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NEW DITERPENES FROM THE GORGONIAN *SOLENOPODIUM EXCAVATUM*

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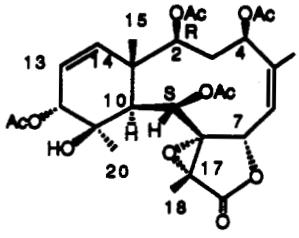
ABSTRACT.—Seven new briariane diterpene lactones were isolated from the gorgonian *Solenopodium excavatum* collected in New Guinea. One structure (**4**) was determined by X-ray diffraction and the remainder were elucidated by spectroscopic analysis. Six of the new briareolides, **4–9**, exist in a conformation that is quite uncommon for representatives of this fairly large family of compounds, namely one in which the bonds joining the 10-membered ring to the 6-membered ring are nearly diaxially oriented. Conformational energy calculations are discussed as are the structural features that are responsible for the uncommon conformation. Mild cytotoxic activity was confirmed for **4**.

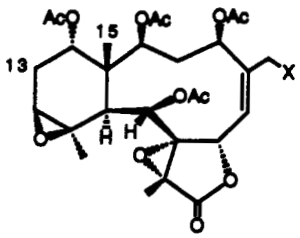
The octocoral genus *Briareum* has been the subject of a number of investigations which have uncovered a variety of oxygenated diterpenes, the majority of which possess the briariane skeleton (1–7), e.g., **1**, although some asbestinin (8–10), cladiellin (3), and cembrane (3) derivatives have also been reported. *Briareum* has been described as being near the transition, both taxonomically and chemically, between the Alcyonacea and Gorgonacea, the two major groups of octocorals (3). The genus *Solenopodium* is morphologically extremely close to *Briareum*. Two earlier studies on species identified as *Solenopodium* sp. have yielded briariane, cladiellin, and cembrane derivatives, thus revealing that *Briareum* and *Solenopodium* are also extremely similar chemically (11,12). Our earlier report (12) on diterpenes from a Great Barrier Reef species classified as *Solenopodium stechei* described briareolides closely related to those isolated (6) from a species classified as *Briareum stechei*, also collected from the great Barrier Reef. Owing to the lack of sharp taxonomic differences between these recorded species, it is possible that the *Solenopodium steckei* referred to in our earlier work (12) and the *Briareum steckei* reported by Bowden *et al.* (6) may be the same species. We have collected *Solenopodium excavatum* (Nutting), Family Briareidae, from New Guinea. [Although our specimen's characteristics matched those of *S. excavatum* best, the key feature distinguishing it from *S. stechei* was color of the calyces (brownish-yellow), and since color is a variable character, the specimen could be *S. stechei*.] We report here the characterization of 7 new briariane derivatives and two known ones. One of the diterpenes showed mild cytotoxic activity. Of most interest is the observation that most of the new briariane derivatives exist in a conformation uncommon for this group of natural products, i.e., with the bonds fusing the 10-membered ring to the 6-membered ring oriented nearly diaxially with respect to the latter. This was established by X-ray analysis in one case and revealed by nOe data and characteristic chemical shifts in others.

RESULTS

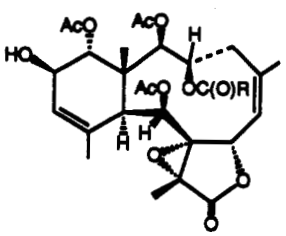
Specimens were frozen after collection and later freeze-dried prior to extraction with a series of solvents: hexane, CH_2Cl_2, and MeOH. From the CH_2Cl_2 extracts, diterpenes **1–9** were isolated by conventional chromatographic procedures (see Experimental). Compounds **1** and **2** were identified by comparison of their ^{1}H-nmr spectra with published data (3,12). The ^{1}H-nmr spectrum of 16-hydroxystecholide C [**3**] was very similar to that of **2**, with the key differences being an upfield shift in the spectrum of **3** of the AB quartet for H-16, -16′, a downfield shift of H-6, and the presence of only 4 acetate methyl groups (Table 1). This was consistent with a C-16 OH substituent in



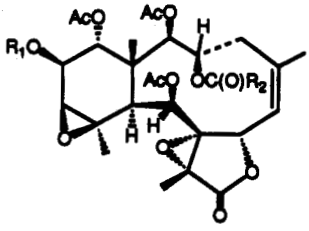
1



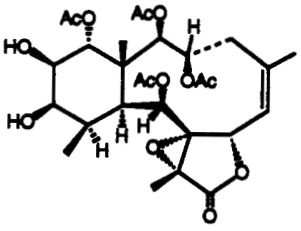
2 X=OAc
3 X=OH



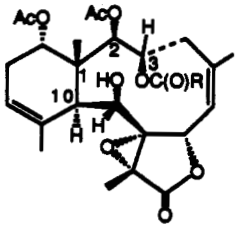
4 R=Pr
5 R=Me



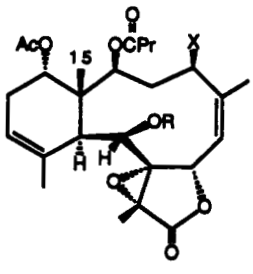
6 R_1=Ac, R_2=Me
7 R_1=H, R_2=Me
8 R_1=H, R_2=Pr



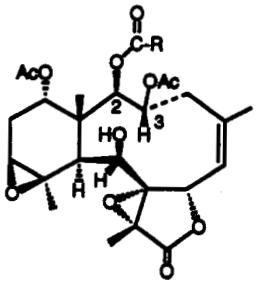
9



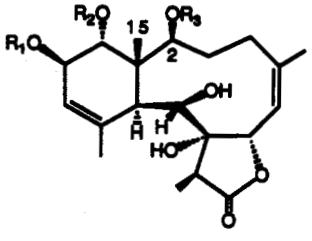
I R=Pr
II R=Me



III R=X=H
IV R=Ac, X=OAC



V R=Me
VI R=Pr



VII R_1,R_2,R_3=Ac, Ac, n-$C_5H_{11}CO$

place of an OAc group. Analysis of the COSY spectrum confirmed the H-2 to H-4, H-9 to H-10, H-12 to H-14 proton coupling sequences and allylic coupling between one of the H-16 protons (4.29 ppm) and H-6. These data, combined with the similarity of the ^{13}C-nmr data of **2** and **3** (Table 2), support the structure assigned to the latter. The nOe data observed for **3** (Table 3) also support this structure and provide evidence for a chair conformation for the cyclohexane ring with the C-14 acetate, C-1 methyl, and C-10 hydrogen all axially oriented, while C-2 and C-9 are equatorially directed. Key determining factors here are the strong H-2/H-10 nOe interaction, the small J's between H-14 and the vicinal H-13 protons, and an nOe between H-14 and H-15.

Stecholide I [**4**], the most abundant metabolite, crystallized nicely, and X-ray analysis established the structure shown. Proton assignments were made from the COSY spectrum which revealed weak correlations between H-13 and H-20 (homoallylic coupling) and also between H-10 and H-20. The X-ray structure revealed a dihedral angle of 160° for C-9/C-10/C-1/C-2 and a flattened chair conformation for the cyclohexane ring with the C-14 acetate group equatorially disposed. Thus **4** has an uncommon conformation for briareolides with the 10-membered ring emanating nearly

TABLE 1. ^{1}H-nmr Data for **2–9** ($CDCl_3$, 300 MHz).

Proton	Compound							
	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**
H-2	4.86 (1H,d,6.5)	4.81 (1H,d,6.5)	5.43 (1H,d,3.5)	5.42 (1H,d,3.4)	5.55 (1H,br s)	5.53 (1H,d,4.0)	5.51 (1H,br s)	5.73 (1H,d,3.1)
H-3	2.86 (1H,dd, 15,13) 2.05 (1H,m)	2.90 (1H,dd, 14.3,13.3) 2.08 (1H,m)	4.74 (1H,br d, 3.5)	4.70 (1H,m)	5.02 (1H,m)	4.99 (1H,br d, 3.2)	5.00 (1H,d,3.7)	4.76 (1H,br d, 3.3)
H-4	5.00 (1H,dd, 12.5,5.2)	5.12 (1H,dd, 13,5.3)	3.22 (1H,dd, 15.6,3.6) 2.08 (1H,m)	3.20 (1H,dd, 15.4,3.3) 2.07 (1H,m)	3.19 (1H,dd, 15.4,3.7) 2.08 (1H,m)	3.18 (1H,dd, 15.6,4.0) 2.08 (1H,m)	3.17 (1H,dd, 15.4,3.8) 2.08 (1H,m)	3.23 (1H,dd, 15.4,3.0) 2.10 (1H,m)
H-6	5.44 (1H,br d, 10.1)	5.78 (1H,br s)	5.37 (1H,d,8.9)	5.36 (1H,d,9.0)	5.38 (1H,d,8.0)	5.38 (1H,d,9.4)	5.36 (1H,d,9.2)	5.33 (1H,d,9.5)
H-7	5.79 (1H,d,10)	5.79 (1H,br s)	5.55 (1H,d,9.0)	5.54 (1H,d,9.2)	5.53 (1H,d,8.4)	5.53 (1H,d,9.0)	5.51 (1H,d,9.2)	5.55 (1H,d,9.3)
H-9	5.73 (1H,d,3.6)	5.75 (1H,d,3.6)	5.46 (1H,d,1.7)	5.46 (1H,s)	5.88 (1H,br s)	5.86 (1H,d,1.2)	5.85 (1H,d,1.3)	5.78 (1H,br s)
H-10	2.40 (1H,d,3.7)	2.37 (1H,d,3.7)	3.14 (1H,s)	3.12 (1H,s)	3.04 (1H,s)	3.01 (1H,s)	3.01 (1H,s)	2.71 (1H,br d, 4.9) 2.15 (1H,m)
H-11								
H-12	3.02 (1H,d,5.3)	3.02 (1H,br s)	5.61 (1H,br s)	5.58 (1H,d,br s)	3.40 (1H,d,2.2)	3.36 (1H,d,2.4)	3.36 (1H,d,2.6)	3.85 (1H,br s)
H-13	2.00 (2H,m)	2.05 (2H,m)	4.21 (1H,br d, 7.6)	4.19 (1H,br d, 6.6)	5.42 (1H,m)	4.16 (1H,dd, 8.7,2.5)	4.15 (1H,dd, 8.7,2.5)	3.78 (1H,dd, 10.2,3.2)
H-14	4.78 (1H,br s)	4.76 (1H,br s)	4.70 (1H,d,7.5)	4.70 (1H,d,6.8)	5.07 (1H,d,9.1)	4.71 (1H,d,8.9)	4.69 (1H,d,8.9)	5.06 (1H,d, 10.2)
H-15	1.01 (3H,s)	1.03 (3H,s)	1.56 (3H,s)	1.59 (3H,s)	1.54 (3H,s)[a]	1.55 (3H,s)[a]	1.53 (3H,s)	1.67 (3H,s)
H-16	5.32 (1H,dd, 16.4,1.9) 4.75 (1H,dd, 16.4,2.1)	4.47 (1H,d,14) 4.29 (1H,d,14.1)	1.79 (3H,s)	1.79 (3H,s)	1.83 (3H,s)	1.83 (3H,s)	1.80 (3H,s)	1.79 (3H,s)
H-18	1.67 (3H,s)	1.68 (3H,s)	1.47 (3H,s)	1.46 (3H,s)	1.55 (3H,s)[a]	1.54 (3H,s)[a]	1.52 (3H,s)	1.54 (3H,s)
H-20	1.37 (3H,s)	1.39 (3H,s)	1.81 (3H,s)	1.79 (3H,s)	1.45 (3H,s)	1.47 (3H,s)	1.46 (3H,s)	1.15 (3H,d,6.9)
Acetate	2.00 (3H,s)	1.98 (3H,s)	2.04 (3H,s)	1.95 (3H,s)	1.97 (3H,s)	2.03 (3H,s)	2.05 (3H,s)	2.00 (3H,s)
Methyl	2.01 (3H,s) 2.04 (3H,s) 2.11 (3H,s) 2.20 (3H,s)	2.04 (3H,s) 2.20 (3H,s)	2.10 (3H,s) 2.23 (3H,s)	2.04 (3H,s) 2.10 (3H,s) 2.20 (3H,s)	1.99 (3H,s) 2.03 (3H,s) 2.11 (3H,s) 2.22 (3H,s)	2.05 (3H,s) 2.13 (3H,s) 2.21 (3H,s)	2.12 (3H,s) 2.22 (3H,s)	2.03 (3H,s) 2.12 (3H,s) 2.17 (3H,s)
Butyrate			2.18 (2H,t,7.5) 1.59 (2H,m) 0.91 (3H,t,7.5)				2.23 (2H,t,7.3) 1.62 (2H,m) 0.94 (3H,t,7.7)	

TABLE 2. ^{13}C-nmr Data for **2–9** ($CDCl_3$, 75 MHz).

Carbon	Compound							
	2	**3**[a]	**4**[a]	**5**[b]	**6**[a]	**7**[b]	**8**[c]	**9**[a]
C-1	45.5	45.9	44.0	43.9	44.3	44.3	44.4	44.7
C-2	73.3	73.6	72.1	72.3	72.1	72.1	71.9	72.6
C-3	38.4	38.8	71.6	71.5	72.3	72.7	72.1	72.9
C-4	69.9	70.3	33.7	33.5	33.0	32.8	33.0	33.8
C-5	147.3	147.7	142.5	142.4	142.2	142.0	142.0	142.5
C-6	125.1	125.5	121.6	121.5	121.4	121.5	121.5	121.8
C-7	73.2	73.7	75.9	75.8	75.6	75.6	75.6	76.0
C-8	70.3	70.9	72.9	72.9	70.4	71.9	72.4	72.9
C-9	70.6	70.7	72.9	72.9	71.6	71.6	71.6	71.0
C-10	42.2	42.6	44.0	43.9	42.3	42.6	42.4	42.4
C-11	63.4	63.8	134.1	134.1	62.4	63.6	63.7	33.0
C-12	61.0	61.4	126.1	126.1	61.1	64.2	64.2	73.3
C-13	24.4	24.8	68.9	69.0	73.9	68.6	68.6	69.0
C-14	73.3	73.7	79.5	79.4	77.2	77.2	77.2	77.2
C-15	15.6	16.0	17.0	16.9	16.7	16.7	16.7	15.6
C-16	66.9	67.3	23.4	23.5	23.5	23.4	23.4	23.4
C-17	62.6	63.0	62.2	62.2	62.8	62.8	62.8	63.0
C-18	10.1	10.5	10.5	10.5	10.4	10.4	10.4	10.8
C-19	171.6	172.0	171.1	170.9	171.0	171.0	171.0	171.5
C-20	25.2	25.5	21.5	20.7	22.9	22.9	23.0	18.2
Acetate	170.7	171.0	171.0	171.0	170.0	170.6	170.6	171.4
	22.0	22.0	20.9	20.9	20.9	20.9	20.9	21.2
	170.6	171.2	169.8	169.7	169.8	170.3	169.2	170.7
	21.5	21.4	20.9	20.7	20.9	20.8	20.8	20.9
	170.4	170.8	169.2	169.2	169.1	169.3	168.2	169.3
	21.5	21.4	20.7	20.8	20.8	20.8	20.8	20.9
	167.3	167.7		169.8	168.2	168.2		168.3
	21.5	21.4		20.9	21.2	20.7		20.8
					170.0			
					20.9			
Butyrate			172.2				172.8	
			35.8				35.8	
			17.9				18.0	
			13.6				13.6	

[a]Assignments by analogy.
[b]Assignments from HETCOR (J=140 Hz).
[c]Assignments by HMQC and HMBC.

diaxially from the cyclohexane ring. That the solution conformation is essentially the same as that of the solid state is evident from nOe (Table 3) and coupling constant data. Thus H-10, which normally shows a large nOe with H-2, exhibited instead a large nOe with H-3 and no noticeable interaction with H-2. On the other hand, H-2 showed a large nOe with H-13. At the same time H-14 and H-13 share a relatively large coupling, 7.5 Hz, consistent with a pseudodiaxial orientation. Furthermore, the small but distinctive nOe between H-10 and H-15 is indicative of a diequatorial orientation of these two groups. These results are all consistent with a chair-like conformation of the cyclohexene ring with C-2 and C-9, diaxially directed therefrom. A similar conformation was deduced earlier from nOe results for two structurally similar briareolides **I** and **II** by Bowden *et al.* (3) who proposed that the ring juncture was oriented such that there was a ca. 90° angle between the bridgehead methyl and H-10. This would imply an overall conformation like that observed for **4**. The H-15 signal of those briareolides, ca. 1.54

TABLE 3. Selected NOe Data for **3**, **4**, **7**, and **9**.

Proton Irradiated	Compound			
	3	**4**	**7**	**9**
	Signals Enhanced (%)			
H-2	H-10 (20.5)	H-3 (6.2), H-13 (12.9)	H-3 (3.8), H-13 (11.6), H-10 (1.2)	H-3 (2.1), H-13 (10.8)
H-3	[a]	[a]	H-2 (8.8), H-10 (19.5), H-16 (4.4)	H-2 (5.1), H-10 (14.5), H-16 (1.8)
H-4	H-2 (5), H-16 (5.9)	[a]	[a]	[a]
H-10	H-2 (16.3), H-9 (7.8), H-20 (5.3)	H-3 (16.9), H-9 (5.2), H-7 (5.1)	H-2 (3.1), H-3 (20%), H-9 (8.5)	H-3 (15.1), H-9 (4.9), H-7 (4.4)
H-13	[a]	H-2 (16), H-12 (8), H-14 (1.3)	H-2 (21%), H-12 (8.4)	[a]
H-14	H-15 (3.6)	H-10 (5.7), H-13 (3.5), H-15 (2.4)	H-9 (1.9), H-15	H-12 (1.7), H-15 (12.8)

[a]No irradiation at this position.

ppm, like that of **4**, is shifted downfield markedly compared to the value reported for the vast majority of briareolides, 1.0–1.30 ppm. The only other briareolides whose H-15 resonance occurs near 1.5 ppm are those with a Δ^{13}-12-keto functionality. Thus it appears that the large H-10/H-3 and H-2/H-13 nOe's, small H-10/H-15 nOe, and the ca. 1.54 ppm shift of H-15 are distinguishing marks for the conformation adopted by **4**.

Stecholide J [**5**], another abundant metabolite, exhibited ^{1}H- and ^{13}C-nmr spectra nearly identical to those of **4** except that the signals for the propyl group of the butyrate ester in **4** were missing and in their place were signals for one additional acetate group. Hence the corresponding structure could be assigned. The protonated carbon chemical shifts were assigned from a HETCOR experiment. Since the proton chemical shift of H-15 and the H-14 coupling constant are almost identical in **4** and **5** they are assumed to have the same conformations.

The ^{1}H-nmr and ^{13}C-nmr data of **6**, stecholide K, clearly indicated that it too belonged to the briariane diterpene class, and a COSY spectrum confirmed the following coupling sequences: H-2 to H-4, H-6/H-7 and H-6/H-16, H-9/H-10, and H-12 to H-14. These correlations substantiated many of the proton shift assignments. The only differences between compounds **5** and **6** lay in epoxidation of the 11,12 double bond and acetylation of the hydroxyl group at C-13. The chemical shifts of the quaternary methyl groups were assigned by analogy with those of **7** for which nOe data was used to confirm assignments. Since H-15 in **6** has a low field resonance like that in **4** and **5**, and H-14 possesses a large coupling constant, **6** must be in the same uncommon conformation as found for **4** and **5**, with H-14 being β and axial. The small coupling between H-12 and H-13 is consistent with an axial/equatorial relationship that would be present if the 11,12-epoxide is β-oriented. The 11,12-β-epoxide configuration is also consistent with the ^{13}C-nmr shifts of C-11 and C-12 (63.4, 61.0), which falls in the range noted for other 11,12-β-epoxy briareolides (12).

The structure of stecholide L [**7**] could readily be assigned by comparison of its ^{1}H- and ^{13}C-nmr data with those of **6**. The multiplicities of all resonances in these two compounds were nearly identical as were the chemical shifts, except for one less acetate methyl signal in the spectrum of **7** and an upfield shift of H-13 and H-14 consistent with

replacing the C-13 acetoxy group of **6** with a hydroxyl group in **7**. NOe data provided a basis for assigning the quaternary methyl resonances. Irradiation of H-14 enhanced the 1.55 ppm signal (H-15) as did irradiation of H-7. The H-7 signal was overlapped by the H-2 signal, but it is assumed that only the former proton contributes to enhancement of H-15. H-2 and H-15 are nearly anti-periplanar. The H-20 protons (1.47 ppm) were enhanced by irradiation of H-12, H-10, H-9, and H-3. The vinyl methyl resonance (1.83 ppm) was enhanced by irradiation of H-6, and an nOe for H-18 was noted upon irradiation of H-9. The nOe noted between H-3 and H-20 argues for an 11,12-β-epoxide configuration and an overall conformation like that of **4**. The 63–64 ppm shift of carbons 11 and 12 is also consistent with an 11,12-β-epoxide (12). The nOe between H-12 and H-13 and the small coupling between these protons argues for a cis (and therefore α) configuration of these protons. Compound **7** can be assigned nearly the same conformation as found for **4–6** since the H-15 resonance is downfield (1.55 ppm) as in the latter set of compounds and the H-14/H-13 coupling is large indicating a 14-axial (and β) hydrogen orientation. The epoxide on the lactone ring is assigned an α orientation by virtue of the nOe between H-18 and H-9 and correspondence of the ^{13}C-nmr resonances for C-8 and C-17 in the group of diterpenes reported here and related compounds (1,3,4,12).

The ^{1}H-nmr spectrum of stecholide M [**8**] was virtually superimposable on that of **7** except that it contained one less acetate methyl signal and instead displayed the three resonances typical of the butyrate moiety as found in **4**. Structure **8** was thus inferred, and this was substantiated by results of HMQC and HMBC experiments which provided ^{13}C-nmr resonance assignments and skeletal connectivity evidence. The HMBC experiment showed correlations between all the acetate methyl groups and their neighboring carbonyl groups, and these carbonyl resonances showed further correlations to H-2, H-9, and H-14. Both H-7 and Me-18 protons showed correlations with the lactone carbonyl (C-19). By difference the butyrate group (carbonyl resonance at 172.8 ppm) was confirmed to be at position 3.

The ^{1}H-nmr resonances for stecholide N [**9**] were nicely resolved, and the results of a COSY spectrum readily confirmed the following proton coupling sequences: H-2 to H-4; H-6 to H-7; H-6 to H-16; H-9 to H-10 to H-11 to H-20; and H-12 to H-14. Although no crosspeaks fror H-11/H-12 were noted in the COSY spectrum, an nOe was noted between the H-12 signal and the methyl doublet due to H-20. These data and the correspondence of most ^{13}C-nmr resonances with those of **6–8** support structure **9**.

The stereochemistry and ring conformations of **9** are predicted to be the same as that established by X-ray analysis for **4**, since the H-15 signal occurs relatively far downfield as in **4** (and **5–8**), H-14 exhibits a large coupling (10.2 Hz) indicative of trans-diaxial coupling with H-13, and strong nOes were observed between H-2/H-13 and H-3/H-10.

X-RAY ANALYSIS OF STECHOLIDE I [**4**].—The final atomic parameters of the non-hydrogen atoms are listed in Table 4. A stereo ORTEP drawing of a single molecule of **4** is shown in Figure 1. The absolute configuration of the molecule is assigned on the basis of other briareins whose absolute configurations were determined by X-ray diffraction (1,13). The crystal structure determination clearly showed that **4** has an unusual conformation about the bridging bond, C-1–C-10, compared to other known briariane derivatives. The C-2/C-1/C-10/C-9 torsion angle, ϕ, is 160° in **4**, while in related briariane derivatives for which X-ray structures were available, ϕ ranges between 56° [briarein A (13)] and 92° [brianthein W (15)]. The conformational difference in **4** may be described as a 90° twist about the bridging bond which resulted in a quite different conformation for the cyclodecene ring (Table 5). The six-membered ring assumes a half-chair conformation.

TABLE 4. Atomic Parameters of the Non-hydrogen Atoms of **4**. ESDs are in parentheses.

Atom	*x*	*y*	*z*	Ueq[a]
O-1	0.1642 (1)	0.10196 (0)	0.5286 (2)	0.0232 (6)
O-2	0.0881 (2)	−0.1038 (4)	0.5711 (3)	0.0485 (9)
O-3	0.0302 (1)	0.1264 (3)	0.3621 (2)	0.0232 (6)
O-4	−0.0068 (2)	−0.0916 (5)	0.2521 (3)	0.060 (1)
O-5	0.2864 (1)	0.4921 (4)	0.0541 (2)	0.0255 (6)
O-6	0.3876 (1)	0.5499 (4)	−0.0489 (2)	0.0373 (8)
O-7	0.2989 (1)	0.1952 (4)	−0.0420 (2)	0.0248 (6)
O-8	0.3986 (1)	0.0926 (4)	0.2556 (2)	0.0227 (6)
O-9	0.4369 (1)	−0.1521 (4)	0.2137 (3)	0.0368 (8)
O-10	0.2999 (1)	−0.4022 (4)	0.4276 (3)	0.0346 (7)
O-11	0.3010 (1)	−0.1080 (4)	0.5707 (2)	0.0254 (6)
O-12	0.4343 (1)	−0.0819 (4)	0.5946 (2)	0.0378 (8)
C-1	0.2543 (1)	0.0375 (4)	0.3788 (3)	0.0189 (7)
C-2	0.1657 (1)	0.0415 (4)	0.4039 (3)	0.0199 (7)
C-3	0.1042 (2)	0.1313 (4)	0.3110 (3)	0.0203 (8)
C-4	0.1206 (2)	0.3058 (4)	0.2812 (3)	0.0236 (8)
C-5	0.1110 (2)	0.3287 (4)	0.1420 (3)	0.0246 (8)
C-6	0.1706 (2)	0.3449 (4)	0.0759 (3)	0.0231 (8)
C-7	0.2584 (2)	0.3585 (4)	0.1217 (3)	0.0211 (8)
C-8	0.3103 (1)	0.2193 (4)	0.0932 (3)	0.0210 (8)
C-9	0.3247 (1)	0.0709 (4)	0.1700 (3)	0.0194 (8)
C-10	0.2511 (1)	0.0233 (4)	0.2336 (3)	0.0188 (7)
C-11	0.2200 (1)	−0.1371 (4)	0.1854 (3)	0.0227 (8)
C-12	0.2197 (2)	−0.2624 (4)	0.2583 (3)	0.0250 (8)
C-13	0.2514 (2)	−0.2666 (4)	0.3942 (3)	0.0241 (8)
C-14	0.2947 (2)	−0.1152 (4)	0.4356 (3)	0.0206 (8)
C-15	0.3016 (2)	0.1809 (4)	0.4383 (3)	0.0223 (8)
C-16	0.0260 (2)	0.3228 (6)	0.0772 (3)	0.038 (1)
C-17	0.3687 (2)	0.2819 (4)	0.0183 (3)	0.0248 (8)
C-18	0.4494 (2)	0.2185 (5)	0.0079 (4)	0.034 (1)
C-19	0.3523 (2)	0.4546 (5)	0.0023 (3)	0.0282 (9)
C-20	0.1869 (2)	−0.1418 (5)	0.0490 (3)	0.031 (1)
C-21	0.1244 (2)	0.0140 (5)	0.6039 (3)	0.0300 (9)
C-22	0.1316 (2)	0.0856 (6)	0.7310 (3)	0.038 (1)
C-23	−0.0188 (2)	0.0042 (5)	0.3279 (3)	0.0313 (9)
C-24	−0.0909 (2)	0.0072 (6)	0.3923 (5)	0.042 (1)
C-27	0.4508 (2)	−0.0284 (4)	0.2652 (3)	0.0263 (9)
C-28	0.5276 (2)	0.0128 (6)	0.3454 (4)	0.040 (1)
C-29	0.3731 (2)	−0.0923 (5)	0.6382 (3)	0.0282 (9)
C-30	0.3678 (3)	−0.0871 (6)	0.7749 (3)	0.044 (1)
C-25a[b]	−0.1722 (0)	−0.0070 (0)	0.3282 (0)	0.030 (1)
C-26a	−0.1905 (0)	0.1363 (0)	0.2426 (0)	0.039 (2)
C-25b	−0.1635 (0)	0.0421 (0)	0.2634 (0)	0.045 (2)
C-26b	−0.2384 (0)	0.0033 (0)	0.3170 (0)	0.046 (2)
OW-1	0.4163 (1)	−0.4772 (4)	0.2741 (3)	0.0382 (8)

[a] $Ueq = \frac{1}{3}\Sigma\Sigma Uij\ a_i^* a_j^*\ \hat{a}_i \cdot a_j$.
[b] Disordered

The solvent H_2O molecule forms intramolecular bridging hydrogen bonds between the acetoxy oxygen on C-9 and the hydroxyl oxygen on C-13: OW-H . . . O-9=2.869 Å, O-10-H . . . OW=2.838 Å (Figure 1). In addition the H_2O molecule bridges two symmetry-related molecules through an intermolecular hydrogen bond: OW-H . . . O-12 [1−x, −½+y, 1−z]=2.858 Å.

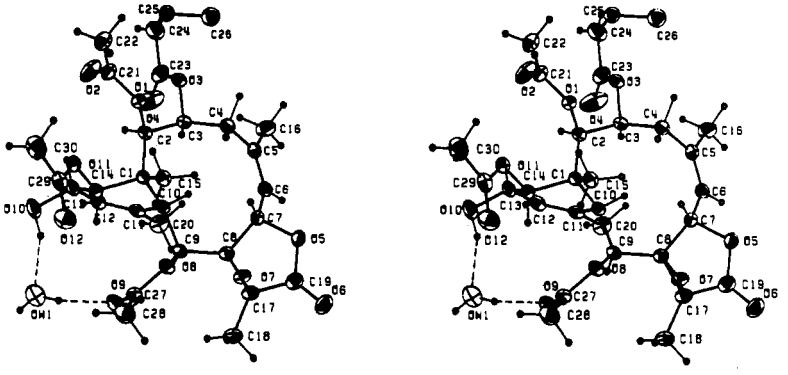
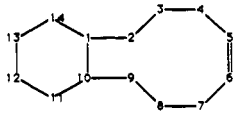


FIGURE 1. A stereoview of an ORTEP drawing of **4** showing the atom numbering. Hydrogen bonds are indicated by dashed lines. Thermal ellipsoids are at the 50% level.

DISCUSSION

The new briareolides described here reconfirm that there is a close chemical as well as taxonomic relationship between the *Briareum* and *Solenopodium* species collected in Palau, New Guinea and the Great Barrier Reef. The chemistry of these species is similar

TABLE 5. Comparison of Endocyclic Torsion Angles in Some Briarein Derivatives with Related Substitution Pattern.



Torsion Angles	Compounds			
	Briareolide B[a]	Brianthein W[b]	Crystal structure of **4**	2nd low energy conformer of **4**
Ten membered ring				
10-1-2-3.....	−82	−64	−38	−32
1-2-3-4.....	163	132	−54	−75
2-3-4-5.....	−57	−55	129	173
3-4-5-6.....	−44	−64	−104	−90
4-5-6-7.....	−9	−4	−6	−2
5-6-7-8.....	112	112	112	56
6-7-8-9.....	−101	−68	−85	−118
7-8-9-10.....	96	63	32	134
8-9-10-1.....	−99	−134	−110	−94
9-10-1-2.....	64	92	160	80
Six membered ring				
1-10-11-12.....	52	1	−19	49
10-11-12-13.....	−48	2	−2	−12
11-12-13-14.....	50	24	−8	−46
12-13-14-1.....	−55	−55	41	68
13-14-1-10.....	54	59	−61	−32
14-1-10-11.....	−53	−30	47	−24

[a]Data are from Pordesino *et al.* (1).
[b]Data are from Cardelline *et al.* (15).

to that of the *Briareum* species from the Caribbean that have been examined. However, it is interesting that thus far no asbestinin-type compounds (8–10) have been found among the Pacific *Briareum* spp. and its close relative, *Solenopodium*. Briariane-type compounds have also been isolated from other gorgonians, soft corals (Alcyonacea), sea pens, and nudibranch molluscs which are presumed to graze on sea pens (7).

The unusual conformation of **4–9** and **I, II** prompted an investigation of the energetics of alternate possible conformations. Energy minimization calculations using the molecular mechanics program MM2 on the crystal structures of **4** and briareolide B (1) and related model compounds showed that the cyclodecene-cyclohexane bicyclic system has two distinct low energy regions with ϕ ranges between 60–90° and 125–165°. Each compound is forced to a conformer within these regions by specific substitution pattern and ring unsaturation. The lowest energy conformer of **4** (ϕ=155°) is very similar to that in the crystal structure, while a second low energy conformer (ϕ=80°) has about 7 Kcal/mol higher steric energy. For briareolide B (1), the lowest energy conformer (ϕ=65°) has about 4.5 Kcal/mol lower energy than the second minimum energy conformer (ϕ=152°).

A review of all reported briariane derivatives (1–7) reveals no evidence (i.e., low field H-15 absorption or reported nOe data) to indicate that the conformation adopted by **4–9** and **I, II** is shared by any other compounds in this family. Since the briareolides display a wide assortment of oxygenation and unsaturation patterns, it was of interest to attempt to discern the features in **4–9** and **I, II** that cause the dramatic conformational change relative to all other briareolides. Two features can be singled out: (a) 2β,3β-disubstitution and (b) a 13-oxygen substituent. Compounds **I, II** (3), and **4–9** share a common substitution pattern in the 10-membered ring, which includes vicinal substitution at C-2, -3, a feature which is uncommon among the briareolides. That this substitution pattern plays a significant role in causing the unusual conformation observed for **I, II, 4–9**, is seen from the fact that 11,12-deoxystecholide E [**III**] (12), which differs from **II** only in the absence of the 3-acyloxy group, has the conventional briareolide conformation. Adding a substituent at C-4 instead of C-3, e.g., 11,12-deoxystecholide A acetate [**IV**] (12), does not cause a change to the uncommon conformation.

Changing the 2β,3β configuration of **4** to 2β,3α and epoxidizing the C-11, -12 double bond (e.g., **V, VI**) results in reversion to the normal conformation. It seems likely that the change in configuration at C-3 is the most important factor in causing this conformational change as the rigidity and geometry of the double bond and epoxide would be quite similar.

What effect does the 13β-OH group in **4–9** have? For a briareolide in the common conformation, a 13β substituent would result in significant steric repulsion with the 15-Me group, a 1,3-diaxial interaction. Hence such substitution would be expected to favor conversion to the uncommon briareolide conformation. However, a 13 substituent by itself is not adequate to cause the conformational change, as may be seen from the fact that **VII** (14) exists in the normal conformation.

In summary, the examples available to date indicate that 2β,3β disubstitution is critical to conversion to the uncommon conformation. The precise nature of functionality needed in the six-membered ring is not so clear, but the steric influence of a 13β-substituent would certainly also operate in favor of the uncommon conformation. The strain in the 6-membered ring caused by a double bond or epoxide at C-11, -12 may also reduce the difference in energy between alternate chair forms and so contribute to the overall lower energy of the uncommon conformation of **4–9** and **I, II**.

Lactone **4** showed weak cytotoxicity against mouse leukemia cells (P-388), ED_{50}=23 μg/ml, whereas all the others were inactive.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr data were obtained at 300 MHz for ^{1}H and 75 MHz for ^{13}C in the solvents specified on a Varian VMR 300 instrument. Optical rotations were obtained on a Rudolph Autopol III digital polarimeter and Ir spectra on a Bio-Rad 3240-SPC FT instrument.

EXTRACTION AND ISOLATION.—Specimens were collected at −15 m using SCUBA in the vicinity of Madang, Papua New Guinea, then frozen and stored at −20°. A voucher specimen is held at the Smithsonian Institution, Washington, D.C., catalogued as *S. excavatum*, USNM 88852. Shortly prior to extraction the specimens were freeze-dried (162 g dry wt) and then soaked in hexane (6 h, 24 h), CH_2Cl_2 (24 h), and MeOH (24 h) to give four extracts: 1.0, 0.31, 2.35, and 2.83 g, respectively. The CH_2Cl_2 extract was resolved by vacuum flash chromatography over Si gel using hexane-EtOAc (1:1) as eluent. Fractions selected on the basis of nmr and tlc analyses were pooled and further purified by normal phase hplc [hexane-EtOAc (4:6)], and fractions therefrom were resolved by C-18 reversed-phase hplc [MeOH-H_2O (6:4)] to give the following 9 compounds.

Compound **1**.—Yield 7.9 mg; mp 103° [lit. (3) glass].

16-Acetoxystecholide C acetate [**2**].—Trace amount.

16-Hydroxysteocholide C acetate [**3**].—Yield 3.5 mg; white powder; $[\alpha]^{20}D$ +56.7° (c=0.12, $CHCl_3$); ir (KBr) 3505 br, 1781, 1740, 1370, 1238, 1220 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; nOe's (H irradiated↦H enhanced) (%) H-2↦H-10 (20.5); H-3↦H-2 (5), H-10 (5.9); H-6/7/9↦H-10, H-18, H-20; H-10↦H-2 (16.3), H-9 (7.8), H-20 (5.3); H-12↦H-20 (6.5); H-14↦H-15 (3.6); hrfabms m/z $[M+Na]^+$ 603.2010 ($C_{28}H_{36}O_{13}Na$ requires 603.2054).

Stecholide I [**4**].—Yield 55 mg; white crystals; mp 116–118°; $[\alpha]^{20}D$ −40.8 (c=2.55, $CHCl_3$); ir ($CHCl_3$) 1782, 1744 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; nOe's (H irradiated↦H enhanced) (%) H-2↦H-3 (6.2), H-13 (12.9); H-6↦H-16 (5.4); H-10↦H-3 (16.9), H-9 (5.2), H-7 (5.1), H-6 (1.4), H-20 (3.8), H-15 (sm); H-13↦H-2 (16), H-12 (8), H-14 (1.3); H-14 (partial irradiation H-3 also)↦H-13 (3.5), H-10 (5.7), H-15 (2.4); hrfabms m/z $[M+Na]^+$ 615.2442 ($C_{30}H_{40}O_{12}Na$ requires 615.2417).

Stecholide J [**5**].—Yield 50 mg; white crystals; mp 204°; $[\alpha]^{20}D$ −39.2° (c=0.5, $CHCl_3$); ir ($CHCl_3$) 3666 (w), 3493, 1792, 1738 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; hrfabms m/z $[M+Na]^+$ 587.2105 ($C_{28}H_{36}O_{13}Na$ requires 587.2104).

Stecholide K [**6**].—Yield 6 mg; clear glass; $[\alpha]^{20}D$ −58.5° (c=0.39, $CHCl_3$); ir ($CHCl_3$) 1781, 1742, 1374 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; hrfabms m/z $[M+Na]^+$ 645.2130 ($C_{30}H_{38}O_{14}Na$ requires 645.2159).

Stecholide L [**7**].—Yield 20 mg; white crystals; mp 148–150°; $[\alpha]^{20}D$ −53.3 (c=2.28, $CHCl_3$); ir ($CHCl_3$) 3568, 1787, 1743, 1448, 1366, 1244 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; nOe's (H irradiated↦H enhanced) (%) H-2/7↦H-3 (3.8), H-13 (11.6), H-4 (4.1), H-10 (1.2), H-15 (5.6); H-3↦H-2 (8.8), H-10 (19.5), H-16 (4.4), Me-20 (5); H-6↦Me-16 (7), H-10 (sm); H-9↦H-10 (2.9), H-14 (2), Me-18 (1.54)(4), Me-20 (1.47)(4); H-10↦H-2 (3.1), H-3 (20), H-6 (sm), H-9 (8.5), Me-20 (1.47)(2.3); H-12↦H-13 (4.5), Me-20 (1.8); H-13↦H-2 (21), H-12 (8.4), H-14 (trace); H-14↦H-9 (1.3), H-15 (small); hrfabms m/z $[M+Na]^+$ 603.2054 ($C_{28}H_{36}O_{13}Na$ requires 603.2054).

Stecholide M [**8**].—Yield 18 mg; white crystals; mp 108°; $[\alpha]^{20}D$ −62.6° (c=0.76, $CHCl_3$); ir ($CHCl_3$) 3687, 1785, 1738, 1602 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; hrfabms m/z $[M+Na]^+$ 631.2370 ($C_{30}H_{40}O_{13}Na$ requires 631.2367).

Stecholide N [**9**].—Yield 3 mg; clear glass; $[\alpha]^{20}D$ −53.0° (c=0.2, $CHCl_3$); ir ($CHCl_3$) 3575, 1783, 1745, 1364, 1240 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; nOe's (H irradiated↦H enhanced) (%) H-2↦H-3 (2.1), H-13 (10.8); H-3↦H-2 (5.1), H-10 (14.5), H-16 (1.8); H-7↦H-4 (8), H-10 (3), H-15 (11.2); H-10↦H-3 (15.1), H-9 (4.9), H-7 (4.4), H-6 (3.4); H-12/H-13↦H-2 (5.4), H-14 (2.1), H-20 (1.8); H-14↦H-12 (1.7), H-15 (12.8); hrfabms m/z $[M+Na]^+$ 605.2188 ($C_{28}H_{36}O_{13}Na$ requires 605.2210).

X-RAY EXPERIMENTAL[1].—Compound **4** crystallized from MeOH solution as colorless blocks. A crystal of size 0.35×0.12×0.10 mm was used for all X-ray measurements. Cell dimensions were determined by a least-squares fit to ±2θ of 52 reflections (15°<θ<30°) measured at −110° using CuKα_1 radiation. Crystal data: $C_{30}H_{40}O_{12}\cdot H_2O$, F.W.=610.7, monoclinic, $P2_1$, a=16.970 (1), b=8.4855 (7), c=10.8381 (6) Å, β=98.565 (7)°, V=1543.3 Å^3, Z=2, D_x=1.314 gm/cm^3, μ (CuKα)=8.7 cm^{-1}, λ=1.54178 Å.

[1]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK.

Intensities of 3400 unique reflections within $0<2\theta<150°$ were collected on an Enraf-Nonius CAD-4 diffractometer fitted with a N_2 low temperature device using CuKα radiation. The ω–2θ scan technique was employed with a variable scan angle ($0.80+0.20 \tan\theta$) and a variable horizontal aperture, ($3.5+0.86 \tan\theta$) mm. Three intensity control monitors were measured every 7200 sec of X-ray exposure time, and they showed a maximum variation of <4%. Intensities were corrected for Lorentz and polarization factors, but no absorption correction was made; 3153 reflections with intensities greater than $2\sigma(I)$ were considered observed, and these were used in the structure refinement.

The structure was solved by the direct methods using the program MITHRIL (16). The structure showed the presence of a solvent H_2O molecule. Preliminary refinement indicated disorder in the propyl chain with two possible orientations for each of the two terminal C atoms. The structure was refined by a full-matrix least-squares routine using the program SHELX76 (17). The quantity $\Sigma\omega\ (Fo-Fc)^2$ was minimized, where ω is the weighting function, $1/\sigma^2(Fo)$. The two disordered atoms were initially refined isotropically with 0.5 occupancy and their positions were kept fixed during the final stages of refinements. All the hydrogen atoms (except those bonded to the two disordered atoms) were located from the difference Fourier map. Hydrogen atom positions were refined with a fixed isotropic thermal parameter. All non-hydrogen atoms (except the disordered ones) were refined anisotropically. The refinement converged to $R=0.039$, $Rw=0.050$, $S=1.9$, $(\Delta/\sigma)\ max=0.03$.

Molecular mechanics calculations were performed with the program MM2 (18) using the normal force field parameters. Endocyclic dihedral drive facility was employed to explore steric energy profile by changing C-2/C-1/C-10/C-9 torsion angle (ϕ) through 0 to 180° in steps of 10°. Each low energy conformer was energy-minimized.

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LITERATURE CITED

1. E.O. Pordesimo, F.J. Schmitz, L.S. Ciereszko, M.B. Hossain, and D. van der Helm, *J. Org. Chem.*, **56**, 2344 (1991).
2. J. Kobayashi, J.F. Cheng, H. Nakamura, Y. Ohizumi, Y. Tomotake, T. Matsuzaki, K.J.S. Grace, R.S. Jacobs, Y. Kato, L.S. Brinen, and J. Clardy, *Experientia*, **47**, 501 (1991).
3. B.F. Bowden, J.C. Coll, and I.M. Vasilescu, *Aust. J. Chem.*, **42**, 1705 (1989).
4. B.F. Bowden, J.C. Coll, I.M. Vasilescu, and P.N. Alderslade, *Aust. J. Chem.*, **42**, 1727 (1989).
5. S.J. Coval, S. Cross, G. Bernardinelli, and C.W. Jefford, *J. Nat. Prod.*, **51**, 981 (1988).
6. B.F. Bowden, J.C. Coll, W. Patalinghug, B.W. Skelton, I. Vasilescu, and A.H. White, *Aust. J. Chem.*, **40**, 2085 (1987).
7. D.J. Faulkner, *Nat. Prod. Rep.*, **9**, 323 (1992).
8. J.J. Morales, D. Lorenzo, and A.D. Rodriguez, *J. Nat. Prod.*, **54**, 1368 (1991).
9. S.J. Selover, P. Crews, B. Tagle, and J. Clardy, *J. Org. Chem.*, **46**, 964 (1981).
10. D.B. Stierle, B. Carte, D.J. Faulkner, B. Tagle, and J. Clardy, *J. Am. Chem. Soc.*, **102**, 5088 (1980).
11. A. Groweiss, S.A. Look, and W. Fenical, *J. Org. Chem.*, **53**, 2401 (1988).
12. S.J. Bloor, F.J. Schmitz, M.B. Hossain, and D. van der Helm, *J. Org. Chem.*, **57**, 205 (1992).
13. J.E. Burks, D. van der Helm, C.Y. Chang, and L.S. Ciereszko, *Acta Crystallogr.*, **B33**, 704 (1977).
14. A. Clastres, P. Laboute, A. Ahond, C. Poupat, and P. Potier, *J. Nat. Prod.*, **47**, 162 (1984).
15. J.H. Cardellina, J.R. James, M.H.M. Chen, and J. Clardy, *J. Org. Chem.*, **49**, 3398 (1984).
16. C.J. Gilmore, "MITHRIL. A Computer Program for the Automatic Solution of Crystal Structures from X-ray Data," Department of Chemistry, University of Glasgow, Scotland, UK, 1983.
17. G.M. Sheldrick, "SHELX76. Program for Crystal Structure Determination," University of Cambridge, Cambridge, England, 1984.
18. N.L. Allinger, "MM2 Program," Quantum Chemistry Program Exchange, University of Indiana, Bloomington, Indiana, 1985.

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