

Structure Elucidation of Briarane Diterpenoids Using Molecular Mechanics Calculations

Yasuto Uchio,*[1] Natsuki K. Kubota,[2] Takeharu Haino,[2] and Yoshimasa Fukazawa*[2]

[1]School of Allied Medical Sciences, Faculty of Medicine, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8544

[2]Department of Chemistry, Graduate School of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8526

Received December 22, 2005; E-mail: uchio@health.nop.kagoshima-u.ac.jp

A combination of NMR analysis and molecular mechanics calculations has succeeded in the determination of the stereostructure of flexible briarane diterpenoids. The molecular mechanics calculations disclosed the multi-conformational nature of the briarane diterpenoids and gave a successful reproduction of the observed ^3J coupling constants to give the correct relative configuration of each stereogenic center in these molecules. An X-ray crystallographic study of these diterpenoids supports the reliable performance of the structure elucidation of these compounds.

The briarane diterpenoids have so far been found only in marine organisms (Coelenterates)[1] and to date a wide variety of new briaranes[2] have been reported with interesting chemistry and bioactivity. The unique diterpenes of the bicyclo[8.4.0]tetradecane skeleton, which may be generated by cyclization of a fourteen-membered cembrane ring,[3] have a flexible 10-membered ring system. This causes some peak broadenings in the NMR spectrum due to slow conformational inversion of the flexible ring as reported[4] for briarane diterpens from an Australian *Briareum* species. The flexible nature of the ring system hampered the structure elucidation. Thus, the stereostructures of briaranes are generally elucidated by X-ray diffraction[3,5] and/or by comparison of NMR spectral data of the compound of interest with that of a molecule determined by the X-ray method. We thought it necessary to develop an efficient method to determine the stereostructure of flexible compounds without the aid of X-ray crystallographic analysis.

In such a flexible molecule there should be several conformers. Determination of significantly populating conformers in a flexible molecule is a matter of long-standing interest. Conformations have been analyzed mainly with NMR spectroscopy by taking advantage of the torsion angle-dependent ^3J coupling constants[6] and distance-dependent NOE.[7] However, ^3J coupling constants in such a flexible system do not give as clear an answer as in a rigid chair cyclohexane. Since the coupling constant is usually a result of the weighted average of several significantly populating conformers, it is not easy to obtain a successful deconvolution of the observed ^3J coupling constant into the segments; hence, it is not simple to determine the stereochemical relationship between some stereogenic centers. Moreover, it is difficult to determine exactly which conformer affords the NOE. Therefore, structure analysis based on the NOE occasionally gives misleading results.[8] Molecular mechanics calculations, however, have been known to overcome these difficulties. In order to solve the conformational multiple minimum problem, a number of different methods have been proposed.[9] We employed the lowmode search[10] algorithm within these methods. It gave a set of conformers and the predicted ^3J coupling constants by which we can determine the relative configurations of several stereogenic centers of some briarane diterpenoids. In this paper, we report on a general method to determine the stereostructure of flexible compounds with a combination of NMR analysis and molecular mechanics calculations without the help of X-ray crystallographic analysis.

Results and Discussion

The briarane diterpenoids (**1**, **2**, **3**, and **4**) analyzed in this work (Fig. 1) have been isolated from the gorgonian coral

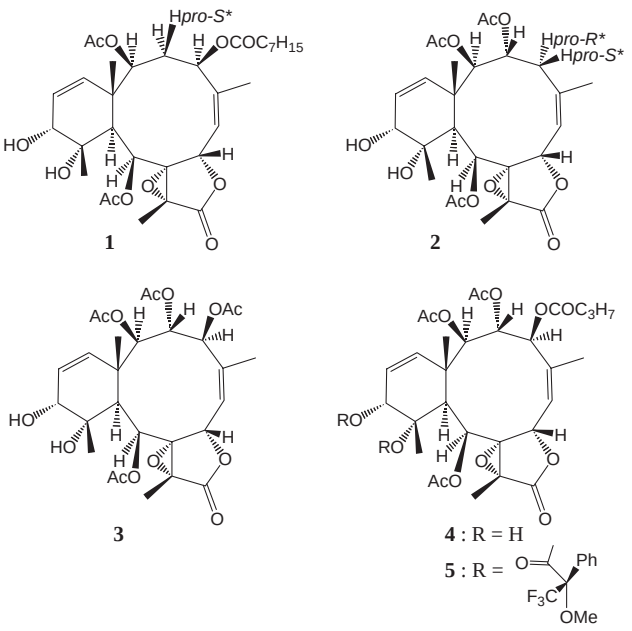

Fig. 1. Structures of briarane diterpenoids.

Published on the web June 6, 2006; doi:10.1246/bcsj.79.914

Table 1. ^{1}H NMR Chemical Shifts[a] and Assignments at 500 MHz in $CDCl_3$ for Compounds **1**–**4**

Position	**1**	**2**	**3**	**4**
2	4.61 (7.0, 1.0)[b]	4.74 (1.0)	4.67 (1.4)	4.68 (1.5)
3	2.09 (14.5, 7.0, 5.5)[c] 2.93 (14.5, 12.5, 1.0)[d]	5.58 (12.5, 5.5, 1.0)	6.09 (10.3, 1.4)	6.09 (10.4, 1.5)
4	5.04 (12.5, 5.5, 1.5)[e]	1.95 (14.1, 12.5)[c] 2.92 (14.1, 5.5, 1.5)[d,e]	5.12 (10.3, 1.1)[e]	5.11 (10.4)[e]
6	5.45 (9.5)[e]	5.41 (9.5)[e]	5.57 (10.0)[e]	5.57 (10.1)[e]
7	5.75 (9.5)	5.69 (9.5)	5.92 (10.0)	5.90 (10.1)
9	5.94 (4.0)	5.92 (4.0)	5.96 (4.1)	5.95 (4.1)
10	2.55 (4.0)	2.59 (4.0)	2.68 (4.1)	2.69 (4.1)
12	3.69 (6.0, 1.0)[f]	3.68 (6.0, 5.0)[f]	3.70 (6.5, 4.3)[f]	3.69 (6.0, 4.5)[f]
13	5.82 (10.5, 6.0)	5.82 (10.0, 6.0)	5.84 (10.3, 6.5)	5.84 (10.3, 6.0)
14	5.39 (10.5)	5.42 (10.0)	5.48 (10.3)	5.48 (10.3)
15	1.18 (s)	1.13 (s)	1.13 (s)	1.13 (s)
16	2.12 (1.5)[e]	1.95 (brs)[e]	2.14 (1.6)[e]	2.16 (1.1)[e]
19	1.69 (s)	1.68 (s)	1.67 (s)	1.67 (s)
20	1.15 (s)	1.13 (s)	1.15 (s)	1.14 (s)
11-OH	2.75 (s)[g]	2.77 (s)[g]	2.73 (brs)[g]	2.73 (brs)[g]
12-OH	2.26 (brs)[g]	2.09 (5.0)[g]	2.30 (brs)[g]	2.18 (4.5)[g]
2-Acetate	2.12 (s)	2.19 (s)[h]	2.16 (s)	2.16 (s)
3-Acetate		2.11 (s)[h]	2.08 (s)	2.07 (s)
4-Acetate			2.01 (s)	
4-Octanoate 2′	2.30 (2H, t, 7.5)		4-Butyrate 2′	2.23 (2H, m)
3′	1.61 (2H, qui, 7.5)		3′	1.59 (2H, sxt, 7.5)
4′–7′	1.20–1.35 (8H, m)		4′	0.91 (3H, t, 7.5)
8′	0.88, 3H (t, 7.5)			
9-OAc	2.23 (s)	2.11 (s)	2.29 (s)	2.29 (s)

a) In ppm; J (Hz) or multiplicity (in parentheses). b) This signal actually appeared as a broad doublet ($J = 7.0$ Hz) with the half-band width of ≈2.7 Hz; sharpened by the irradiation at δ 2.93. Inversely, the irradiation at δ 4.61 sharpened the signal at δ 2.93. Therefore, we estimated the small vicinal coupling at least 1.0 Hz between H-2 and H-$3_{pro\text{-}S^*}$. c) *pro-R**. d) *pro-S**. e) Small allylic couplings of H_3-16 (at 2.12 (**1**), 1.95 (**2**), 2.14 (**3**), and 2.16 (**4**)) and H-4 (at 5.04 (**1**), 2.92 (**2**), 5.12 (**3**), and 5.11 (**4**)) were removed by irradiation of H-6 signal (5.45 (**1**), 5.41 (**2**), 5.57 (**3**), and 5.57 (**4**)). f) The smaller coupling of the two disappeared by the addition of D_2O. g) These protons disappeared by the addition of D_2O. h) These values may be interchanged.

Pachyclavularia violacea.[11] However, these diterpenoids were found also by Iwagawa and co-workers in a *Briareum* sp. from the same area in 1997.[12] They deduced the relative stereochemistries of this series of briaranes from an X-ray analyzed compound and the NMR spectral similarities among them. They also reported the structure[12b] of **1** from the spectral similarities; however, it cannot be conclusive evidence of the structure, especially the relative configuration of the C-4. Hence, we independently analyzed the stereochemistries on our own compounds by a new method.

The planar structures of the four compounds were elucidated by ^{1}H and ^{13}C NMR spectroscopic studies assisted by 2D NMR experiments (COSY, HMBC, and COLOC), thus making unambiguous assignments of ^{1}H (Table 1) and ^{13}C NMR spectra (experimental) of the briaranes.

Structure of 1. The relative stereochemistry of **1** was elucidated from NOESY experiments. In the NOESY experiments, correlations were observed for the pairs H-9/H_3-19, H-9/H_3-20, and H_3-20/H-12. In addition, one of the protons of C-3 showed NOESY correlations with H_3-15 and H-7. As per convention on the analysis, H-10, H_3-15, and H_3-20 were assigned to the α, β, and β faces, respectively, since no NOE interactions from H-10 to H_3-15 and H_3-20 were found. Furthermore, direct NOE observation between H_3-15 and H_3-20 was unsuccessful because the chemical shifts of these protons are so close to each other. When we considered these NOE correlations it was found that the protons (H_3-15, H_3-19, H-7, H_3-20, and H-12) were located on the same face of the molecule and assigned as on the β side. These experiments disclosed the relative stereochemistry of the basic skeleton of **1** excluding three acyloxy substituents from the 10-membered ring (Fig. 2).

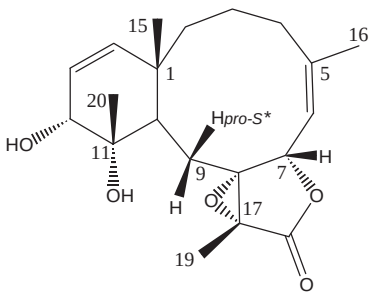

Fig. 2. Basic structure of **1**.

The assignment of the relative stereochemistry of the stereogenic centers of oxygen carrying carbons on a 10-membered ring is not straightforward since the 10-membered ring is be-

lieved to be flexible in solution. In order to solve this problem, a molecular mechanics calculation was carried out for this basic skeleton.[13] A conformational search (lowmode) of the basic skeleton was carried out using Amber* implemented within Macromodel package V.6.5. A large number of structures were obtained within an energy window of 10 kcal mol^{-1} of the global energy minimum. To reduce the number of conformers, we employed the conformational distance[14] of the ring atoms as a criterion. The conformational distance (unit in degree) is defined as the root-mean-square differences in the corresponding torsion angles along the backbone ring between the two conformations being compared. This pair is judged to be identical below the threshold value (10°).[15] In these procedures, 6 conformers were obtained.

The observed NOE correlation of H-9 to H_3-19 and H_3-20 in **1** gave valuable information on the relative stereochemistry of C-9. Table 2 shows the average distances from C-9 methylene protons (*pro-R** and *pro-S**) in the 6 structures of the basic skeleton to one of the three protons of H_3-19 and H_3-20, respectively. The *pro-R** proton has shorter distances than the *pro-S** proton and the average distances of the former are sufficiently small to expect the observed NOE correlations. From this analysis, we can determine unambiguously that the *pro-S** proton should be substituted to acetate and the *pro-R** proton should be left unchanged.

There are two stereogenic centers (C-2 and C-4) whose relative configurations should be given. Similar conformational analyses of 4 isomers (2*S**4*R**, 2*S**4*S**, 2*R**4*R**, and 2*R**4*S**) were carried out. In these cases, we selected vicinal coupling constants as the criterion. In the case of the 2*S**4*R** isomer, there is one other conformer within the energy window of 2.7 kcal mol^{-1} of the global energy minimum conformer. The vicinal coupling constants in the 10-membered ring for these conformers were calculated by using Altona's equation.[16] The relative population of the two conformers, estimated from their steric energies (the most stable conformer exists in 95% and the second stable one in 5%), was utilized for the estimation of the weighted average of the calculated vicinal coupling constants. These coupling constants were compared to the observed values. Excellent correlation of these data was obtained in a linear regression analysis: [for the 2*S**4*R** isomer, the range of the observed vicinal coupling constants 1.0–12.5 Hz, $J_{obs} = a \cdot J_{cal} + b$; $a = 1.046$, $b = 0.42$, $R^2 = 0.905$, rms = 1.16 Hz]. Table 3 shows the results of the comparison together with those of the other isomers.

Table 2. Interatomic Distances (Å) from H-9 to Selective Protons

Proton	H-9$_{pro\text{-}R^*}$	H-9$_{pro\text{-}S^*}$
H_3-19	2.45 ± 0.3	3.63 ± 0.3
H_3-20	2.26 ± 0.1	3.11 ± 0.4

Only the 2*S**4*R** isomer could reproduce the observed vicinal coupling constants satisfactorily. The calculated structure of the most stable conformer is shown in Fig. 3. The calculated interatomic distances between the selected protons having key NOE correlations in the most stable conformation of the 2*S**4*R** isomer are shown in Table 4. The observed NOE correlations can be reasonably explained in the most stable conformation of this isomer. From these analyses the relative configurations of the two stereogenic centers of **1** were determined unambiguously.

Structure of 2. The structure elucidation of **1** can be successfully carried out by using the NOE correlation and coupling data with the aid of molecular mechanics calculations. In order to confirm the reliability of this method for structure elucidation, it was applied for the structure elucidation of compound **2**. Since the aforementioned NOE correlation of H-9 to H_3-19 and H_3-20 was also found in **2**, the relative configuration of the C-9 of **2** is identical to that of **1**. A similar conformational analysis and the estimation of the vicinal coupling constants were carried out for 4 isomers (2*R**3*R**, 2*R**3*S**, 2*S**3*R**, and 2*S**3*S**). Table 5 summarizes the results of the comparison of the observed and calculated coupling constants for the 4 isomers.

Only the 2*R**3*R** isomer can reproduce the observed vicinal coupling constants by the calculation satisfactorily. Also, in this case, there is one more conformer within the energy window of 2.7 kcal mol^{-1} of the global energy minimum conformer. The population of the most stable conformer of this isomer

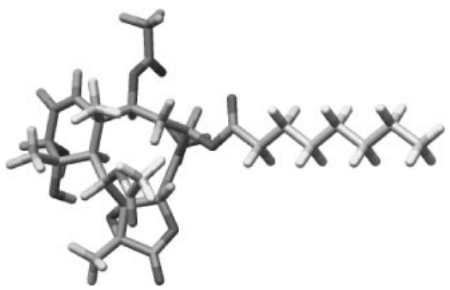

Fig. 3. Calculated structure of the most stable conformer of **1**.

Table 4. Calculated Distances (Å) between Selective Protons Having Key NOE Correlations of **1**

H/H	Distance	H/H	Distance
H_3-15/H-3$_{pro\text{-}S^*}$	2.321	H-2/H-10	2.248
H-2/H_3-16	2.496	H-9/H_3-19	2.295
H-4/H_3-16	2.484	H-9/H_3-20	2.223
H-6/H_3-16	2.222	H-12/H_3-20	2.439
H-3$_{pro\text{-}S^*}$/H-7	2.084	H-14/H_3-15	2.343

Table 3. Comparison of Coupling Constants of **1**

Isomers	$J_{H2\text{–}H3_{pro\text{-}R^*}}$	$J_{H2\text{–}H3_{pro\text{-}S^*}}$	$J_{H4\text{–}H3_{pro\text{-}R^*}}$	$J_{H4\text{–}H3_{pro\text{-}S^*}}$	$J_{H6\text{–}H7}$	$J_{H9\text{–}H10}$	R^2	rms
obsd.	7.0	1.0	5.5	12.5	9.5	4.0		
2*R**4*R**	4.0	4.4	8.8	1.1	11.3	6.8	0.020	5.36
2*R**4*S**	4.4	4.0	1.1	8.6	11.3	6.8	0.377	3.16
2*S**4*R**	6.7	2.2	4.7	10.9	11.3	4.4	0.905	1.16
2*S**4*S**	1.6	11.1	11.6	2.8	8.5	0.6	0.114	6.77

Table 5. Comparison of Coupling Constants of **2**

Isomers	J_{H2-H3}	$J_{H3-H4_{pro-R^*}}$	$J_{H3-H4_{pro-S^*}}$	J_{H6-H7}	J_{H9-H10}	R^2	rms
obsd.	1.0	12.5	5.5	9.5	4.0		
$2R^*3R^*$	2.1	10.8	4.5	11.2	4.5	0.903	1.28
$2R^*3S^*$	1.0	2.2	10.2	10.9	6.1	0.033	5.18
$2S^*3R^*$	0.9	7.7	0.9	11.4	8.8	0.364	3.76
$2S^*3S^*$	4.7	1.3	8.6	11.2	7.3	0.027	5.70

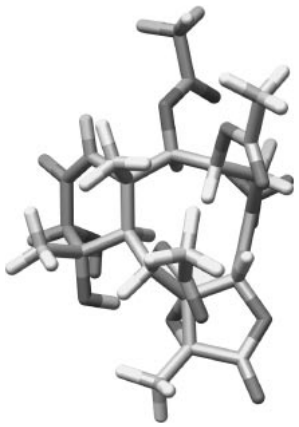

Fig. 4. Calculated structure of the most stable conformer of **2**.

Table 6. Calculated Distances (Å) between Selective Protons Having Key NOE Correlations of **2**

H/H	Distance	H/H	Distance
H_3-15/H-3	2.379	H-2/H-10	2.278
H-2/H-4_{pro-R^*}	2.899	H-9/H_3-19	2.290
H-2/H_3-16	2.446	H-9/HO-11	2.401
H-4_{pro-R^*}/H_3-16	2.547	H-9/H_3-20	2.231
H-6/H_3-16	2.263	H-12/H_3-20	2.444
H-3/H-7	2.073	H-14/H_3-15	2.348

was more than 96% estimated from its steric energy in comparison to that of the second one. The contribution of the other conformer is very small. The calculated structure of the most stable conformer is shown in Fig. 4. The calculated distances between the selected protons having key NOE correlations in the most stable conformation of the $2R^*3R^*$ isomer are shown in Table 6. The observed NOE correlations can be reasonably explained in the most stable conformation of this isomer. From these analyses the relative configurations of the three stereogenic centers were determined unambiguously.

Structures of 3 and 4. The ^{1}H NMR spectrum of **4** is very similar to that of **3** except for the presence of the signals of the butyrate group. The observed NOE correlations in **4** are also found in **3**, indicating that the 4-acetate of **3** is substituted to 4-butyrate in **4**. Since the structures of the two compounds are very similar to each other, the molecular mechanics calculations were carried out for **3**. The NOE correlation of H-9 to H_3-19 and H_3-20 was also found in **3**; hence, the relative configuration of the C-9 of **3** is identical to that of **1**. In this compound, 8 isomers are possible with respect to the three contiguous stereogenic centers of C-2, C-3, and C-4. Similar conformational analyses and the estimation of the vicinal coupling constants were carried out for 8 isomers ($2R^*3R^*4R^*$, $2R^*3R^*4S^*$, $2R^*3S^*4R^*$, $2R^*3S^*4S^*$, $2S^*3R^*4R^*$, $2S^*3R^*4S^*$, $2S^*3S^*4R^*$, and $2S^*3S^*4S^*$). Table 7 summarizes the results of the comparison of the observed and calculated coupling constants for the 8 isomers.

Table 7. Comparison of Coupling Constants of **3**

Isomers	J_{H2-H3}	J_{H3-H4}	J_{H6-H7}	J_{H9-H10}	R^2	rms
obsd.	1.4	10.3	10.0	4.1		
$2R^*3R^*4R^*$	8.4	2.3	9.2	1.3	0.005	5.52
$2R^*3R^*4S^*$	1.7	9.3	11.3	4.7	0.953	0.87
$2R^*3S^*4R^*$	1.1	9.9	9.2	0.5	0.911	1.86
$2R^*3S^*4S^*$	1.1	1.6	3.1	1.3	0.507	5.73
$2S^*3R^*4R^*$	1.4	1.4	10.8	8.3	0.072	4.94
$2S^*3R^*4S^*$	5.7	9.5	10.9	4.0	0.760	2.23
$2S^*3S^*4R^*$	4.0	7.1	10.8	7.6	0.561	2.73
$2S^*3S^*4S^*$	5.0	0.8	11.4	7.3	0.001	5.37

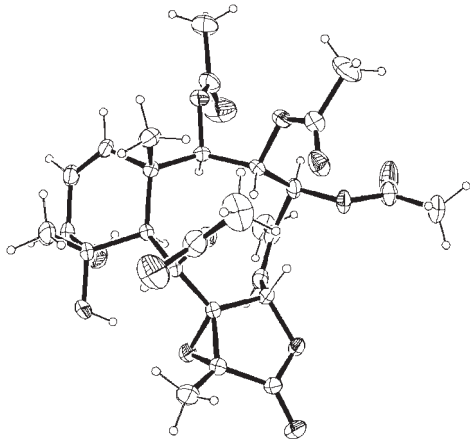

Fig. 5. ORTEP drawing of **3**.

Linear regression analyses between the observed and calculated vicinal coupling constants for these 8 isomers gave the respective correlation coefficients (R^2). In these values, both the $2R^*3R^*4S^*$ and $2R^*3S^*4R^*$ isomers gave high correlation and both isomers are possible candidates for the structure. However, the value of rms is smaller in the $2R^*3R^*4S^*$ isomer than in the $2R^*3S^*4R^*$ isomer, suggesting that the most probable candidate for the structure is the former. Further support of the $2R^*3R^*4S^*$ isomer was given by the calculated distance between H-3 and H-7, between which the NOE correlation was detected in **3** and **4**. In the case of the $2R^*3R^*4S^*$ isomer, there is no other conformer within the energy window 2.7 kcal mol^{-1} of the most stable structure and it gives a small distance (2.09 Å) between the two protons. By contrast, in the case of the $2R^*3S^*4R^*$ isomer, two other conformers are present within the energy window of the most stable structure, and none of them give a small distance between the two protons (4.75 ± 0.26 Å, the average of the three structures), which provides evidence that the latter isomer does not satisfy the observed NOE correlation. The final structural proof of **3** was given by an X-ray crystallographic study of **3** (Fig. 5). The calculated structure of the most stable conformer of the $2R^*3R^*4S^*$ isomer of **3** is found to be identical to the X-ray structure.

Compound **4** was converted to its bis-MTPA ester **5** by the condensation reaction of **4** and (*R*)-(−)-α-methoxy-α-(trifluo-

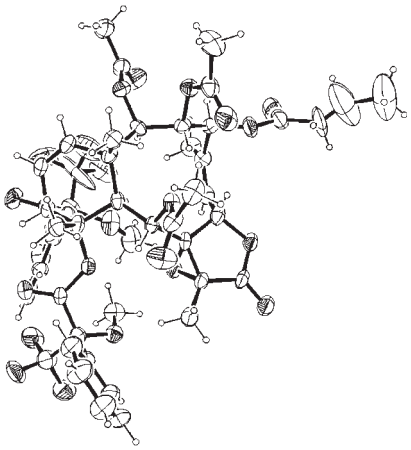

Fig. 6. ORTEP drawing of **5**.

romethyl)phenylacetyl chloride. An X-ray crystallographic analysis of **5** (Fig. 6) disclosed its absolute structure. The structures of these compounds were thus elucidated unequivocally with the aid of the molecular mechanics calculations.

Conclusion

The structures of the briarane diterpenoids have been determined without the aid of X-ray crystallographic studies. The molecular mechanics calculations gave successful reproductions of the observed vicinal coupling constants to give the correct relative configuration of each of the stereogenic centers in the molecules. The observed NOE correlations found in **1**, **2**, and **3** can be reasonably explained with the proton–proton distances observed in the respective most stable structures obtained from the molecular mechanics calculations. These findings support that the molecular mechanics calculation is a method of choice for the elucidation of the structure in highly flexible medium ring compounds.

Experimental

General. Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a JASCO FT/IR-420S infrared spectrometer. NMR spectra were recorded on a JEOL GSX-500 spectrometer at 27 °C in $CDCl_3$. Chemical shifts were referenced to the residual $CHCl_3$ at δ_H 7.26 and $CDCl_3$ at δ_C 77.0. EIMS and FABMS spectra were measured on a JEOL SX102A spectrometer. Preparative HPLC was carried out with a Waters 515 pump connected to a Waters 410 differential refractometer. Silica gel (Kieselgel 60 H, Merck) was used for column chromatography, and plastic-backed plates coated with silica gel 60 F_{254} were used for TLC chromatography.

Isolation and Purification. A sample of *Pachyclavularia violacea* (Quoy & Gaimard, 1833) was collected in Akasaki Bay, Bonotsu near Kagoshima, on May 29th, 1995 at a depth of 2–3 m. The organism was identified by Prof. Yehuda Benayahu of the Department of Zoology of Tel Aviv University, Israel, where a taxonomic specimen has been deposited. The gorgonian coral was frozen upon collection and freeze-dried (867 g), then was crushed by a heavy mortar, followed by extraction several times with CH_2Cl_2 to afford a crude extract (17 g). Rapid chromatography[17] of the extract was carried out on a SiO_2 column by stepwise elution with a hexane/EtOAc mixture (v/v, 3:1, 2:1, and 1:1). The resultant fractions were examined by TLC and ^{1}H NMR spectroscopy. Fractions eluted with hexane/ethyl acetate (2:1) were shown to contain a mixture of briarane diterpenoids by ^{1}H NMR and they were rechromatographed on SiO_2 with 3% MeOH in CH_2Cl_2 to give fractions containing each terpenoid abundantly. Further purification was achieved by repeated HPLC separation on a reversed-phase column (Waters, SymmetryPrep C18, 7 μm, 19 × 150 mm) with H_2O/MeOH (1:4) to afford **1** (19 mg), **2** (14 mg), **3** (32 mg), and **4** (34 mg).

(1*S,2*S**,4*R**,5*Z*,7*S**,8*S**,9*S**,10*S**,11*S**,12*R**,13*Z*,17*R**)-2,9-Diacetoxy-8,17-epoxy-11,12-dihydroxy-4-octanoyloxybriara-5,13-dien-18-one**[8] **(1):** Viscous oil, $[\alpha]^{25}_D$ −8.4° (*c* 1.9, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3600, 3545, 1780, 1740, 1250, 1220 cm^{-1}; ^{1}H NMR ($CDCl_3$) see Table 1; ^{1}H NMR (C_6D_6) δ 4.90 (1H, brd, J = 7.5 Hz, H-2), 2.20 (1H, ddd, J = 15.0, 7.5, 5.5 Hz, H-3$_{pro\text{-}R}$), 3.16 (1H, dd, J = 15.0, 12.5 Hz, H-3$_{pro\text{-}S}$), 5.42 (1H, ddd, J = 12.5, 5.5, 1.0 Hz, H-4), 5.70 (1H, brd, J = 9.5 Hz, H-6), 6.09 (1H, d, J = 9.5 Hz, H-7), 6.28 (1H, d, J = 4.0 Hz, H-9), 2.82 (1H, d, J = 4.0 Hz, H-10), 3.48 (1H, brd, J = 6.0 Hz, H-12), 5.56 (1H, dd, J = 10.5, 6.0 Hz, H-13), 5.30 (1H, d, J = 10.5 Hz, H-14), 1.10 (3H, s, H-15), 2.29 (1H, d, J = 1.5 Hz, H-16), 1.85 (3H, s, H-19), 1.23 (3H, s, H-20), 2.99 (1H, s, 11-OH, disappeared by D_2O exchange), 2.30 (1H, brs, 12-OH, disappeared by D_2O exchange), 1.66 (3H, s, Acetate at C-2), 1.67 (3H, s, Acetate at C-9), and octanoate at C-4: 2.23 (2H, t, J = 7.5 Hz, H-2′), 1.62 (2H, qui, J = 7.5 Hz, H-3′), 1.15–1.30 (8H, m, H-4′–H-7′), and 0.88 (3H, t, J = 7.0 Hz, H-8′); ^{13}C NMR ($CDCl_3$) δ 9.8 (C-19), 15.2 (C-15), 21.4 (C-20), 25.7 (C-16), 38.3 (C-3), 43.4 (C-10), 46.7 (C-1), 64.5 (C-17), 65.7 (C-9), 70.2 (C-12), 71.1 (C-8), 72.1 (C-4), 73.7 (C-7), 73.7 (C-11), 77.5 (C-2), 122.9 (C-6), 124.8 (C-13), 138.7 (C-14), 144.1 (C-5), 168.2 and 21.6 (Ac at C-9), 170.3 and 21.1 (Ac at C-2), 170.7 (C-18), and octanoate at C-4: 172.9 (C-1′), 34.2 (C-2′), 24.8 (C-3′), 29.0 (C-4′ or C-5′), 28.9 (C-4′ or C-5′), 31.6 (C-6′), 22.6 (C-7′), and 14.0 (C-8′); HRMS(FAB) $[M + H]^+$, Found: m/z 607.3134. Calcd for $C_{32}H_{47}O_{11}$: 607.3118.

(1*S,2*R**,3*R**,5*Z*,7*S**,8*S**,9*S**,10*S**,11*S**,12*R**,13*Z*,17*R**)-2,3,9-Triacetoxy-8,17-epoxy-11,12-dihydroxybriara-5,13-dien-18-one (2):** White powder, $[\alpha]^{25}_D$ +2.1° (*c* 1.4, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3600, 3550, 1780, 1740, 1255, 1235 cm^{-1}; ^{1}H NMR ($CDCl_3$) see Table 1; ^{13}C NMR ($CDCl_3$): δ 9.9 (C-19), 15.4 (C-15), 21.3 (C-20), 27.1 (C-16), 34.6 (C-4), 43.5 (C-10), 46.8 (C-1), 64.8 (C-17), 65.7 (C-9), 70.2 (C-12), 71.6 (C-3), 71.6 (C-8), 73.7 (C-11), 74.6 (C-7), 77.3 (C-2), 121.7 (C-6), 124.9 (C-13), 138.2 (C-14), 139.7 (C-5), 168.9 and 21.2 (Ac at C-9), 170.3 and 21.2 (Ac at C-3), 170.3 and 21.2 (Ac at C-2), 170.7 (C-18); HRMS(FAB) $[M + H]^+$, Found: m/z 523.2160. Calcd for $C_{26}H_{35}O_{11}$: 523.2179.

(1*S,2*R**,3*R**,4*S**,5*Z*,7*S**,8*S**,9*S**,10*S**,11*S**,12*R**,13*Z*,17*R**)-2,3,4,9-Tetraacetoxy-8,17-epoxy-11,12-dihydroxybriara-5,13-dien-18-one (3):** Colorless prisms (benzene), mp 201–202 °C, $[\alpha]^{25}_D$ +55.9° (*c* 3.2, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3600, 3550, 1780, 1745, 1245, 1225 cm^{-1}; ^{1}H NMR ($CDCl_3$) see Table 1; ^{13}C NMR ($CDCl_3$) δ 9.8 (C-19), 15.3 (C-15), 21.1 (C-20), 25.3 (C-16), 42.9 (C-10), 46.8 (C-1), 64.4 (C-17), 65.5 (C-9), 70.1 (C-12), 71.0 (C-3), 71.4 (C-8), 73.4 (C-7), 73.9 (C-11), 76.1 (C-4), 76.8 (C-2), 125.4 (C-6), 125.5 (C-13), 138.4 (C-14), 140.4 (C-5), 169.1 and 20.8 (Ac at C-3), 169.9 and 21.3 (Ac at C-9), 170.1 and 20.7 (Ac at C-4), 170.7 and 20.5 (Ac at C-2), 170.9 (C-18); HRMS(FAB) $[M + H]^+$, Found: m/z 581.2258. Calcd for $C_{28}H_{37}O_{13}$: 581.2234.

(1*S*,2*R*,3*R*,4*S*,5*Z*,7*S*,8*S*,9*S*,10*S*,11*S*,12*R*,13*Z*,17*R*)-2,3,9-Tri-

acetoxy-4-butyryloxy-8,17-epoxy-11,12-dihydroxybriara-5,13-dien-18-one (4): White plates, mp 188–189 °C, $[\alpha]^{25}_{D}$ +55.7° (*c* 0.97, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3600, 3450, 1780, 1745, 1740, 1245, 1225 cm^{-1}; ^1H NMR ($CDCl_3$) see Table 1; ^{13}C NMR ($CDCl_3$) δ 9.9 (C-19), 15.5 (C-15), 21.2 (C-20), 25.4 (C-16), 43.1 (C-10), 46.8 (C-1), 64.5 (C-17), 65.5 (C-9), 70.1 (C-12), 70.9 (C-3), 71.5 (C-8), 73.4 (C-7), 73.9 (C-11), 75.9 (C-4), 76.9 (C-2), 125.2 (C-6), 125.4 (C-13), 138.5 (C-14), 140.5 (C-5), 168.8 and 20.9 (Ac at C3), 169.7 and 21.3 (Ac at C-9), 169.9 and 20.6 (Ac at C-2), 170.8 (C-18), and butyrate at C-4: 172.6 (C-1′), 36.1 (C-2′), 18.2 (C-3′), and 13.6 (C-4′); HRMS(FAB) $[M+H]^+$, Found: *m/z* 609.2557. Calcd for $C_{30}H_{41}O_{13}$: 609.2547.

(1*S*,2*R*,3*R*,4*S*,5*Z*,7*S*,8*S*,9*S*,10*S*,11*S*,12*R*,13*Z*,17*R*)-2,3,9-Triacetoxy-4-butyryloxy-8,17-epoxy-11,12-bis((*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropionyloxy)briara-5,13-dien-18-one (5). To a solution of **4** (15 mg) and 4-(dimethylamino)pyridine (4 mg) in a 1:1 mixture of CCl_4 and pyridine (2 mL) was added (*R*)-(−)-MTPA chloride (10 μL), and the resulting reaction mixture was stirred at room temperature for 48 h. Excess reagent was destroyed by the addition of water, and extracted with EtOAc. The organic solution was sequentially washed with water, dil $CuSO_4$, brine, and then dried over Na_2SO_4 and filtered. The filtrate was evaporated in vacuo to afford a crude product. The crude product was passed through a short SiO_2 column eluted with hexane/EtOAc (3:1) to give, after recrystallization from diethyl ether/hexane, **5** (11 mg). **5**: Colorless plates, mp 224–225 °C; $[\alpha]^{25}_{D}$ −11.2° (*c* 0.72, $CHCl_3$); EIMS *m/z* 982 ($M^+ - OCOCH_3$); ^1H NMR ($CDCl_3$) δ 0.75 (3H, s, H-15), 0.89 (3H, t, $J = 7.3$ Hz, –OCOCH$_2$-CH$_2$CH$_3$), 1.21 (3H, s, H-20), 1.58 (2H, sxt, $J = 7.3$ Hz, –OCO-CH$_2$CH$_2$CH$_3$), 1.71 (3H, s, H-19), 2.06 (3H, s, Ac), 2.07 (3H, brs, H-16), 2.16 (3H, s, Ac), 2.18 (3H, s, Ac), 2.22 (2H, m, –OCOCH$_2$-CH$_2$CH$_3$), 3.04 (1H, d, $J = 2.7$ Hz, H-10), 3.49 (3H, s, –OCH_3), 3.50 (3H, s, –OCH_3), 4.89 (1H, brs, H-2), 5.02 (1H, d, $J = 10.7$ Hz, H-4), 5.41 (1H, d, $J = 2.7$ Hz, H-9), 5.57 (1H, brd, $J = 10.4$ Hz, H-6), 5.67 (1H, d, $J = 10.4$ Hz, H-14), 5.89 (1H, d, $J = 10.4$ Hz, H-7), 6.02 (1H, brd, $J = 10.7$, H-3), 6.22 (1H, dd, $J = 10.4$, 6.7 Hz, H-13), 6.41 (1H, d, $J = 6.7$ Hz, H-12), and 7.36–7.48 (10H, complex, aromatic Hs on the MTPA ester).

Crystal Structure Analysis. Crystals of **3** and **5** suitable for X-ray diffraction experiments were grown by slow evaporation of solutions of **3** in benzene and **5** in diethyl ether/hexane. Data were collected at 297 K on a Mac Science DIP2030 imaging plate equipped with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Unit cell parameters were determined by autoindexing several images in each data set separately with the program DENZO. For each data set, rotation images were collected in 3° increments with a total rotation of 180° about ϕ. Data were processed by using SCALEPACK (The programs DENZO and SCALEPACK are available from Mac Science Co., Z. Otwinowski, University of Texas, Southwestern Medical Center.). Structures were solved by direct methods using the program SIR-97[19] and refined by full-matrix least-squares refinement of F^2 using the program SHELXL-97.[20] All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed using AFIX instructions. Crystal Data for **3**: $2(C_{28}H_{36}O_{13})\cdot C_6H_6$, Colorless prisms, orthorhombic, space group $P2_12_12_1$, a = 13.762(2), b = 38.725(6), c = 12.725(2) Å, V = 6591.4(18) Å^3, $Z = 4$, 6792 unique reflections, R indices; $R_1 = 0.0482$, $wR_2 = 0.1246$. Crystal Data of **5**: $C_{50}H_{50}F_6O_{17}$, monoclinic, space group $P2_1$, a = 12.623(8), b = 12.387(11), c = 11.803(7) Å, β = 95.31(5)°, V = 2579(3) Å^3, $Z = 2$, 3974 unique reflections, R indices; R_1 = 0.1174, $wR_2 = 0.2069$. Crystallographic Data have been deposited with Cambridge Crystallographic Data Center as supplementary publication Nos. CCDC-292038 and -292039. Copies of the data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB2 1EZ, UK; Fax: +44 1223 336033; or e-mail: deposit@ccdc.cam.ac.uk).

Conformational Search and Energy Minimization. We carried out a conformational search of compounds **1–3** using the Amber* force-field implemented within the Macromodel version 6.5 software package. We used the lowmode method, which has been proven very efficient for the conformational search of flexible compounds. First, we randomly generated a structure as the starting point such that the bond lengths and angles were consistent with the equilibrium values, whereas the dihedral angles assume random values also ensuring the ring structure of the backbone is formed and it is energetically minimized to give less than 1.0 kJ mol^{-1} Å^{-2} of the gradient using the truncated Newton methods. We next carried out calculations using the lowmode method. Each structure generated by this method was then subjected to energy minimization. The resulting structure was compared and eliminated if it was identical to the stored one(s). This procedure was repeated 2000 times to give many unique structures. We next ordered the structures according to energy and structures within the region of 20 kJ mol^{-1} of the global energy minimum were stored. Within these stored structures we used the conformational distance of the ring atoms as the criterion of the second comparison of the structures. The threshold value (10°) was employed to give the important structures.

References

1 a) W. Fenical, Diterpenoids, in *Marine Natural Products: Chemical and Biological Perspectives*, ed. by P. J. Scheuer, Academic Press, New York, **1978**, Vol. 2, Chap. 3, pp. 173–245. b) P. W. Sammarco, J. C. Coll, The Chemical Ecology of Alcyonarian Corals, in *Bioorganic Marine Chemistry*, ed. by P. J. Scheuer, Springer-Verlag, New York, **1988**, Vol. 2, pp. 87–116. c) A. D. Rodoriguez, *Tetrahedron* **1995**, *51*, 4571.

2 J. W. Blunt, B. R. Copp, M. H. G. Munro, P. T. Northcote, M. R. Prinsep, *Nat. Prod. Rep.* **2005**, *22*, 15.

3 D. B. Stierle, B. Carte, D. J. Faulkner, B. Tagle, J. Clardy, *J. Am. Chem. Soc.* **1980**, *102*, 5088.

4 a) B. F. Bowden, J. C. Coll, I. M. Vasilescu, *Aust. J. Chem.* **1989**, *42*, 1705. b) B. F. Bowden, J. C. Coll, I. M. Vasilescu, P. N. Alderslade, *Aust. J. Chem.* **1989**, *42*, 1727.

5 J. E. Burks, D. van der Helm, C. Y. Chang, L. S. Ciereszko, *Acta Crystallogr., Sect. B* **1977**, *33*, 704.

6 a) M. Karplus, *J. Chem. Phys.* **1959**, *30*, 11. b) E. Garbish, Jr., *J. Am. Chem. Soc.* **1964**, *86*, 5561. c) S. Sternhell, *Q. Rev., Chem. Soc.* **1969**, *23*, 236. d) C. Jaime, E. Osawa, Y. Takeuchi, P. Camps, *J. Org. Chem.* **1983**, *48*, 4514. e) E. J. Corey, J. W. Ponder, *Tetrahedron Lett.* **1984**, *25*, 4325. f) S. Masamune, P. Ma, R. E. Moore, T. Fujiyoshi, C. Jaime, E. Osawa, *J. Chem. Soc., Chem. Commun.* **1986**, 261.

7 a) F. A. Anet, A. J. R. Bourn, *J. Am. Chem. Soc.* **1965**, *87*, 5025. b) R. A. Bell, J. K. Saunders, *Can. J. Chem.* **1970**, *48*, 1114. c) L. D. Hall, K. M. Sanders, *J. Am. Chem. Soc.* **1980**, *102*, 5703. d) R. M. Scheek, N. Russo, R. Boelens, R. Kaptein, J. H. van Boom, *J. Am. Chem. Soc.* **1983**, *105*, 2914. e) D. Neuhaus, R. N. Sheppard, I. R. C. Bick, *J. Am. Chem. Soc.* **1983**, *105*, 5996.

8 a) C. Landis, V. S. Allured, *J. Am. Chem. Soc.* **1991**, *113*, 9493. b) P. Cuniasse, I. Raynal, A. Yiotakis, V. Dive, *J. Am.*

Chem. Soc. **1997**, *119*, 5239.

9 M. Saunders, K. N. Houk, Y.-D. Wu, W. C. Still, M. Lipton, G. Chang, W. C. Guida, *J. Am. Chem. Soc.* **1990**, *112*, 1419.

10 I. Kolossváry, W. C. Guida, *J. Am. Chem. Soc.* **1996**, *118*, 5011.

11 a) Y. Uchio, T. Haino, S. Usui, Y. Fukazawa, H. Fujitaka, 70th National Meeting of the Chemical Society of Japan, Tokyo, March, **1996**, Abstr., No. 1C211. b) Y. Uchio, T. Haino, S. Usui, Y. Fukazawa, 72th National Meeting of the Chemical Society of Japan, Tokyo, March, **1997**, Abstr., No. 1G341. c) Y. Uchio, T. Haino, S. Usui, Y. Fukazawa, 39th Symposium on the Chemistry of Natural Products, Sapporo, **1997**, Symposium Papers, p. 625.

12 a) T. Iwagawa, N. Takenoshita, H. Okamura, M. Nakatani, M. Doe, K. Shibata, M. Shiro, *Heterocycles* **1998**, *48*, 123. b) T. Iwagawa, K. Takayama, H. Okamura, M. Nakatani, M. Doe, *Heterocycles* **1999**, *51*, 1653. They reported two vicinal coupling constants of C_4-H (12.6 and 5.3 Hz), one of which is similar to the corresponding value of the X-ray analyzed compound. c) T. Iwagawa, K. Takayama, H. Okamura, M. Nakatani, M. Doe, K. Takemura, M. Shiro, *Heterocycles* **1999**, *51*, 2619.

13 There have been several reports on molecular mechanics calculations for brianolides. a) J.-F. Cheng, S. Yamamura, Y. Terada, *Tetrahedron Lett.* **1992**, *33*, 101. b) F. J. Schmitz, M. M. Schulz, J. Siripitayananon, M. B. Hossain, D. van der Helm, *J. Nat. Prod.* **1993**, *56*, 1339. c) G. Chiasera, A. Guerriero, M. D'Ambrosio, F. Pietra, *Helv. Chim. Acta* **1995**, *78*, 1479. d) P.-J. Sung, W.-P. Hu, S.-L. Wu, J.-H. Su, L.-S. Fung, J.-J. Wang, J.-H. Sheu, *Tetrahedron* **2004**, *60*, 8975.

14 a) M. Saunders, *J. Comput. Chem.* **1991**, *12*, 645. b) H. Goto, *Tetrahedron* **1992**, *48*, 7131.

15 H. Goto, Y. Kawashima, M. Kashimura, S. Morimoto, E. Osawa, *J. Chem. Soc., Perkin Trans. 2* **1993**, 1647.

16 C. A. G. Haasnoot, F. A. A. M. de Leeuw, C. Altona, *Tetrahedron* **1980**, *36*, 2783.

17 J. C. Coll, B. F. Bowden, *J. Nat. Prod.* **1986**, *49*, 934.

18 We named the compounds isolated in this investigation semi-systematically as derivatives of briarane: B. N. Ravi, J. F. Marwood, R. J. Wells, *Aust. J. Chem.* **1980**, *33*, 2307, and as reported in the Ref. 4a.

19 SIR97, Program for the Solution of Crystal Structures: A. Altomare, M. C. Burla, M. Camalli, G. L. Cascarano, C. Giacovazzo, A. Guagliardi, A. G. G. Moliterni, G. Polidori, R. Spagna, *J. Appl. Crystallogr.* **1999**, *32*, 115.

20 G. M. Sheldrick, *SHELXL-97, Program for the Refinement of Crystal Structures*, University of Göttingen, Germany, **1997**.