	3, 14							2.71	dd	3, 14
H_6	3.41	dd	3, 10	4.78*	br s		4.07*	br s				
H_8	2.14 (α)	dd	3, 14							3.48	dd	7, 13
	1.78 (β)	dd	3, 14	1.98	dd	5, 14				1.76	d	13
H_9	4.12	dd	3, 6	4.18	dd	5, 6	4.17	dd	3, 5	4.06	dd	4, 7
H_{10}	2.14	m								1.78	m	
H_{11}	5.27	dd	3, 5	5.37	dd	2, 5	5.32	dd	3, 5	5.22	dd	1, 4
H_{12}	1.57	m					1.59	m		1.60	m	
H_{13}	0.75 (α)	ddd	2, 3, 13	0.75	dd	3, 13	0.74	dd	3, 13	0.74	ddd	3, 4, 13
	1.25 (β)	ddd	10, 13, 13	1.25	m		1.25	ddd	10, 13, 13	1.24	ddd	10, 13, 13
H_{14}	1.86	dddd	4, 4, 9, 10							1.50	m	
H_{15}	1.30	m		1.57	m					1.22	m	
H_{16}	3.65 (α)	d	13	3.47	d	13	3.52	d	13	3.42	d	12
	3.44 (β)	dd	3, 13	3.36	dd	2, 13	3.34	dd	3, 13	3.28	dd	3, 12
Me_{17}	0.86	d	7	0.81	d	7	0.84	d	7	0.88	d	7
Me_{18}	1.54	s		1.34	s		1.40	s		1.34	s	
Me_{19}	1.15	s		{5.45*	br s		{5.31*	br s		{5.27	br s	
				{5.28*	br s		{4.92*	br s		{4.93	br s	
Me_{20}	0.91	d	7	1.01	d	7	0.86	d	7	0.88	d	7
$(CH_2)_{22}$	2.10	t	7	2.12	t	7	2.15	t	7	2.06	t	7
$(CH_2)_{23}$	1.55	m		1.57	m		1.32	m		1.54	m	
Me_{24}	0.82	t	7	0.81	t	7	0.82	t	7	0.77	t	7
Me_{26}	1.74	s		1.82	s		1.75	s		1.76	s	
				1.84	s							

[a] Asterisk indicates assignments that can be switched. [b] The number of hydrogens is given in parentheses.

Scheme I



$C_{26}H_{40}O_6$). Cleavage of an acetate and a butyrate side chain from **4** or **5** with $LiAlH_4$ afforded a crystalline diol, **6** or **7** (M^+, m/e 336, $C_{20}H_{32}O_4$). Comparison of the ^1H and ^{13}C NMR spectra of **4** and **6** with that of the known *Briareum* metabolite asbestinin-1 and its diol derivative (mp 154–156 °C)[6] suggested their identity; however, two factors complicated a straightforward comparison. Crystallization of **6** yielded two polymorphic forms: needles, mp 141–142 °C, and cubes, mp 156–158 °C. Initial X-ray examination of the latter showed that the space group and unit cell were different than previously observed for the diol of asbestinin-1,[6] but the refined X-ray results showed that these two compounds were identical. Selective cleavage of the acetate in **4** by reaction with L-Selectride at −78 °C yielded alcohol **8**, thereby completing the proof that **4** was identical with asbestinin-1.[6] That **5** was simply a double bond isomer of **4** and thus identical with the known asbestinin-2[6] was shown by isomerization of **4** to **5** with acetic acid.

The NMR properties of **4–7** were useful in establishing structures of the new asbestinins **2** and **3** (vide infra) and will be discussed briefly. Chemical shifts were determined for all 18 ring protons (Table I) of **4–7** (see Scheme I) after extensive spin-decoupling experiments at 360 MHz in benzene-d_6 and provided insights into several elements of stereochemistry. For example, the vicinal J values for key protons of **4** such as H_1, H_5, H_{10}, and H_{14} indicated that its solution conformation was identical with that reported for the crystal state.[6] Pinpointing the differing C_6–C_7 double bond stereochemistry in **4** vs. **5** (or **6** vs. **7**) were the relative shifts of H_4, $H_{5\alpha}$, $H_{5\beta}$, and H_6 along with J values for H_6. That the stereochemistry for **4–7** at the chiral centers 1, 2, 9, 10, 11, and 14 was identical could be shown by the similarity of the J values involving protons at these sites.

Unambiguous assignments of all 26 carbons of **4** were made from ^{13}C spectra (completely coupled, off resonance, and NOE suppressed) at 90 MHz. This was needed in order to obtain stereochemical information at other sites, including carbons 3, 7, 12, and 15. Two useful features not easily seen in the 25-MHz carbon spectra but quite obvious at 90 MHz were doublets of doublets in the off-resonance spectra for CH_2's with diastereotopic H's and accenuated differences in J_R (residual J_{CH} coupling[9a]) observable among the sp^3 carbons. The latter along with the ^1H shifts from Table I provided a basis for the exact assignments shown in Table II. A test of the quality of the data given by such a J_R analysis can be obtained from the linear correlation of $[J_R/J_{CH}]$ vs. $\delta(^1H)$. Use of the ratio J_R/J_{CH} conveniently normalizes any changes in J_{CH} due to polar substituent effects.[9a] A second and more powerful use of such a graph is in locating hidden ^1H shifts when it is possible to measure some residual and exact J_{CH} values. This procedure requires only two ^{13}C spectra, but it is most effective at superconducting fields. Finally, it is complementary to and perhaps simpler to apply than the usual technique of using either a series of heteronuclear single-frequency decoupled spectra or a series of off-resonance a graphical approach to cross correlate ^{13}C and ^1H shifts.[9b]

The *E* or *Z* geometry of the double bonds of **4** and **5** is clearly shown by the chemical shift difference[10] of Me_{19} (δ 18.7 vs. 29.6). The near identity of the other methyl shifts in **4** vs. **5** at Me_{17} (δ 11.5 vs. 11.0), Me_{18} (δ 19.7 vs. 19.6), and Me_{20} (δ 18.4 vs. 18.5) is in line with the identical stereochemistry of these groups.[11]

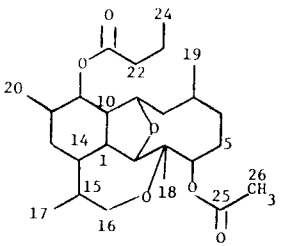
Asbestinin epoxide (**2**) was isolated from the Hog Island crude oil. The mass spectrum (M^+, m/e 464, $C_{26}H_{40}O_7$) and the ^{13}C data demonstrated the structural similarities and differences between **2** and **4** or **5**. The absence of olefinic carbons and the presence of an epoxide [δ 63.5 (d), 58.5 (s)] were clearly evident (Table II). The stereochemistry at Me_{17} (δ 11.0), Me_{18} (δ 18.8), and Me_{20} (δ 18.3) was unchanged. Comparison of the J values of **2** and **5** at H_1, H_4, H_{10}, H_{11}, and H_{14} showed that the other common stereochemistry elements, excepting that of the epoxide

(9) (a) Wehrli, F. W.; Wirthlin, T. "Interpretation of Carbon-13 NMR Spectra"; Heydon and Son, Inc.: Philadelphia, PA, 1976; Chapter 3. (b) Gray, G. A. *Anal. Chem.* **1975**, *47*, 546A.

(10) Crews, P.; Kho-Wiseman, E. *J. Org. Chem.* **1977**, *42*, 2812.

(11) Crews, P.; Kho-Wiseman, E. *Tetrahedron Lett.* **1978**, 2483.

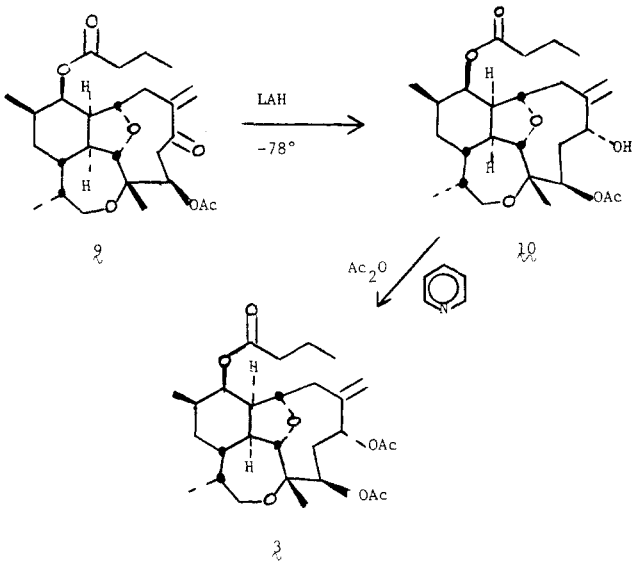
Table II. ^{13}C NMR Data (Benzene-d_6)



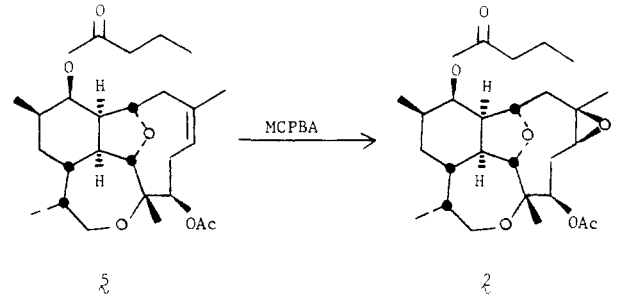
	4[a]				5[c]			2			10			3		
C	δ	OR mult	J_{CH}, Hz	J_R,[b] Hz	δ	OR mult	J_R, Hz	δ	OR mult	J_R	δ	OR mult	J_R, Hz	δ	OR mult	J_R, Hz
1	38.5	d	127	14	38.1	d	67	37.8	d	53	38.9	d	57	38.8	d	53
2	94.7	d	140	20	91.7	d	75	91.5	d	75	93.3	d	75	93.5	d	75
3	79.3	s			77.1	s		77.2	s		76.8	s		76.9	s	
4	72.8	d	142	43	79.4	d	86	75.0	d	82	73.9	d	75	76.0	d	82
5	29.7	dd	130	5, 15	33.9	t	45	35.4	t	53	37.2	t	50	37.3	t	53
6	125.5	d	153	40	127.3	d	90	63.5	d	82	72.2	d	80	70.1	d	86
7	129.2	s			131.7	s		58.5	s		148.4	s		144.4	s	
8	44.4	dd	127	12, 20	37.1	t	60	40.9	t	60	39.2	t	50	38.8	t	53
9	81.0	d	140	20	82.0	d	78	81.0	d	78	83.3	d	80	83.6	d	82
10	48.7	d	127	20	45.4	d	60	44.7	d	67	46.0	d	60	46.7	d	56
11	73.4	d	140	36	73.3	d	86	73.0	d	90	73.6	d	77	73.4	d	86
12	31.2	d	127	23	31.7	d	60	31.7	d	53	31.5	d	50	31.5	d	53
13	31.6	dd	125	28, 37	30.0	t	38	31.7	t	45	31.5	t	45	31.5	t	45
14	38.5	d	127	14	38.8	d	60	38.8	d	53	38.9	d	57	38.8	d	53
15	37.5	d	122	27	41.1	d	60	39.8	d	60	37.2	d	50	34.9	d	53
16	67.6	t	138		67.6	t	71	67.6	t	71	67.3	t	70	67.9	t	67
Me_{17}	11.5	q	127	37	11.0	q	45	11.0	q	48	11.0	q	50	11.0	q	48
Me_{18}	19.7	q	125	29	19.6	q	53	18.8	q	53	17.7	q	50	17.4	q	53
Me_{19}	18.7	q	122	25	29.6	q	45	28.1	q	53	115.5	t	90	117.5	t	90
Me_{20}	18.4	q	125	36	18.5	q	45	18.3	q	48	17.7	q	50	17.4	q	53
$O{=}C_{21}$	173.2	s			173.1	s		173.1	s		173.1	s		173.1	s	
$(CH_2)_{22}$	36.6	t	125	14	36.6	t	60	36.6	t	53	36.6	t	55	36.6	t	53
$(CH_2)_{23}$	18.7	t	127	23	18.8	t	45	18.8	t	53	18.7	t	55	18.6	t	53
Me_{24}	13.7	q	122	36	13.8	q	45	13.7	q	48	13.6	q	50	13.7	q	45
$O{=}C_{25}$	169.9	s			169.8	s		170.1	s		171.5	s		169.0	s	
Me_{26}	21.1	q	128	21	21.2	q	53	21.1	q	56	21.1	q	60	21.0	q	48
OAc														20.8	q	56
														168.8	s	

[a] 90 MHz. [b] ^1H off-resonance frequency = δ 3.0. [c] 25 MHz.

Scheme II



ring, were also unchanged. The stereochemistry of the latter was established by epoxidation of **5** with MCPBA whch gave **2**, presumably by attack from the unhindered β face. By contrast, all attempts to epoxidize **4** yielded only complex mixtures.[12]



Two known compounds, asbestinin-4 (**9**)[6] and asbestinin-5 (**10**)[6] (Scheme II), were isolated by repeated chromatography of the crude extracts from Anthony's Key or Lost Paradise (Roatan Island). The ^{1}H NMR spectrum of **10** showed broad lines for the H's at positions 4, 19, and 19′ which sharpened somewhat upon warming, whereas all of the signals of **9** were sharp at room temperature. The planar enone chromophore apparently imparts rigidity to the ten-membered ring of **9**, whereas the same ring in **10** seems to quite flexible. Compound **9** was converted into **10** by reaction with $LiAlH_4$ at −78 °C with the hydride again entering from the unhindered β face to give an α-oriented C_6 hydroxyl. A third, more polar compound isolated from both Roatan Island collections was asbestinin-5 acetate **3** ($C_{28}H_{42}O_8$; *m/e* 506, (M^+). Its ^{1}H NMR spectrum was reminiscent of that observed for **10**. The near identity of ^{13}C shifts in **3** and **10** for Me_{17}, Me_{19}, and Me_{20} as well as those at most other carbons further suggested that **10** and **3** differed only in replacement of the C_6 OH by OAc. Indeed, **10** could be acetylated to yield **3**. The α-oxygen stereochemistry at C_6 of both **3** and **10** suggests that the unstable β-epoxide derivative expected from **4** (see Experimental Section) could serve as a relay between the endocyclic olefins (i.e., **4**) and the exocyclic ones (i.e., **3**, **9**, **10**).

(12) For similar problems in a related system see: Kazlauskas, R.; Murphy, P. T.; Wells, R. J.; Schonholzer, P. *Tetrahedron Lett.* **1977**, 4643.

(13) Jacobs, R., University of California at Santa Barbara, unpublished results.

Several of the above asbestinin derivatives show pharamcological activity. Compounds **4**, **6**, and **10** all show the ability to antagonize the effects of acetylcholine on guinea pig ileum preparations at a respective level of 13%, 14%, and 38% at a concentration of 16 μg/mL.[13] Compounds **6** and **10** also exhibit histamine antagonism in this same assay at levels of 30% and 40%, respectively, at the same concentration.

Experimental Section

The NMR spectra were recorded on a JEOL FX-100 PFT spectrometer operating at 99.55 MHz for ^{1}H and 25.0 MHz for ^{13}C and on a HXS-360 spectrometer operating at 360 MHz for ^{1}H and 90 MHz for ^{13}C at Stanford University. The *J* values are given in hertz. Gas chromatography/mass spectral data were obtained on a Finnigan 4000 system equipped with a $^1/_8$ in. × 4 ft glass column packed with 3% OV-17 on Chromasorb Q and temperature programmed from 100 to 220 °C at 10 °C min^{-1}. High-performance liquid chromatography (LC) was done on a Waters ALC 201 using Waters μ-Porasil or Whatman Partisil columns. Rotations were measured on a JASCO ORD/CD with a 0.1-dm cell (0.5 mL). All solvents were reagent grade and distilled for high-performance LC use. Low-boiling petroleum ether was used in all instances. Spectral grade solvents were used for NMR (Me_4Si standard) determinations.

Collections and Extractions. *B. asbestinum* was collected from various locations in the Bay Islands of Honduras, Central America, during June and July of 1979 or March and April of 1978. Collections from different locations were kept separate, and all samples were kept frozen until extraction.

Anthony's Key (Roatan Island). *B. asbestinum* (7.5 kg, 3.76 kg dry weight) was extracted for 48 h with methanol in a percolator to yield 25.78 g (0.69% yield, based on dry weight) of crude extract. When the mixture was allowed to stand, 500 mg (0.02%) of a mixture of briarein A and B crystallized out. Flash chromatography (4:1 benzene/ethyl acetate) followed by repeated high-performance LC (4:1 petroleum ether/ethyl acetate) of the remaining oil yielded **3** (300 mg, 0.01%), **4** (3.2 g, 0.08%), **5** (1.1 g, 0.03%), **9** (300 mg, 0.01%), and **10** (600 mg, 0.02%).

Lost Paradise (Roatan Island). *B. asbestinum* (0.90 kg, 481 g dry weight) was extracted as above to yield 24.05 g (5.0% yield, based on dry weight) of crude extract. Chromatography as above yielded **3** (360 mg, 0.07%), **4** (3.6 g, 0.75%), **5** (200 mg, 0.04%), **9** (100 mg, 0.02%), and **10** (100 mg, 0.02%).

Pelican Point (Hog Islands). *B. asbestinum* (4.0 kg, 2.2 kg dry weight) was extracted as above to yield 41.3 g (1.8% yield, based on dry weight) of crude extract. Chromatography as above yielded **2** (100 mg, 0.0045%), **4** (4.30 g, 0.196%), **5** (610 mg, 0.028%), and **10** (3.20 g, 0.146%).

Asbestinin Epoxide (2). High-performance LC (4:1 petroleum ether/ethyl acetate) of the seventh fraction from flash chromatograhy of the Pelican Point extract gave **2** (fourth column volume) as a colorless oil: $[\alpha]^{25}_D$ −21° (*c* 0.3, CH_3OH); ^{1}H and ^{13}C NMR in Tables I and II; mass spectrum, *m/e* 464, 447, 426, 411, 404, 376 (exact mass 464.2759 vs. 464.2774 calcd for $C_{26}H_{40}O_7$); IR 1735 cm^{-1}.

Asbestinin-5 Acetate (3). High-performance LC (3:1 petroleum ether/ethyl acetate) of fractions 10–12 from flash chromatography of either the Anthony's Key or Lost Paradise extracts gave **3** (fifth column volume) as a colorless oil: $[\alpha]^{25}_D$ −12° (*c* 0.2, CH_3OH); ^{1}H and ^{13}C NMR in Tables I and II; mass spectrum, *m/e* 506, 446, 418, 404, 386, 376, 358, 316, 307, 298, 219 (base peak) (exact mass 506.2906 vs. 506.2880 calcd for $C_{28}H_{42}O_8$); IR 1735 cm^{-1}.

Asbestinin-1 (4). Flash chromatography of the Pelican Point extract (4:1 benzene/ethyl acetate) gave **4** as a colorless oil (second fraction). Flash chromatography followed by high-performance LC of the Anthony's Key and Lost Paradise extracts also gave **4** as a colorless oil whose spectral properties including ^{1}H and ^{13}C NMR (Tables I and II) and mass spectra were similar to those reported in the literature.[6]

Asbestin-2 (5). High-performance LC (8:1 petroleum ether/ethyl acetate) of the third fraction from flash chromatography gave **5** (eighth column volume) as a colorless oil whose spectral properties including ^{1}H and ^{13}C NMR (Tables I and II) and mass

spectra were similar to those reported in the literature.[6]

Asbestinin-4 (9). High-performance LC (4:1 petroleum ether/ethyl acetate) of fractions 10–12 from flash chromatography of either the Anthony's Key or Lost Paradise extracts gave **9** (sixth column volume) as a colorless oil, whose spectral properties including ^1H and ^{13}C NMR (Tables I and II) and mass spectra were similar to those reported in the literature.[6]

Asbestinin-5 (10). High-performance LC (2.5:1 petroleum either/ethyl acetate) of fractions 14 and 15 from flash chromatography of either the Anthony's Key or Lost Paradise extracts gave **10** (tenth column volume) as a colorless oil whose spectral properities including ^1H and ^{13}C NMR (Tables I and II) and mass spectra were similar to those reported in the literature.[6]

Briarein-A (1). Repeated fractional crystallization of the crude solid gave **1** as colorless crystals: (mp 240–245 °C); ^1H NMR (360 MHz, $CDCl_3$) δ 6.07 (d, J = 9), 5.92 (br d, J = 10), 5.76 (dd, J = 9, 10), 5.67 (ddd, J = 2, 2, 7), 5.60 (d, J = 2), 5.53 (s), 5.43 (dd, J = 2, 2), 5.05 (dd, J = 4, 7), 4.80 (m, 2 H), 3.78 (s), 3.09 (q, J = 7), 3.06 (s), 2.31 (ddd, J = 2, 2, 15), 2.20 (s, 3 H), 2.01 (ddd, J = 2, 2, 15), 1.97 (s, 3 H), 1.96 (s, 6 H), 1.93 (s, 3 H), 1.50 (s, 3 H), 1.36 (s, 3 H), 1.33 (d, 3 H, J = 7); ^{13}C (25 MHz, C_6D_6) δ 176.3, 169.9, 169.8, 168.6, 168.2 (2 C), 137.1, 130.2, 127.7, 115.6, 84.1, 83.1, 79.2 (2 C), 72.1, 71.4, 64.6, 48.9, 45.8, 38.3, 25.6, 21.7, 21.1, 20.5 (2 C), 19.4, 17.9, 15.7, 13.5, 10.5.

Briarein B. The mother liquors from the above crystallizations were enriched in briarein B, whose structure is proposed as the tetraacetate monobutyrate analogue of briarein A: ^1H NMR (100 MHz, $CDCl_3$) δ 6.05 (d, J = 9), 5.90 (d, J = 10), 5.80–5.0 (m, 5 H), 4.80 (d, J = 3), 3.76 (s), 3.05 (m, 2 H), 2.20 (t, 2 H, J = 7), 2.20 (s, 3 H), 1.95 (s, 3 H), 1.93 (s, 6 H), 1.50 (s, 3 H), 1.40 (s, 3 H), 1.36 (d, 3 H, J = 7), 0.95 (t, 3 H, J = 7).

Asbestinin-1 (4) to Diol 6. Asbestinin-1 (644 mg) in 2 mL of dry diethyl ether was added to a slurry of excess LAH in 50 mL of dry diethyl ether cooled to −78 °C and maintained under an inert atmosphere. This mixture was stirred for $^1/_2$ h and allowed to warm to 0 °C. The reaction was immediately quenched by the dropwise addition of NH_4Cl solution (saturated). The organics were extracted with benzene (2 × 50 mL), combined, and dried over $MgSO_4$. The solvent was removed under vacuum to give 340 mg (66% yield) of diol **6**, which was recrystallized from CCl_4 (needles, mp 141 °C) or benzene/hexanes (cubes, mp 156–158 °C; lit.[6] mp 154–156 °C) and whose spectral properties (Tables I and II) were similar to those reported in the literature.[6]

Asbestinin-2 (5) to Diol 7. By employment of the above procedure, **5** (88 mg) gave **7** (60 mg, 90% yield), which was recrystallized from CCl_4 (needles, mp 172–174 °C) or hexane (cubes, mp 184–186 °C) and had spectral properties (Tables I and II) similar to those in the literature.[6]

X-ray Crystallographic Study of Asbestinin-1 Diol 6. A crystal of **6** recrystallized from benzene/hexanes was selected. Preliminary X-ray photographs showed orthorhombic symmetry and accurate lattice parameters of a = 12.361 (9), b = 11.580 (17), and c = 13.033 (12) Å. Systematic extinctions and the presence of chirality and density considerations were uniquely accomodated by space group $P2_12_12_1$ with one molecule of $C_{20}H_{32}O_4$ in the asymmetric unit. All diffraction maxima with $2\theta \leq 114°$ were recorded on a computer-controlled, four-circle diffractometer using a variable-speed, 1° ω scan and graphite-monochromated Cu Kα radiation (λ 1.541 78 Å). Of the 1470 reflections investigated, 1300 were considered observed after correction for Lorentz, polarization, and background effects.

A phasing model was arrived at by using a multisolution, weighted, tangent formula approach and the resulting E synthesis revealed the entire nonhydrogen structure.[14] Full-matrix, least-squares refinements were carried out to a conventional crystallographic residual of 0.08 at which point it was clear that the connectivity, relative stereochemistry, and conformation were essentially identical with those published.[6] A forthcoming paper will deal with a further comparison of these two structures.

Asbestinin-1 (4) to Alcohol 8. To compound **4** (64 mg, 0.14 mmol) in 4 mL of dry diethyl ether at −78 °C under an inert atmosphere was added 0.5 mL of a 1 M solution (0.5 mmol, 3 equiv) of lithium tri-*sec*-butyl borohydride (L-Selectride). After being stirred for $^1/_2$ h and warmed to 0 °C, the reaction mixture was quenched by dropwise addition of saturated NH_4Cl. The organics were extraced with benzene (2 × 50 mL), combined, and dried over $MgSO_4$. The solvent was removed under vacuum and the residue chromtographed on high-performance LC (3:1 petroleum ether/ethyl acetate, μ-Porasil column) to give **8**: 35 mg (50%); ^1H NMR (100 MHz, $CDCl_3$) δ 5.38 (m, 2 H, H_6, H_{11}), 4.31 (dd, J = 4, 11, H_4), 3.83 (d, J = 9, H_2), 3.75 (m, H_9), 3.63 (d, J = 13, H_{16}), 3.43 (dd, J = 2, 13, H_{16}), 2.90 (ddd, J = 9, 9, 9, H_1), 2.26 (t, 2 H, J = 7, H_{22}), 1.99 (s, Me_{19}), 1.60 (m, 2 H, H_{23}), 1.27 (s, Me_{18}), 0.95 (t, J = 7, Me_{24}), 0.90 (d, 6 H, J = 7, Me_{17}, Me_{20}); ^1H NMR (100 MHz, C_6D_6) δ 5.52 (br s, H_6), 5.22 (dd, J = 2, 5, H_{11}), 4.30 (dd, J = 4, 11, H_4), 3.75 (m, 2 H, H_2, H_9), 3.25 (br s, 2 H, H_{16}), 2.05 (t, 2 H, J = 7, H_{22}), 1.80 (s, Me_{19}), 1.40 (s, Me_{18}), 0.80 (d, 6 H, J = 7, Me_{17}, Me_{20}), 0.74 (t, J = 7, Me_{24}).

Asbestinin-2 (5) to Asbestinin Epoxide 2. Compound **5** (25 mg, 0.056 mmol) in 1 mL of dry CH_2Cl_2 was added to a solution of 25 mg (0.12 mmol) of *m*-chloroperbenzoic acid in 5 mL of dry CH_2Cl_2 stirring over 20 mg of $NaHCO_3$. This was stirred for 1 h at room temperature and quenched with $NaHSO_3$ solution (saturated). The organics were extracted with benzene (2 × 20 mL), combined, washed with $NaHCO_3$ solution (saturated), and dried over $MgSO_4$. The solvent was removed under vacuum to yield **2** (16 mg, 60% yield) whose spectral properties were identical with those of an authentic sample.

Attempted Epoxidation fo 4. Compound **4** (900 mg) was dissolved in 5 mL of chloroform and the mixture added to a mixture of 620 mg of *m*-chloroperoxybenzoic acid in 50 mL of chloroform stirring over 300 mg of $NaHCO_3$ at −78 °C. This was stirred for $^1/_2$ h and allowed to warm to 0 °C, at which point the reaction was quenched by the addition of saturated Na_2SO_4 solution. An additional 50 mL of chloroform was added, and the mixture was washed with saturated $NaHCO_3$ solution (2 × 100 mL), 100 mL of water, and 100 mL of saturated NaCl solution. The organic layer was dried with $MgSO_4$, and the solvent was removed under vacuum to give an oily mixture (910 mg, 95%) which was a complex mixture by ^{13}C NMR. The ^{13}C NMR (25 MHz, C_6D_6) showed 52 lines: δ 207.0, 173.4, 173.0, 170.0, 169.6, 164.4, 134.5, 133.1, 130.1, 129.3, 128.3, 126.1, 125.5, 94.8, 81.7, 80.9, 80.1, 79.3, 78.5, 78.0, 77.7, 73.2, 71.7, 67.9, 67.7, 48.7, 47.8, 44.5, 42.6, 42.0, 38.5, 37.6, 37.1, 36.7, 35.1, 34.7, 31.7, 30.1, 29.7, 27.5, 25.9, 23.0, 21.2, 20.8, 19.8, 18.7, 17.5, 16.0, 14.3, 13.9, 11.5, 11.3.

Asbestinin-4 (9) to Asbestinin-5 (10). Compound **9** (3.0 mg) in 1 mL of dry diethyl ether was added to a slurry of 15 mg of LAH in 1 mL of dry diethyl ether cooled to −78 °C under an inert atmosphere. This mixture was stirred for $^1/_2$ h and 1 mL of NH_4Cl solution (saturated) was added dropwise. The solution was allowed to warm to room temperature. The organics were extracted with benzene (2 × 20 mL), combined, and dried over $MgSO_4$. The solvent was removed under vacuum, and the residue was passed through a silica gel pad with benzene to give **10** (2.2 mg, 70%) whose spectral properties were identical with those of an authentic sample.

Asbestinin-5 (10) to Asbestinin-5 Acetate 3. To compound **10** (2.0 mg) in 1 mL of dry pyridine was added 10 drops of freshly distilled acetic anhydride. This was stirred for 18 h at room temperature and diluted with 10 mL of benzene. It was washed with several portions of 1% HCl followed by $NaHCO_3$ solution (saturated). The organic layer was dried with $MgSO_4$, and the solvent was removed under vacuum. The residue was chromatographed on high-performance LC (4:1 petroleum ether/ethyl acetate, μ-Porasil column) to give **3** (1.2 mg, 53% yield) whose spectral properties were identical with those of an authentic sample.

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Registry No. 1, 62681-06-5; 2, 75961-66-9; 3, 75961-67-0; 4, 74219-41-3; 5, 74145-73-6; 6, 74145-74-7; 7, 74183-98-5; 8, 74145-72-5; 9, 74145-71-4; 10, 74145-70-3; briarein B, 75961-58-9.

Synthesis of C_{19}-Functionalized 7-Dehydrocholesteryl Derivatives. Photochemical Transformation to Vitamin D_3 Analogues

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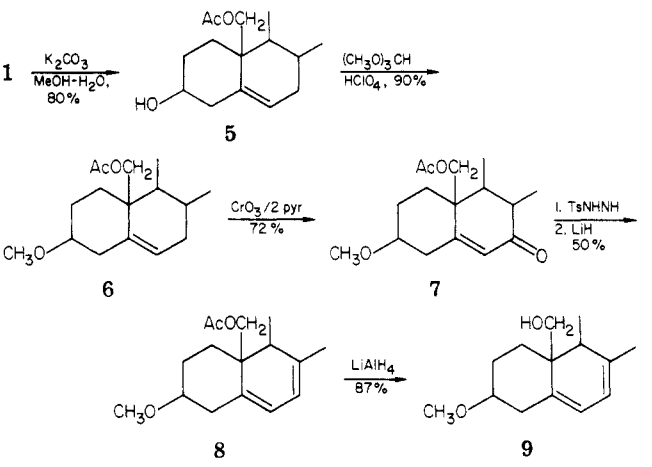
A series of C_{19}-substituted 7-dehydrocholesterol derivatives has been prepared in which the C_{19} substituent is hydroxyl, acetoxyl, methoxyl, or aldehydo. These compounds are cholesta-5,7-diene-3β,19-diol (4), cholesta-5,7-diene-3β,19-diol diacetate (3), cholesta-5,7-diene-3β,19-diol 3-acetate 19-methyl ether (19) and 3β-methoxycholesta-5,7-dien-19-al (10). In each case the synthesis proceeded from a Δ^5-steroid which was converted to the 7-keto-Δ^5 system. Then the derived tosylhydrazone was decomposed with lithium hydride to introduce the $\Delta^{7,8}$ double bond to complete the ring-B diene synthesis. Irradiation of 3 followed by thermally induced hydrogen migration yields the vitamin D_3 analogue with *E* stereochemistry at the C_{19} position. Likewise, photochemical ring-opening of 19 followed by the thermal hydrogen transfer yielded purely the C_{19} *E* isomer. This stereoselectivity is discussed. Irradiation of 4 proceeded with loss of the C_{19} functionality to yield 19-norcholesta-5(10),7-dien-3β-ol. The chiroptical effects of the homoannular cisoid dienes occurring in this study are discussed in terms of the diene quadrant rule.

The past decade has witnessed impressive advances in the biochemistry of vitamin D.[1] The hepatic metabolism of vitamin D_3 to its 25-hydroxylated derivative followed by renal 1α-hydroxylation to the active hormonal metabolite 1α,25-dihydroxyvitamin D_3 constitutes the basic enzymatic conversions of the vitamin D endocrine system.[2-5] Like other steroid hormones, the biochemical action of vitamin D_3 is believed to be regulated by a nuclear mechanism of gene expression and de novo protein synthesis.[6] A crucial step in this process is the binding of the steroid hormone 1α,25-dihydroxyvitamin D_3 to tissue specific intracellular binding proteins and translocation of this complex to the nucleus where it initiates the hormonal response.

Other steroidal endocrine systems have shown a great deal of sensitivity toward structural analogues of the active hormone. These effects can be directly attributed to variations in the ligand receptor protein interaction.[7] Analogues of greater binding affinity exhibit enhanced or selective biochemical activity while derivatives which bind to the receptor molecule without promoting translocation or nuclear activation possess "antihormonal" or antagonistic properties.

Scheme I



A clinically useful vitamin D antagonist has not been discovered, although effective antagonists of mineralocorticoids, estrogens, and androgens are used clinically for a wide range of disorders.[8,9] A great deal of synthetic work has accompanied the biochemical advances in the vitamin D field, but few reported examples exist of analogues which possess unique biological activities.[10] Furthermore, most synthetic work has been directed toward metabolites, i.e., A-ring- and side-chain-hydroxylated derivatives. We considered it of interest to synthesize C_{19}-functionalized analogues of vitamin D_3 and to determine their biological

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