raphy on silica gel, eluting with pentane, gave essentially pure **14** (19 mg, 32%). Further purification could be afforded by preparative TLC with petroleum ether 40–60 °C. Compound **14**: mp 176–180 °C; mass spectrum, *m/e* 230.1095 (calcd for $C_{18}H_{14}$, 230.1096); ^1H and ^{13}C NMR spectra, see discussion; electronic spectrum, see discussion.

Hydrogenation of 16a. The diester **16a** (226 mg, 0.64 mmol) was dissolved in EtOAc (8 mL) and 10% $Pd/CaCO_3$ (100 mg) was added. The mixture was stirred under a H_2 atmosphere and the uptake of H_2 was followed. After the uptake of 56 mL of H_2 had occurred the reaction was stopped, the mixture filtered through Celite, and the filtrate concentrated under vacuo. The resulting oil (230 mg) was subjected to ^1H NMR and mass spectral analysis. Mass spectrum, *m/e* 354.1829 (calcd for $C_{22}H_{26}O_4$, *m/e* 354.1831), *m/e* 356.2006 (calcd for $C_{22}H_{28}O_4$, *m/e* 356.2024), *m/e* 358.2153 (calcd for $C_{22}H_{30}O_4$, *m/e* 358.2162).

Treatment of 6b with Sodium Sulfide. The dibromide **6b** (325 mg, 1.03 mmol) and $Na_2S{\cdot}9H_2O$ (990 mg, 4.1 mmol) were suspended in 95% EtOH (6 mL) and the mixture was stirred and heated under reflux for 6 h. The mixture was cooled, water (15 mL) was added, and the resultant mixture was allowed to stand overnight in a refrigerator. The precipitated solid was removed by filtration, washed with water, and air dried. CH_2Cl_2 (5 mL) was added and the resulting solution, containing some undissolved material, was filtered through a short column of neutral alumina. The filtrate was evaporated under reduced pressure to give a pale orange solid (179 mg, 92%). Sublimation at 90–100 °C (0.2 mmHg) gave **22**: 158 mg (0.84 mmol, 81%); mp 150–157 °C dec; mass spectrum, *m/e* 188.0645 (calcd for $C_{12}H_{12}S$, *m/e* 188.0659), 189 (14), 188 (100), 187 (50), 173 (43), 172 (18), 171, (17), 128 (18), 115 (18); ^1H NMR 4.07 (br s, 4 H), 3.13 (br s, 8 H).

Oxidation of 22. The sulfide **22** (150 mg, 0.8 mmol) was dissolved in dry CH_2Cl_2 (10 mL), *m*-chloroperoxybenzoic acid (660 mg, 3.2 mmol) was added, and the mixture was stirred at room temperature for 43 h. The resulting solution was washed with $NaHCO_3$ solution (10 mL) and dried ($MgSO_4$). The solvent was removed under reduced pressure and the resulting white solid chromatographed by preparative TLC, eluting with CH_2Cl_2 to give **23**: 145 mg (0.66 mmol, 83%); mp 198–200 °C dec: mass spectrum, *m/e* 220.0496 (calcd for $C_{12}H_{12}SO_2$, *m/e* 220.0558), 220 (10), 157 (14), 156 (100), 155 (11), 141 (21), 139 (14), 128 (13), 115 (17); ^1H NMR 4.17 (br s, 4 H), 3.15 (br s, 8 H).

Pyrolysis of 23. The sulfone **23** (50 mg, 0.23 mmol) was dissolved in CH_2Cl_2 (2 mL) in a 10-mL round-bottomed flask and the solvent removed by a stream of N_2. The flask was then attached to the pyrolysis apparatus and the system evacuated to 0.005 mmHg. The furnace was heated to 320 °C and maintained at this temperature for 2 h. The flask in the preoven was then heated to 90–100 °C when **23** slowly sublimed into the furnace over ca. 3 h. The product was collected in a cold trap at −190 °C. The product was dissolved in CH_2Cl_2 and chromatographed on alumina, eluting with pentane–CH_2Cl_2 (7:3) to give 16.5 mg of **23**, and **21**: 12.6 mg (0.08 mmol, 35%, 53% based on recovered **23**); mp 143–144 °C (lit.[18] 141–142 °C); mass spectrum, *m/e* 156 (100), 155 (20), 153 (10), 141 (36), 128 (15), 115 (22), 105 (23), 91 (36); ^1H NMR 3.12 (s); electronic spectra λ_{max} (cyclohexane) 208, 222 (ε6000), 252 (225), 265 sh (200), 274 sh (170), 290 nm sh (100).

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Registry No. **1a**, 72040-07-4; **1b**, 89165-73-1; **1c**, 89165-74-2; **2**, 33954-15-3; **3**, 69573-29-1; **4**, 72040-01-8; **5**, 72040-02-9; **6a**, 72040-03-0; **6b**, 72040-04-1; **8a**, 70359-11-4; **8b**, 74942-83-9; **8c**, 72039-99-7; **9a**, 72040-05-2; **9b**, 89165-66-2; **9c**, 89165-67-3; **10a**, 72040-06-3; **10b**, 89165-68-4; **10c**, 89165-69-5; **11a**, 89165-70-8; **11b**, 89165-71-9; **11c**, 89165-72-0; **14**, 80229-24-9; **15**, 83248-47-9; **16a**, 80229-21-6; **16b**, 80229-25-0; **17**, 80229-22-7; **18**, 80229-23-8; **19**, 89165-75-3; **20**, 89165-78-6; **21**, 60323-52-6; **22**, 89165-76-4; **23**, 89165-77-5; $MeO_2CC{\equiv}CCO_2Me$, 762-42-5.

Calyculones, New Cubitane Diterpenoids from the Caribbean Gorgonian Octocoral *Eunicea calyculata*

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Three new representatives of the rare cubitane class of rearranged diterpenoid molecules have been isolated as minor metabolites from *Eunicea calyculata*, a sea whip collected in the Caribbean Sea. The structure of the crystalline metabolite, calyculone A (**3**) was solved by single-crystal X-ray diffraction analysis. Structures could then be proposed for calyculones B and C (**4** and **5**) by comprehensive ^1H NMR analyses involving difference decoupling and nuclear Overhauser enhancement difference spectrometric methods.

In connection with our investigations of biologically active and structurally novel secondary metabolites from Caribbean gorgonian octocorals (Cnidaria, Gorgonacea), we have examined the sea whip *Eunicea calyculata* (Ellis and Solander) (Plexauridae).[1] While a number of shallow-water *Eunicea* species have been chemically investigated,[2] *E. calyculata* is found in abundance only below 20 meters and had not been previously studied. Recently, we described the structures of two new bicarbocyclic diterpenoid derivatives, **1** and **2** (Chart I), as the major secondary metabolites of *E. calyculata*.[3] In this paper we report the structures of three new monocarbocyclic diterpenoids, calyculones A–C (**3–5**), which were isolated as minor constituents of *E. calyculata*. These compounds are new examples of the rearranged cubitane class of diterpenoids. Cubitene (**6**) was reported as a component of the defensive secretion of the East African termite, *Cubitermes umbratus*.[4] Since then, no compounds of this

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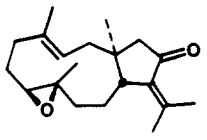
Chart I



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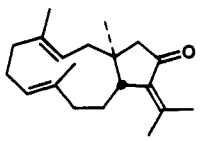

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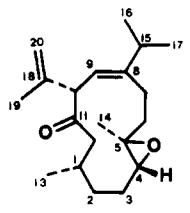

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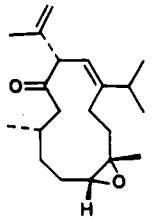

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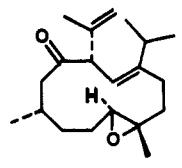

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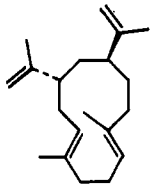

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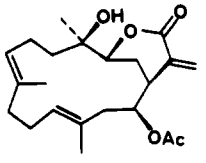

7

apparently rare ring system have been isolated from any other source. In addition, the cembranoid diterpenoid crassin acetate (**7**)[5] was found as a minor metabolite of *E. calyculata*. Crassin acetate had been previously isolated only from plexaurids of the genus *Pseudoplexaura*.[6]

Collections of *E. calyculata* were made in deeper water (−30 m) along the barrier reef of Belize and in various habitats in the Bahamas Islands. Freshly collected animals were either frozen or stored in 2-propanol and later extracted with chloroform/methanol (2/1). Calyculones A–C (**3–5**) were isolated by rapid elution silica gel chromatography of the crude extract and purified by HPLC from approximately the same nonpolar fractions containing the dolabellane derivative **2**. The three isomeric calyculones **3–5** were minor components of the crude extract, each

Table I. ^{13}C NMR Assignments for Calyculones A–C[a]

C	calyculone A (3)	calyculone B (4)	calyculone C (5)
1	29.1	29.3	29.2
2	26.3	23.8	24.1
3	32.5	32.3	31.3
4	60.4	63.2	67.5
5	61.2	60.7	59.9
6	24.8	25.5	25.4
7	36.1	37.1	33.9
8	145.5[b]	148.6[b]	147.7[b]
9	119.7	119.8	117.1
10	53.9	59.5	59.0
11	209.8	210.1	209.8
12	51.0	49.7	48.9
13	20.1[c]	21.3[c]	20.4[c]
14	18.6	17.2	15.3
15	30.1	31.7	27.5
16	20.2[c]	20.2[c]	20.4[c]
17	23.0	23.6	20.8[c]
18	143.1[b]	143.3[b]	142.8[b]
19	20.9	21.4	22.8
20	112.8	113.0	112.6

[a] ^{13}C NMR spectra were recorded at 50 MHz in $CDCl_3$ solution. Multiplicities were determined from single-frequency off-resonance decoupling and assignments were made based upon J_R values when applicable and/or a comparison to models. The δ values are in ppm downfield from Me_4Si. [b,c] Signals within a column may be reversed.

comprising slightly less than 0.5% of the organic extract.

Calyculone A (**3**), the most polar of the three isomeric cubitanes, crystallized from diethyl ether after extensive purification by HPLC. Data from high-resolution mass and ^{13}C NMR spectrometry (Table I) established a molecular formula of $C_{20}H_{32}O_2$ for this compound. Evaluation of spectral information established the presence of a trisubstituted epoxide and a ketone, which accounted for the two oxygen atoms in the molecular formula. ^{13}C NMR signals at δ 60.4 (d) and 61.2 (s) along with ^1H NMR resonances (Table II) at δ 2.96 (1 H, dd, J = 8.9, 4.0 Hz) and 1.14 (3 H, s) showed that the epoxide in **3** was methyl substituted. A ^{13}C NMR band at δ 209.8 (s), coupled with an infrared absorption at 1700 cm^{-1} further indicated that calyculone A possessed a nonconjugated ketone. The presence of an isopropenyl group in **3** was next established based upon signals in the ^{13}C NMR spectrum at δ 112.8 (t) and 143.1 (s) or 145.5 (s)], in conjunction with bands in the ^1H NMR spectrum at δ 4.85 (1 H, t, J = 1.3 Hz) and 4.79 (1 H, br s). These latter proton bands were allylically coupled to a broadened resonance at δ 1.70 (3 H, br s), assigned to an olefinic methyl. Two other resonances in the ^{13}C NMR spectrum of **3** at δ 145.5 (s) [or 143.1 (s)] and 119.7 (d), as well as a signal in the ^1H NMR spectrum at δ 5.57 (1 H, d, J = 10.6 Hz), showed the molecule to possess one other nonconjugated, trisubstituted olefin. The olefin proton of this group was coupled to a highly deshielded proton at δ 4.54 (1 H, d, J = 10.6 Hz) that was apparently bounded by two quaternary centers since it was not further coupled.

Further consideration of the ^1H NMR spectrum of calyculone A showed the presence of three doublet methyls at δ 1.00 (3 H, d, J = 6.8 Hz), 1.06 (3 H, d, J = 6.4 Hz), and 1.12 (3 H, d, J = 6.7 Hz). Irradiation of a one-proton multiplet at δ 2.39 caused two of the doublet methyls (δ 1.12 and 1.00) to collapse to singlet resonances, thereby establishing their assignment in an isopropyl group. In order to position this group, a proton NMR difference decoupling spectroscopy (DDS)[7] experiment was per-

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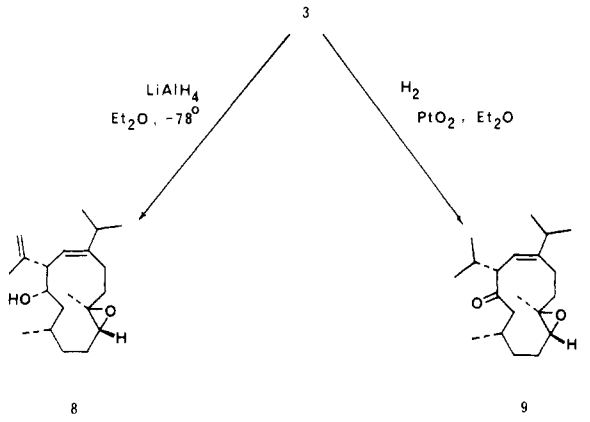
Table II. ^{1}H NMR Assignments for Calyculones A–C and Derivatives 8 and 9[a]

C	calyculone A (3)	calyculone B (4)	calyculone C (5)	8	9
1	2.31 (1 H, m)[n]	2.32 (1 H, m)[n]	2.26 (1 H, m)[n]	2.04 (1 H, m)[n]	2.35 (1 H, m)[n]
2	1.27 (2 H, m)[n]	1.40 (1 H, m)[n] 1.70 (1 H, m)[n]	1.39 (2 H, m)[n]	1.61 (2 H, m)[n]	*d*
3	2.05 (1 H, m),[b,n] 1.27 (1 H, m)[c,n]	1.93 (1 H, m),[b,n] 1.45 (1 H, m)[c,n]	1.89 (1 H, m),[b,n] 1.32 (1 H, m)[c,n]	1.87 (1 H, m),[b,n] 1.35 (1 H, m)[c,n]	1.96 (1 H, m),[b,n] (d)[c]
4	2.96 (1 H, dd, J = 8.9, 4.0)	2.63 (1 H, dd, J = 8.8, 2.5)	2.90 (1 H, dd, J = 9.0, 2.9)	2.84 (1 H, dd, J = 9.2, 2.5)	2.90 (1 H, dd, J = 9.6, 2.8)
5					
6	(d),[e] 1.27 (1 H, m)[f,n]	2.17 (1 H, m),[e,n] 1.33 (1 H, m)[f,n]	1.72 (1 H, m),[n] 1.40 (1 H, m)[n]	2.38 (1 H, m),[e,n] 1.35 (1 H, m)[f,n]	*d*
7	2.20 (1 H, ddd, J = 2.2, 3.4, 12.6),[g] 2.61 (1 H, ddd, J = 3.9, 13.6, 13.8)[h]	2.06 (1 H, m),[g,n] 2.43 (1 H, m)[h,n]	2.20 (1 H, m),[n] 1.58 (1 H, m)[n]	2.20 (1 H, m),[g,n] 2.66 (1 H, ddd, J = 3.9, 14.4, 14.0)[h]	2.24 (1 H, ddd, J = 2.6, 2.2, 14.6),[g] 2.63 (1 H, ddd, J = 3.6, 13.9, 13.8)[h]
8					
9	5.57 (1 H, d, J = 10.6)	5.37 (1 H, d, J = 9.9)	5.33 (1 H, d, J = 10.3)	5.17 (1 H, d, J = 9.5)	5.17 (1 H, d, J = 10.5)
10	4.54 (1 H, d, J = 10.6)	4.02 (1 H, d, J = 9.9)	4.04 (1 H, d, J = 10.3)	3.08 (1 H, dd, J = 8.9, 9.3) 3.65 (1 H, ddd, J = 2.1, 8.9, 7.3)	3.48 (1 H, dd, J = 8.4, 10.7)
11					
12	2.03 (1 H, dd, J = 10.8, 12.7),[i] 2.64 (1 H, dd, J = 5.0, 12.9)[k]	2.18 (1 H, dd, J = 11.8, 6.2),[i] 2.46 (1 H, d, J = 12.4)[k]	2.28 (1 H, m),[i,n] 2.39 (1 H, d, J = 9.1)[k]	1.61 (1 H, m),[n] 1.35 (1 H, m)[n]	*d*
13	1.06 (3 H, d, J = 6.4)	0.94 (3 H, d, J = 6.7)	0.96 (3 H, d, J = 4.8)	0.93 (3 H, d, J = 6.7)	1.00 (3 H, d, J = 4.6)
14	1.14 (3 H, s)	1.43 (3 H, s)	1.15 (3 H, s)	1.24 (3 H, s)	1.16 (3 H, s)
15	2.39 (1 H, m)[n]	2.28 (1 H, m)[n]	2.99 (1 H, m)[n]	2.46 (1 H, m)[n]	2.43 (1 H, m)[n]
16	1.12 (3 H, d, J = 6.8)	1.02 (3 H, d, J = 6.9)	1.07 (3 H, d, J = 6.9)[o]	1.07 (3 H, d, J = 6.7)[o]	1.09 (3 H, d, J = 6.7)[o]
17	1.00 (3 H, d, J = 6.8)	1.08 (3 H, d, J = 6.9)	0.97 (3 H, d, J = 6.9)[o]	0.97 (3 H, d, J = 6.7)[o]	1.01 (3 H, d, J = 6.7)[o]
18					1.96 (1 H, m)[n]
19	1.70 (3 H, br s)	1.71 (3 H, s)	1.78 (3 H, s)	1.76 (3 H, s)	0.79 (3 H, d, J = 6.8)[p]
20	4.85 (1 H, t, J = 1.3), 4.79 (1 H, br s)	4.90 (1 H, s), 4.75 (1 H, s)	5.00 (1 H, d, J = 0.9), 4.91 (1 H, s)	4.97 (1 H, s), 4.86 (1 H, s)	0.91 (3 H, d, J = 6.8)[p]

[a] ^{1}H NMR spectra were recorded in $CDCl_3$ solution at 360 MHz. Assignments were aided by spin decoupling, DDS, T_1 IR, and NOEDS experiments. J values are reported in Hz and the chemical shifts are given in δ units (ppm downfield from Me_4Si). [b] The methylene proton that points out of the ring and is coupled by 2–4 Hz to the C-4 epoxide methine. [c] The methylene proton that points below and more into the ring which is coupled to the C-4 epoxide methine by 8–10 Hz. [d] Nonassignable proton resonances. [e] The methylene proton that points out more below the ring. [f] The methylene proton that is above the ring. [g] The methylene proton that points out of the ring. [h] The methylene proton that points more in toward the center of the ring. [i] The pseudoaxial methylene proton that is down, pointing outward from and below the ring. [k] The pseudoequatorial methylene proton that points up, more above the ring. [n] Coupling constants were not determined. [o,p] Signals within a column may be reversed.

Scheme I



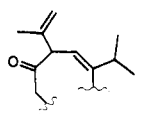
formed by irradiating the isopropyl methine proton at δ 2.39. This proton was found to be further coupled only to the olefinic proton at δ 5.57 by a small (<1 Hz) allylic coupling constant. The reverse DDS experiment, involving irradiation of the olefinic proton at δ 5.57, also showed this allylic coupling, thus establishing the isopropyl group as a substituent of the trisubstituted olefinic bond.

From these data, four of the five degrees of unsaturation in the molecular formula of **3** could be accounted for, demonstrating that calyculone A was monocarbocyclic. Furthermore, the ring was determined to be 12-membered via elimination of the recognized carbon substituents. However, the presence of these latter defined functional groups, and especially the presence of two isopropyl equivalents, excluded regularly isoprenoid ring systems for the carbon skeleton of calyculone A.

Pseudopterolide, a recently reported diterpenoid from the Caribbean gorgonian *Pseudopterogorgia acerosa*,[8] is an example of 12-membered monocyclic ring system that contains two isopropyl groups oriented 1,7. The cubitane carbon skeleton is the only other known example of a 12-membered monocyclic diterpenoid ring system that contains two isopropyl groups. Both of these diterpenoid ring systems were considered as possibilities for the carbon skeleton of calyculone A (**3**).

Two reactions yielded derivatives of **3** which proved the relative positions of the ketone, trisubstituted olefin, isopropyl group, and isopropenyl group in calyculone A. Reduction of **3** with $LiAlH_4$ (2 h, −78 °C) gave a single product, alcohol **8** (Scheme I), in high yield. Analysis of the ^1H NMR spectrum of alcohol **8** (Table II) revealed the presence of a new one-proton band at δ 3.65 (1 H, dd, J = 8.9, 7.3 Hz), which was assigned to the methine proton on the carbon bearing the newly formed hydroxyl group (C-11). Further analysis of the ^1H NMR data showed that the resonance seen in the spectrum of calyculone A at δ 4.54 was shifted upfield to δ 3.08 (1 H, dd, J = 8.9, 9.3 Hz) in the spectrum of **8**. Irradiation of this proton simplified a signal at δ 5.17 (1 H, d, J = 9.5 Hz) that was assigned to the olefinic proton and also decoupled the signal at δ 3.65 that was assigned to the α-hydroxyl methine proton. The trisubstituted olefin in **3** was therefore positioned β,γ to the carbonyl, with the isopropyl substituent placed at the γ carbon.

Next, catalytic hydrogenation of calyculone A yielded a dihydro derivative which gave spectral evidence to prove the relative positions of the isopropyl and isopropenyl groups. Hydrogenation of **3** selectively saturated the terminal olefin to give a single product, the dihydro derivative, **9**. The ^1H NMR spectrum of **9** (Table II) showed two new doublet methyls at δ 0.91 (3 H, d, J = 6.8 Hz) and 0.79 (3 H, d, J = 6.8 Hz), and the methine proton, observed at δ 4.54 in **3**, was found to be shifted upfield to δ 3.48 (1 H, dd, J = 8.4, 10.7 Hz) in **9**. Irradiation of this latter methine proton simplified the olefinic proton at δ 5.17 (1 H, d, J = 10.5 Hz) and also a complex signal at δ 1.96. When the one-proton resonance at δ 1.96 (1 H, m) was irradiated, the doublet methyls at δ 0.91 and 0.79 collapsed to singlets, thereby indicating an isopropyl group. The methine signal at δ 3.48 was also found to be reduced to a doublet resonance (J = 10.7 Hz). This experiment demonstrated that the isopropyl groups in derivative **9** were oriented 1,3 as in the cubitane carbon skeleton. The isopropyl group in **3**, was therefore positioned on the carbon α to the carbonyl and adjacent to the trisubstituted olefin. The resonance observed at δ 4.54 in the ^1H NMR spectrum of **3** was assigned to the diallylic proton at C-10 adjacent to the trisubstituted olefin and the ketone. Considering the connectivity established from spin-decoupling studies with derivatives **8** and **9**, a partial structure could be drawn as in a that established the relative



(a)

orientation of eleven carbon atoms in calyculone A. This partial structure also accounted for a deshielded carbon, observed in the ^{13}C NMR spectrum of **3** at δ 51.0 (t), assigned to a methylene carbon adjacent to the ketone.

While derivatives **8** and **9** established the 1,3 orientation of the isopropyl and isopropenyl groups in calyculone A, the relative positions and stereochemistries of the other substituents in **3** could not be ascertained. Hence, the final structure of compound **3** was solved by using X-ray crystallographic methods. The X-ray drawing of calyculone A is shown in Figure 1 and is thus fully defined as 4-(R*),5(R*)-epoxy-11-keto-1(S*),10(S*)-cubata-8(E),18-(20)-diene. The absolute stereochemistry of **3** was not determined in the X-ray experiment.

Once the structure of calyculone A was fully defined, structures for the other two isomeric cubitanes could be proposed based upon comparisons with compound **3**.

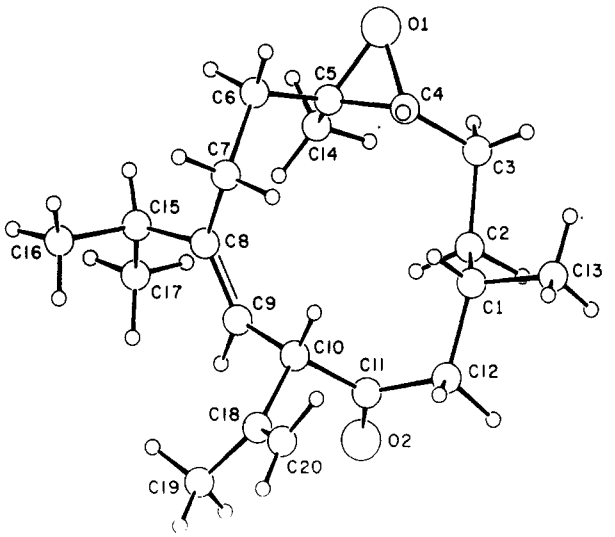
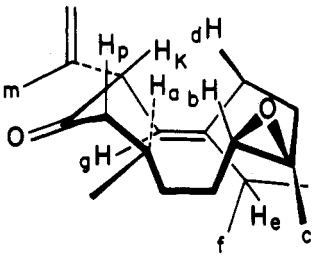


Figure 1. Final X-ray drawing of calyculone A indicating relative stereochemistry only.

(8) Bandurraga, M. M.; Fenical, W.; Donovan, S. F.; Clardy, J. *J. Am. Chem. Soc.* **1982**, *104*, 6463.



3

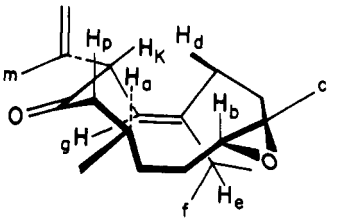
Proton(s) irradiated	Proton(s) enhanced	Estimated internuclear distances (Å)
a	b,d,k	2.0, 1.9, 1.9
b	a,d,k	2.0, 1.8, 2.5
c	e	2.1
g	f,m	1.8, 2.4
k	a,d,m,p	1.9, 1.6, 2.8, 2.6

Figure 2. Results of a ^1H NMR difference nuclear Overhauser enhancement (NOEDS) experiment with calyculone A.

Calyculones B and C (**4** and **5**) analyzed for $C_{20}H_{32}O_2$ by high-resolution mass spectrometry and showed infrared absorptions that suggested the presence in each of a carbonyl, similar to that found in calyculone A. The NMR features of these isomeric compounds (Tables I and II) also compared favorably to calyculone A, showing the presence in each isomer of similar functional groups and substituents. ^1H NMR spin-decoupling studies allowed the chemical shifts, multiplicities, and coupling constants for all protons to be readily defined. Assignments are given in Table II. Detailed analyses of these data revealed stereochemical similarities between the three isomers. First, the coupling constant between the diallylic methine at C-10 and the olefinic proton at C-9 was approximately equivalent (9.5–10.6 Hz) in all three isomers. This suggested that the dihedral angle between these protons and, hence, between the isopropenyl group at C-10 and the plane of the trisubstituted olefin, was identical in the three isomers. Secondly, the resonances due to the methine proton at C-1 and the C-13 doublet methyl were shifted only slightly in compounds **4** and **5** from their positions in the ^1H NMR spectrum of calyculone A, again suggesting similar stereochemical environments. Analysis of the ^{13}C NMR data also revealed, in comparison to compound **3**, that the resonances due to the C-13 secondary methyl and the methine carbon (C-1) were shifted only slightly in isomers **4** and **5**. On the basis of these favorable comparisons, the stereochemistry at C-1 and C-10 was proposed to be identical in all three isomers. Calyculones B and C were, therefore, concluded to be geometric isomers of calyculone A about the trisubstituted olefin and/or the epoxide functionalities.

Nuclear Overhauser enhancement difference[9] spectral (NOEDS) studies were performed with each isomer in order to confirm the stereochemical assignments of the asymmetric centers at C-10 and C-1, and to determine the structural differences between the three isomers. First, ^1H NMR experiments involving NOEDS were performed with calyculone A for the purpose of determining the relative spatial arrangement of all substituents, and at the same time, the conformation of the 12-membered ring in

(9) Richarz, R.; Würthrich, K. *J. Magn. Reson.* **1978**, *30*, 147.



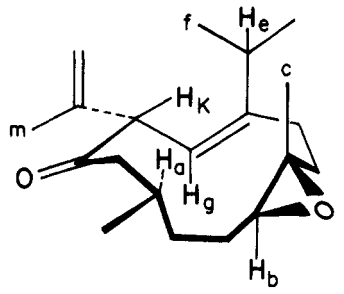
4

Proton(s) irradiated	Proton(s) in $CDCl_3$ enhanced (internuclear distances in Å)	Proton(s) in C_6D_6 enhanced (internuclear distances in Å)
a	b,k (1.7, 2.4)	k (2.4)
b	c,k (2.4, 2.8)	a,c,k (1.7, 2.4, 2.8)
c	b,k (2.4, 2.0)	a,b,k (2.8, 2.4, 2.0)
e	g (2.4)	f,g (2.2, 2.4)
f	e,g (2.2, 1.6)	--
g	e,f,m (2.4, 1.6, 2.3)	--
k	a,b,c,d,m (2.4, 2.8, 2.0, 1.6, 2.4)	a,b (2.4, 2.8)
m	k (2.4)	k (2.4)

Figure 3. Results of a ^1H NMR difference nuclear Overhauser enhancement (NOEDS) experiment with calyculone B.

solution. The results of these experiments, summarized in Figure 2, formed the basis for comparison to similar studies with isomers **4** and **5**. No enhancement of the epoxide proton (C-4) was observed when the associated methyl resonance (C-14) was irradiated, thus reinforcing the trans relationship already shown by X-ray crystallography. The *E* stereochemistry of the trisubstituted olefin could also be observed by measurement of the enhancement of one of the isopropyl methyls when the olefinic proton at C-9 was irradiated. More importantly, the spatial arrangement of the isopropyl group and the epoxide methyl on the same α-face of the molecule was clearly apparent. The methine proton at C-10 (H_k, Figure 2), the epoxide proton, and the proton at C-1 (H_a) were found to be within NOE proximity on the opposite β-face of the molecule. On the basis of these results and the X-ray structure of **3** in the solid state, calyculone A was assigned the solution conformation shown in Figure 2.

The results of similar experiments with calyculone B are summarized in Figure 3. Overlap of the signals due to the C-1 methine proton (H_a) and C-15 proton (H_e) in the ^1H NMR spectrum of calyculone B (**4**) (in $CDCl_3$) made it necessary to perform the NOEDS experiments in two solvents, $CDCl_3$ and C_6D_6. Since the olefinic proton (H_g), the isopropyl methine proton at C-15 (H_e), and one of the isopropyl methyls (H_f) were observed to be within NOE proximity, the trisubstituted olefin in calyculone B (**4**) was assigned the *E* configuration, as already defined in calyculone A. Since enhancement of the epoxide proton (H_b) was observed when the epoxide methyl (H_c) was irradiated, the epoxide was assigned a cis geometry in compound **4**. The spatial arrangement of the epoxide proton, the epoxide methyl, the C-10 methine proton (H_k), and the proton at C-1 (H_a) on the β-face of the molecule was also etablished in this manner. Finally, as in the case of calyculone A, the olefinic proton and the isopropyl group were shown to be oriented on the opposite α-face of the molecule. These NOE results demonstrated that the sole difference between calyculones A and B was in the geometry of the epoxide ring. Based on these studies, the structure of calyculone B (**4**) was assigned as 4(*R**),5(*S**)-epoxy-11-keto-1-(*S**),10(*S**)-cubata-8(*E*),18(20)-diene.



5

Proton(s) irradiated	Proton(s) enhanced	Estimated internuclear distances (Å)
b	g	2.7
c	a,e	2.4, 2.0
e	a,c,f,k,m	2.8, 2.0, 2.2, 1.8, 2.3
f	e	2.2
g	b	2.7
k	a,e,m	2.0, 1.8, 2.4
m	k	2.4

Figure 4. Results of a ^1H NMR difference nuclear Overhauser enhancement (NOEDS) experiment with calyculone C.

A similar investigation using ^1H NMR NOE difference spectroscopy allowed the relative spatial arrangement of all substituents to be defined and also suggested the conformation of the 12-membered ring in calyculone C (**5**). The results of these experiments are given in Figure 4. In this metabolite, one of the isopropyl methyls, the C-15 isopropyl methine proton (H_e), and the proton at C-10 (H_k), were found to be within NOE proximity. Therefore, in contrast to calyculones A and B, the stereochemistry of the trisubstituted olefin was defined as *Z* in compound **5**. Since no enhancement was observed in the epoxide proton (H_b) when the epoxide methyl (H_c) was irradiated, a trans geometry was assigned to this group. Overall, these experiments further established the spatial arrangement of the proton at C-10, the epoxide methyl, the isopropyl group, and the proton at C-1 on the β-face of the molecule. These studies established that calyculone C (**5**) differed from calyculone A in the geometry of the epoxide (also trans, but opposite) and the trisubstituted olefin. From these results, the structure of calyculone C (**5**) was assigned as 4(*S**),5(*S**)-epoxy-11-keto-1(*S**),10(*S**)-cubata-8-(*Z*),18(20)-diene.

Crassin acetate (**7**) was isolated and purified by HPLC from relatively polar fractions of the initial chromatography. This metabolite was a minor component of the gorgonian, representing only approximately 0.6% of the organic extract. Since very little NMR data had been published for this natural product,[6] it was necessary to isolate authentic compound **7** from a *Pseudoplexaura* species for spectral comparison. Hence, the originally quoted sources[5] for this metabolite were collected and crassin acetate was obtained. Spectral characteristics of the compound isolated from *E. calyculata* were determined to be identical in all respects with those of authentic crassin acetate.

The cubitane skeleton was first observed in a metabolite of the termite, *Cubitermes umbratus*.[4] Cubitene (**6**) was found to be a major constituent of the defensive secretion released from the frontal glands of *C. umbratus* soldiers. Since the isolation of cubitene, the cubitane skeleton has not been reported from any other source.

Although numerous cembranoids have been reported from several other *Eunicea* species,[10] crassin acetate has been previously isolated only from several species of the genus *Pseudoplexaura*.[5] Isolation of crassin acetate from a *Eunicea* species is not surprising, however, since both *Eunicea* and *Pseudoplexaura* are taxonomically within the same family Plexauridae.[1]

The cembranoid carbon skeleton has been proposed as a logical biosynthetic precursor to the irregularly isoprenoid cubitane ring system.[4] Prestwich et al.[4] found that cubitene (**6**) co-occurred with two related cembranoid derivatives. Although no direct chemical relationship can be envisioned for the conversion of crassin acetate (**7**) to any of the calyculones isolated from *E. calyculata*, the isolation of both diterpenoid ring systems from the same organism supports this proposed relationship.

Experimental Section

The general experimental conditions and instruments used in this investigation were described in a recent paper.[3] The decoupling difference (DDS) and NOE difference spectroscopy (NOEDS) experiments were performed, in general, as outlined by Hall and Sanders.[7] However, in the DDS and NOEDS experiments, the various irradiations were manually performed sequentially since the decoupler used was not under computer control. All samples prepared for NOEDS were degassed by bubbling Ar through the solution for 45–60 min and the tubes were sealed with parafilm. Solutions were made in $CDCl_3$ such that after degassing and a loss of a significant volume of $CDCl_3$, the final concentration was 0.03–0.05 M.

Collection and Extraction. *Eunicea calyculata* (Ellis and Solander) (collection number B-79-46) was collected by hand using scuba at 25–30 m depth in May, 1979 along the barrier reef near Carrie Bow Cay, Belize. The collection was stored and transported in 2-propyl alcohol (IPA). Upon workup, the IPA was decanted, the animal was homogenized, and the gorgonian cake was repeatedly extracted with $CHCl_3$/MeOH (2/1). After filtration, the combined IPA and $CHCl_3$/MeOH extracts were evaporated under vacuum to give a residue which was partitioned between saturated brine and $CHCl_3$. The $CHCl_3$ extract was subsequently dried over $MgSO_4$, filtered, and evaporated to yield 17 g of a crude extract (from 500 gm, dry weight of the gorgonian). A second collection of *E. calyculata* was made in September, 1981 in the Bahamas Islands. The gorgonian was again found in deeper waters and collected by hand using scuba. The animal was stored frozen and an organic extract was produced as described above except that IPA was not used. From both collections, compounds **1** and **2** were the major constituents eluted from a silica gel column with 25% and 15% EtOAc in isooctane, respectively. Calyculones A–C were isolated from the same relatively nonpolar fractions of the initial chromatography (15–20% EtOAc in isooctane). Crassin acetate (**7**) was isolated from a more polar fraction of the initial chromatography (50% EtOAc in isooctane). All metabolites were further purified by silica gel HPLC.

Calyculone A (3): 4(*R),5(*R**)-Epoxy-11-keto-1(*S**),10-(*S**)-cubata-8(*E*),18(20)-diene.** Calyculone A (**3**) crystallized from diethyl ether after extensive purification by silica HPLC (μ-Porasil, 15% EtOAc in isooctane). Recrystallization gave 50 mg (0.3% of the crude extract) of **3**, mp 101–102 °C. Calyculone A showed $[\alpha]^{20}_D$ +76 (*c* 0.61, $CHCl_3$) and exhibited the following spectral characteristics: IR ($CHCl_3$) 2950, 2900, 1700, 1460, 1420, 1220 cm^{-1}; HRMS M^+, *m/z* (relative intensity) obsd 304.2399 (2.4); (M^+; calcd for $C_{20}H_{32}O_2$, 304.2402).

(10) Hossain, M. B.; Nicholas, A. F.; van der Helm, D. *J. Chem. Soc., Chem. Commun.* **1968**, 385. van der Helm, D.; Enwall, E. L.; Weinheimer, A. J.; Karns, T. K. B.; Ciereszko, L. S. *Acta Crystallogr., Sect. B* **1976**, 1558. Chang, C. Y.; Ciereszko, L. S.; Hossain, M. B.; van der Helm, D. *Ibid.* **1980**, 731. Ealick, S. E.; van der Helm, D.; Weinheimer, A. J. *Ibid.* **1975**, 1618. Weinheimer, A. J.; Matson, J. R.; van der Helm, D.; Poling, M. *Tetrahedron Lett.* **1977**, 1295. Gross, R. A. Ph.D. thesis, University of Oklahoma, 1974.

Calyculone B (4): 4(*R),5(*S**)-Epoxy-11-keto-1(*S**),10-(*S**)-cubata-8(*E*),18(20)-diene.** Calyculone B (4) was isolated as an oil. Purification by silica HPLC (μ-Porasil, 15% EtOAc in isooctane) gave 70 mg (0.4% of crude extract) of 4. Calyculone B showed $[\alpha]^{20}_D$ −28 (*c* 1.10, $CHCl_3$) and exhibited the following spectral features: IR ($CHCl_3$) 2960, 2920, 1700, 1455, 1380 cm^{-1}; HRMS M^+, *m/z* (relative intensity) obsd 304.2393 (0.64); (M^+; calcd for $C_{20}H_{32}O_2$, 304.2402); 261.1845 ($C_{17}H_{25}O_2$, 5); 203.1426 ($C_{14}H_{19}O$, 1); 121.1010 (C_9H_{13}, 100); ^1H NMR (C_6D_6) δ 5.41 (1 H, d, *J* = 9.9 Hz) (C-9), 4.89 (1 H, s) (C-20), 4.85 (1 H, s) (C-20), 3.99 (1 H, d, *J* = 9.9 Hz) (C-10), 2.52 (1 H, dd, *J* = 9.1, 2.5 Hz) (C-4), 2.21 (1 H, m) (C-1), 1.94 (1 H, m) (C-15), 1.77 (3 H, s) (C-19), 1.23 (3 H, s) (C-14), 0.89 (3 H, d, *J* = 6.9 Hz) (C-17), 0.83 (3 H, d, *J* = 6.8 Hz) (C-16), 0.75 (3 H, d, *J* = 6.6 Hz) (C-13).

Calyculone C (5): 4(*S),5(*S**)-Epoxy-11-keto-1(*S**),10-(*S**)-cubata-8(*Z*),18(20)-diene.** Calyculone C (5) was isolated as an oil. Purification by silica HPLC (μ-Porasil; 15% EtOAc in isooctane) gave 50 mg (0.3% of crude extract) of calyculone C (5) which showed $[\alpha]^{20}_d$ +95 (*c* 0.99, $CHCl_3$) and displayed the following spectral characteristics: IR ($CHCl_3$) 3010, 2960, 2920, 2400, 1700, 1510, 1420, 1210, 1040, 920 cm^{-1}; HRMS M^+, *m/z* (relative intensity) obsd 304.2384 (2); (M^+; calcd for $C_{20}H_{32}O_2$, 304.2402); 136.1253 ($C_{10}H_{16}$, 72); 121.1022 (C_9H_{13}, 92); 93.0712 (C_7H_9, 86).

Single-Crystal X-ray Diffraction Analysis of Calyculone A (3). Preliminary X-ray photographs showed monoclinic symmetry and accurate lattice constants, determined from a least-squares fit of fifteen 2θ values, were *a* = 8.699 (2) Å, *b* = 10.771 (5) Å, *c* = 10.436 (2) Å, and β = 104.21 (2)°. The systematic extinctions, presence of chirality, and estimated density were uniquely accommodated by space group $P2_1$ with one molecule of $C_{20}H_{32}O_2$ forming the asymmetric unit. All unique diffraction maxima with 2θ ≤ 114° were collected by using a variable speed, 1° ω scan and graphite monochromated Cu Kα radiation (1.54178 Å). After correction for Lorentz, polarization, and background effects, 1324 (97%) of the 1361 reflections were judged observed ($I > 3\sigma(I)$) and used in subsequent calculations. The structure was solved uneventfully by direct methods.[11] Block diagonal least-squares refinements have converged to a standard crystallographic residual of 0.044 for the observed data. Additional crystallographic details can be found in the supplementary material described at the end of this paper.

Reduction of Calyculone A (3) To Yield Alcohol 8. Over a period of 15 min, a solution of calyculone A (3) (4.5 mg, 0.015 mmol) in dry diethyl ether (2 mL) was added with stirring to a suspension of lithium aluminum hydride ($LiAlH_4$) in dry diethyl ether (5 mL) cooled to −78 °C. After stirring for an additional 1.75 h at −78 °C, excess reagent was destroyed by addition of ethyl acetate followed by dropwise addition of 0.1 N HCl. The ether layer was then separated and the aqueous layer was repeatedly extracted with diethyl ether. The combined ether layers were dried over $MgSO_4$ and filtered, and the solvent was removed under vacuum to obtain the crude alcohol 8 (4.2 mg, 0.014 mmol, 91% from 3). Compound 8 showed $[\alpha]^{20}_D$ −5 (*c* 0.42, $CHCl_3$) and exhibited the following spectral features: IR ($CHCl_3$) 3500, 2950, 1460, 1060 cm^{-1}; MS M^+, *m/z* 306 for $C_{20}H_{34}O_2$ (low-resolution), 288 ($M^+ - H_2O$).

Catalytic Hydrogenation of Calyculone A (3) To Yield the Dihydro Derivative 9. Calyculone A (3) (3.1 mg, 0.01 mmol) was dissolved in anhydrous diethyl ether (15 mL) and added to a 25 mL filter flask containing a catalytic amount of PtO_2 and a stirring bar. A balloon and septum were attached to the reaction vessel. The flask was then purged with hydrogen and the balloon was filled. After stirring at 25 °C for 1 h, the hydrogen was removed and the solution was filtered through Celite. Evaporation of the ether gave the dihydro derivative 9 (2.9 mg, 0.009 mmol, 95% from 3) as an oil which showed $[\alpha]^{20}_D$ −137 (*c* 0.29, $CHCl_3$) and exhibited the following spectral characteristics: IR ($CHCl_3$) 2950, 1700, 1450, 1360, 1270 cm^{-1}; MS M^+, *m/z* 306 for $C_{20}H_{34}O_2$ (low-resolution).

Collection and Extraction of *Pseudoplexaura* sp. (F-39). *Pseudoplexaura* sp. (voucher number F-39) was collected by hand using scuba in July, 1980 in the Florida Keys. Crassin acetate (7), the major metabolite, was eluted from a silica gel column with 35% diethyl ether in petroleum ether and crystallized from the crude fraction. Repeated recrystallization from diethyl ether gave 3.9 gm (9% from 43 gm of crude extract chromatographed) of 7, mp 144.5–146 °C. Compound 7 showed $[\alpha]^{20}_D$ +55 (*c* 0.61, $CHCl_3$) and exhibited the following spectral features: UV λ_{max}^{MeOH} 215 nm (ε 5000); IR ($CHCl_3$) 3500, 2950, 1735–1745, 1615, 1480, 1460, 1370, 1120, 1090 cm^{-1}; MS M^+, *m/z* 376 for $C_{22}H_{32}O_5$ (low-resolution); ^1H NMR (220 MHz, $CDCl_3$) δ 6.47 (1 H, d, *J* = 2.0 Hz), 5.70 (1 H, d, *J* = 2.0 Hz), 5.39 (1 H, m), 5.27 (1 H, m), 5.08 (1 H, br t), 3.95 (1 H, d, *J* = 11.0 Hz), 3.67 (1 H, s), 2.70 (1 H, m), 2.49 (1 H, m), 1.96 (3 H, s), 1.72 (3 H, s), 1.61 (3 H, s), 1.43 (3 H, s); ^{13}C NMR (20 MHz, $CDCl_3$) 170.2 (s), 167.0 (s), 136.8 (s), 135.0 (s), 130.0 (s), 128.2 (d), 127.4 (t), 125.5 (d), 82.2 (d), 73.8 (s), 73.0 (d), 41.6 (t), 39.9 (t), 39.1 (t), 37.4 (d), 24.5 (t), 24.0 (t), 22.2 (t), 20.8 (2 C, q), 14.7 (q), 14.5 (q).

Crassin Acetate (7) Isolated from *E. calyculata*. Crassin acetate was isolated as an amorphous solid following purification over Florisil and medium-pressure and high-performance liquid chromatography (with μ-Bondapak C_{18} reverse phase using 80% MeOH in H_2O). Approximately 100 mg (0.6% of the crude extract) of 7 were obtained. Crassin acetate isolated from *E. calyculata* was identical in all respects with the authentic metabolite isolated from collection F-39.

Acknowledgment. This paper is contribution number 135 of the IMSWE (Inshore Marine Shallow Water Ecosystem) Reef and Mangrove Investigation in Carrie Bow Cay, Belize. The IMSWE program is sponsored by the Smithsonian Institute and supported, in part, by the Exxon Corporation. We thank Dr. Klaus Ruetzler, program director, for the kind invitation to participate. Mass spectra were kindly provided by the Mass Spectrometry Resource Center, School of Pharmacy, University of California, San Francisco (A.L. Burlingame, Director), supported by NIH grant number RR00719. Support for research at Cornell University was provided under NIH Grant CA-24487, the New York Sea Grant Program, and the NSF under Grant INT-81-17327. Research at the Scripps Institution was supported by the NSF, Chemistry Division, under Grant CHE81-11907. We wish to thank Drs. Katy Muzik and Frederick M. Bayer for their identification of *Eunicea calyculata*.

Supplementary Material Available: Tables of fractional coordinates, thermal parameters, bond distances, bond angles for calyculone A (5 pages). Ordering information is given on any current masthead page.

(11) All crystallographic calculations were done on a PRIME 850 computer operated by the Cornell Chemistry Computing Facility. Principal programs employed were: REDUCE and UNIQUE, data reduction programs by M.E. Leonowicz, Cornell University, 1978; MULTAN 78, a system of computer programs for the automatic solution of crystal structures from x-ray diffraction data (locally modified to perform all Fourier calculations including Patterson syntheses) written by P. Main, S. E. Hull, L. Lessinger, G. Germain, J. P. Declercq, and M. M. Woolfson, University of York, England, 1978; NQEST, CBER 173 version of the negative quartets figure of merit estimate written by C. M. Weeks at the Medical Foundation of Buffalo, Inc., 1976; BLS78A, an anisotropic block diagonal least-squares refinement written by K. Hirotsu and E. Arnold, Cornell University, 1980; CRYSTALS, a crystallographic system written by D.J. Watkin and J.R Carruthers, Chemical Crystallography laboratory, University of Oxford, 1981; ORTEP, a crystallographic illustration program written by C.K. Johnson, Oak Ridge National Laboratory (ORNL-3794) 1970; PLUTO78, a crystallographic illustration program by W.D.S. Motherwell, Cambridge Crystallographic Data Centre, 1978; and BOND, a program to calculate molecular parameters and prepare tables written by K. Hirotsu, Cornell University, 1978.