Four Novel Diterpenoids: Clavirolides B, C, D, and E from the Chinese Soft Coral Clavularia viridis

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J. Nat. Prod., **1991**, 54 (2), 380-385• DOI:
10.1021/np50074a005 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 2, pp. 380-385, Mar-Apr 1991

FOUR NOVEL DITERPENOIDS: CLAVIROLIDES B, C, D, AND E FROM THE CHINESE SOFT CORAL *CLAVULARIA VIRIDIS*

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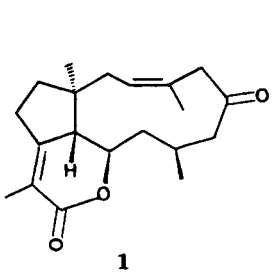
ABSTRACT.—Four new dolabellane diterpenoids, clavirolides B, C, D, and E, have been isolated from the soft coral *Clavularia viridis* collected off the Xisha Islands in the South China Sea. Their structures have been determined as **2**, **3**, **4**, and **5**, respectively.

In previous papers on the constituents of *Clavularia viridis* Quoy and Gaimard (Clavulariidae), we reported the isolation and structure elucidation of two diterpenoids, clavudiol A and clavirolide A [**6**] (1), and a new sterol, clavisterol A (2). In the course of our continuing investigation on the constitutents of this animal, we obtained four new diterpene lactones. This paper describes the isolation and structure elucidation of these diterpenoids.

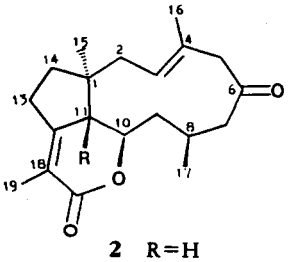
The MeOH-$CHCl_3$ (10:1) extract of the dried specimens collected from the Xisha Islands in the South China Sea was partitioned by an EtOAc/H_2O solvent system. The EtOAc phase was chromatographed over Si gel columns repeatedly to afford the known clavulactone [**1**] (3) and four new compounds, clavirolides B [**2**], C [**3**], D [**4**], and E [**5**].

The molecular formulae, $C_{20}H_{28}O_3$ or $C_{20}H_{30}O_3$, suggested the diterpenoid nature of these metabolites. Their nmr spectral data (Tables 1 and 2) revealed that they possessed the dolabellane skeleton, rare among octacorals.

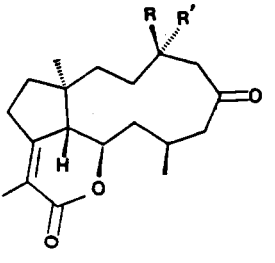
Compound **1**, obtained as colorless prisms, exhibited in hrms a molecular ion at *m/z* 316.2036 ($C_{20}H_{28}O_3$, calcd 316.2011). All spectral data of this compound coincided in every respect with those of clavulactone [**1**] (Tables 1 and 2), which has previously been isolated from an unidentified species of *Clavularia*. The absolute stereochemistry of clavulactone was determined by single-crystal X-ray analysis and cd spectroscopy (3). Catalytic hydrogenation of **1** afforded a mixture of two diastereoisomers. In the ^{1}H-nmr



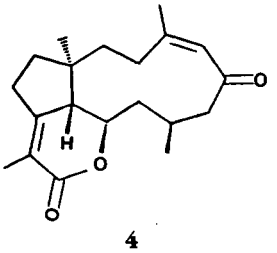
1



2 R=H
6 R=OH



3 R=H, R′=Me
7 R=Me, R′=H



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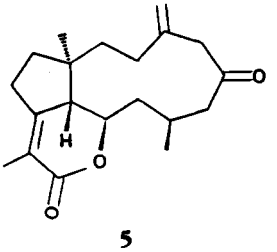

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TABLE 1. ^{1}H-nmr Partial Data of Clavirolides **1–5** in $CDCl_3$.[a]

Proton	Compound				
	1	**2**	**3**	**4**	**5**
H-3	5.42, 1H, bd (12.0)	5.58, 1H, m			
H-5				5.94, 1H, s	
H-10	4.15, 1H, t (8.0)	4.27, 1H, m	4.17, 1H, t (10.8)	4.28, 1H, m	4.20, 1H, m
H-15	0.96, 3H, s	0.97, 3H, s	0.87, 3H, s	0.82, 3H, s	0.86, 3H, s
H-16	1.92, 3H, s	1.70, 3H, s	0.95, 3H, d (7.3)	1.82, 3H, s	5.03, 1H, bs 4.92, 1H, bs
H-17	1.08, 3H, d (7.0)	1.10, 3H, d (8.0)	1.14, 3H, d (6.8)	1.17, 3H, d (7.6)	1.18, 3H, d (5.7)
H-19	1.82, 3H, s	1.83, 3H, s	1.81, 3H, dd (3.8, 2.0)	1.83, 3H, s	1.82, 3H, s

[a]At 90 MHz, δ in ppm, J (in parentheses) in Hz.

spectrum, the signals at δ 1.92 (3H, s, Me-16) and 0.92 (3H, s, Me-15) of **1** were replaced by two sets of doublets at δ 1.09 (1.8H, d, J = 7 Hz), 0.96 (1.2H, d, J = 7 Hz) and 0.81 (1.8H, s), 0.87 (1.2H, s), respectively (Table 3). A similar result was observed for clavulactone (3). Gc-ms analysis gave two peaks in the ratio of 64.7:35.3; their ms data are given in the Experimental section.

TABLE 2. ^{13}C-nmr Data of Clavirolides **1–6**.[a]

Carbon	Compound						
	1	**2**[b]	**3**[b,c]	**3**[b,d]	**4**[b]	**5**[b]	**6**
C-1	45.3 (s)	46.4 (s)	44.6 (s)	44.8 (s)	44.6 (s)	45.0 (s)	50.7 (s)
C-2	37.0 (t)	40.2 (t)[e]	46.5 (t)[e]	46.2 (t)[e]	37.1 (t)[e]	37.1 (t)[e]	33.2 (t)
C-3	122.3 (d)	127.4 (d)	32.8 (t)[f]	32.4 (t)[f]	27.8 (t)[f]	30.5 (t)	127.7 (d)
C-4	131.6 (s)	128.5 (s)	26.2 (d)	26.8 (d)	147.6 (s)	142.1 (s)	130.0 (s)
C-5	42.8 (t)	50.7 (t)	53.2 (t)	53.4 (t)	129.7 (d)	48.3 (t)	54.0 (t)
C-6	207.5 (s)	206.7 (s)	212.0 (s)	211.6 (s)	206.0 (s)	210.1 (s)	207.6 (s)
C-7	55.7 (t)	55.1 (t)	53.2 (t)	53.4 (t)	48.5 (t)	53.0 (t)	54.3 (t)
C-8	25.8 (d)	29.6 (d)	28.8 (d)	29.1 (d)	30.6 (d)	28.5 (d)	30.9 (d)
C-9	43.0 (t)	44.2 (t)	43.0 (t)[e]	43.1 (t)[e]	41.2 (t)	43.1 (t)	37.4 (t)
C-10	77.9 (d)	78.7 (d)	78.0 (d)	77.9 (d)	79.4 (d)	78.4 (d)	82.6 (d)
C-11	51.5 (d)	54.3 (d)	52.3 (d)	52.5 (d)	53.3 (d)	52.1 (d)	78.2 (d)
C-12	161.3 (s)	159.5 (s)	160.3 (s)	160.5 (s)	158.8 (s)	156.0 (s)	162.0 (s)
C-13	27.0 (t)	26.3 (t)	27.1 (t)	27.1 (t)	26.4 (t)[f]	27.0 (t)	25.3 (t)
C-14	38.0 (t)	41.2 (t)[e]	37.7 (t)[f]	37.4 (t)[f]	38.7 (t)[e]	36.3 (t)[e]	39.1 (t)
C-15	23.0 (q)	20.6 (q)	22.6 (q)	22.4 (q)	22.1 (q)	22.1 (q)	21.4 (q)
C-16	24.6 (q)	15.1 (q)	21.3 (q)	21.3 (q)	22.8 (q)	117.1 (t)	16.7 (q)
C-17	20.1 (q)	18.7 (q)	19.9 (q)	19.9 (q)	18.3 (q)	21.9 (q)	20.7 (q)
C-18	120.1 (s)	119.6 (s)	119.9 (s)	119.7 (s)	119.1 (s)	120.0 (s)	121.3 (s)
C-19	12.5 (q)	12.4 (q)	12.4 (q)	12.3 (q)	12.1 (q)	12.5 (q)	12.5 (q)
C-20	166.1 (s)	165.0 (s)	166.2 (s)	165.8 (s)	164.8 (s)	166.0 (s)	165.4 (s)

[a]22.5 MHz, in $CDCl_3$, in ppm.

[b]Assignments were made by comparison with data for **1** and **6**.

[c]Data for compound **3** obtained by subtraction of the spectrum of compound **7** from that of mixture of compounds **3** and **7**.

[d]Data for compound **3** isolated from *Clavularia viridis*.

[e,f]Values in the same column with the same superscript may be interchanged.

TABLE 3. Selected ^{1}H-nmr Data of Hydrogenation Products of Compounds **1**, **2**, **4** in $CDCl_3$.

Proton	Hydrogenation product of		
	1	**2**	**4**
Me-15	0.87, 1.2H, s		0.86, 1.8H, s
	0.81, 1.8H, s	0.81, 3H, s	0.81, 1.2H, s
Me-16	0.96, 1.2H, d (7.3)		0.95, 1.8H, d (7.3)
	1.09, 1.8H, d (7.3)	1.08, 3H, d	1.08, 1.2H, d (7.3)
Me-17	1.15, 3H, d (6.8)	1.15, 3H, d (6.8)	1.15, 3H, d (6.8)
Me-19	1.82, 3H, s	1.81, 3H, s	1.81, 3H, s
H-10	4.12, 1H, m	4.09, 1H, m	4.10, 1H, m

[a]At 90 MHz, δ in ppm, J (in parentheses) in Hz.

Clavirolide B [**2**] exhibited a molecular ion at *m/z* 316.2034 in high resolution fabms, corresponding to a molecular formula of $C_{20}H_{28}O_3$. The uv absorption of **2** at 228 nm ($\epsilon = 10600$), together with ir absorptions at 1693 and 1125 cm^{-1} and ^{13}C-nmr signals at δ 165.0 (s), 159.5 (s), and 119.6 (s), suggested the presence of a conjugated lactone system. In addition, the ir band at 1706 cm^{-1} and the ^{13}C-nmr signal at δ 206.7 (s) indicated a ketone function. Signals in the ^{13}C-nmr spectrum at δ 128.5 (s) and 127.4 (d), together with a signal accounting for one vinylic hydrogen in the ^{1}H-nmr spectrum at δ 5.58 (1H, bt, $J = 8.3$ Hz), established the presence of one trisubstituted double bond. The *E* configuration of this olefinic bond was indicated by the chemical shift of the vinyl methyl group (δ 15.1, q) (4). Comparison of the ^{13}C-nmr chemical shifts of **2** with those of clavirolide A [**6**] (1) (Table 2) clearly showed that **2** might be the 11-deoxy derivative of **6**. The chemical shift of C-11 (δ 54.3, d) was shielded in comparison to the corresponding signal of **6** (δ 78.2, s), and the significant upfield shifts of C-1, C-10, and C-12 were accounted for by the lack of the 11-OH group (5). The signal of C-2 in **2** showed a downfield shift, due to the absence of a γ effect of the 11-OH group.

In contrast with the hydrogenation of **1**, that of **2** afforded a single product **7** identical to the major hydrogenation product of **1**, as supported by its ^{1}H-nmr data (Table 3) and a gc-ms experiment. The 11-membered ring in the dolabellane skeleton is more rigid than expected (6), and the stereospecific hydrogenation might be due to steric hindrance to attack on the si-si prochiral face of the trans double bond. Inspection of a Drieding model of **7** suggested that it has a 4*R* configuration.

The similarities of the ^{1}H and ^{13}C nmr of **1–5** led to the hypothesis that all these compounds had a dolabellane skeleton with similar functionalities. The eims of clavirolides **1–5** suggested that these compounds, except for **3**, might be double-bond isomers.

Clavirolide C [**3**] gave satisfactory elemental analysis for a $C_{20}H_{30}O_3$ formula, in agreement with the molecular ion at *m/z* 318 in its ms. The ^{1}H-nmr and ^{13}C-nmr spectra of **3** exhibited closely comparable data with those of **1**. However, in the ^{1}H-nmr spectrum of **3**, the signal for the olefinic proton was absent, and the signal at δ 1.92 (s) for the vinylic methyl group was replaced by a signal at δ 0.95 (d) (>CH-CH_3). Furthermore, the ^{13}C-nmr spectrum showed only one C=C [δ 165.8 (s) and 119.7 (s)] in the conjugated lactone moiety and lacked the two sp^2 carbons for the isolated double bond, suggesting that **3** is one of the hydrogenation products of **1**. The ^{1}H-nmr spec-

tral data (Table 1) and the gc retention time, 52:50 min, of **3** were identical with those of the minor hydrogenation product of **1**, confirming this structural assignment and establishing the 4*S* stereochemistry of **3**.

The molecular formula $C_{20}H_{28}O_3$ of clavirolide D [**4**] was established by high resolution fabms. The spectral data of **4** indicated the presence of an α,β-unsaturated lactone moiety. In addition, a broad ir absorption band between 1668 and 1710 cm^{-1} indicated the presence of a supplementary α,β-unsaturated ketone. When compared with **1** and **2**, the signal of the vinyl proton of **4** appeared as a sharp singlet (5.94, 1H, s) instead of a multiplet and was deshielded due to the anisotropy of the ketone. Thus, this double bond is located at C-4–C-5. NOe difference spectroscopy indicated the *Z* stereochemistry of this double bond. Irradiation of H-5 (δ 5.94) gave an enhancement of the signal of Me-16, and irradiation of Me-16 (δ 1.82) caused a 23% enhancement of the signal at δ 5.94 for the vinyl proton.

Catalytic hydrogenation of **4** afforded a mixture of **3** and **7**, with **3** as the major product. The ratio of the products analyzed by gc-ms is opposite to that observed for clavulactone [**1**]. Inspection of a Drieding model of **4** shows that the si-si prochiral face of the cisoid conjugated double bond is the less hindered side, giving **3** as the major product; the re-re prochiral face is more hindered due to the Me-15. Thus, the structure and stereochemistry of clavirolide D were confirmed.

The fifth compound, clavirolide E [**5**], $C_{20}H_{28}O_3$ by lrms coupled with a ^{13}C-nmr DEPT spectrum, was obtained in a small amount. Spectral data showed that **5** was a double-bond isomer of **2** in which the trisubstituted double bond [δ_C 127.4 (d), 128.5 (s); δ_H 5.58 (1H, m)] and the accompanying vinyl methyl group [δ_C 15.1 (q); δ_H 1.70 (3H, s)] of **2** were replaced by an exocyclic methylene [δ_C 142.1 (s) and 117.1 (s)]. This allowed us to propose the structure of clavirolide E as **5**. In consideration of the biogenetic relationships, these double-bond isomers can be assumed to be derived via a common C-4 carbonium ion, followed by loss of a proton. Thus the structure of clavirolide E can be confirmed as **5**.

The biological properties of **1**, **2**, **3**, and **4** have been described elsewhere (7). In summary, cytotoxicity assays showed that clavulactone [**1**] was the most active compound on cultured Ehrlich ascites carcinoma (EAC) cells (**1**, IC_{50} 8 μg/ml; **4**, 11.7 μg/ml; **3**, 27 μg/ml; and **2**, 21 μg/ml). Experiments with rabbit isolated smooth muscles established that **2**, **3**, and **4** block Ca^{++} channels (8,9); observed PD'_2 values were 4.68, 5.48, and 4.79, respectively. In addition, **3** showed 50% negative inotropic activity on isolated guinea pig atria muscles at 36 μM and a 43.7% bradycardia on isolated guinea pig heart together with decreasing of the blood pressure of rats (24 mm Hg) at 45 μM/kg.

EXPERIMENTAL

INSTRUMENTAL.—Optical rotations were measured with a Perkin-Elmer 241 polarimeter. ^1H- and ^{13}C-nmr were recorded on a JEOL FX-90Q instrument. A VG analytical ZAB-HF-3F and an MAT 731 mass spectrometer were used; gc-ms was carried out on a Finnigan Mat TSQ70B GC/MS/MS Q3MS system operating in the electron impact mode. A DB-5 column (30 m × 0.25 mm i.d.) was used with temperature program 150°–290° at 2°/min, with He at 30 cm^3/min as carrier. A Nicolet 5DX Ft-ir spectrometer and a Perkin-Elmer 240C automatic elemental analyzer were used.

ANIMAL MATERIAL.—The soft coral *C. viridis* was collected from the Xisha Islands in the South China Sea in April 1988. A voucher specimen is deposited in the Research Center of Organic Natural Products, Zhongshan University, Guangzhou, China.

EXTRACTION AND ISOLATION.—Chopped dry specimens (3 kg) were immersed in MeOH containing 10% $CHCl_3$ at room temperature. The extract was concentrated and partitioned between EtOAc and H_2O. The EtOAc phase (120 g) was chromatographed over a column of Si gel, eluting with a stepwise gradient of EtOAc in cyclohexane. Pure compounds were obtained by rechromatography and final recrystalli-

zation. The fraction eluted with 9% EtOAc gave clavulactone; the 18% EtOAc eluate was chromatographed again to give clavirolides A, B, C, and E. The more polar fractions eluted with 25% EtOAc gave clavirolide D.

Fraction 8 eluted with 9% EtOAc contained two major components. Further chromatography over a basic Al_2O_3 column [Et_2O-petroleum ether (1:1)] and recrystallization from Me_2CO/petroleum ether gave clavulactone [**1**] (500 mg, 0.017%): mp 195.0–197.0° [lit. (3) 188–190°], $[\alpha]^{20}D$ −238.5 (c = 0.035, MeOH) [under the same condition, the authentic sample of clavulactone gave the specific optical rotation: $[\alpha]^{20}D$ −232.0 (c = 0.031, MeOH)], $[\alpha]^{30}D$ −33.1 (c = 0.068, $CHCl_3$) [lit. (3) $[\alpha]^{30}D$ −32.3 (c = 1.81, $CHCl_3$)]; uv λ max (MeOH) 232 nm (ε = 13000); ir (KBr) 1707, 1698, 1664, 1454, 1377, 1300, 1159 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; ms *m/z* (rel. int.) $[M]^+$ 316 (36), 301 (6), 288 (23), 273 (12), 247 (27), 229 (12), 218 (23), 203 (32), 187 (37), 175 (27), 163 (29), 162 (35), 148 (35), 135 (55), 119 (20), 105 (27), 91 (63), 79 (46), 69 (100), 55 (41).

Fraction 9 eluted with 18% EtOAc was shown by tlc [EtOAc-$CHCl_3$ (6:1)] to contain at least five components. Rechromatography over a basic Al_2O_3 column furnished fraction 2, eluted with Et_2O-petroleum ether (1:1), and that was further subjected to preparative tlc performed on $AgNO_3$-Si gel F_{254} (0.25 thick) using Me_2CO/petroleum ether as solvent. Clavirolide C [**3**] (60 mg) was obtained as colorless needles: mp 124.5–126.5°; $[\alpha]^{26.5}D$ −38.5 (c = 0.018, MeOH); uv λ max (MeOH) 232 nm (ε = 9500); ir (KBr) 1706, 1698, 1647, 1450, 1385, 1310, 1120 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; gc-ms retention time 52:50 min, ms *m/z* (rel. int.) 317.8 (48), 299.8 (36), 284.2 (7), 271.1 (23), 254.6 (12), 243.2 (10), 218.4 (14), 205.3 (27), 200.9 (40), 187.2 (26), 177.2 (23), 163.2 (38), 161.3 (62), 159.0 (50), 134.9 (100), 120.9 (69), 107.1 (47), 94.5 (38), 91.0 (47), 78.9 (32), 69.0 (51), 55.1 (37). *Anal.* found C 74.90, H 9.57; calcd for $C_{20}H_{30}O_3$, C 75.43, H 9.49.

Clavirolide E [**5**] (10 mg, 0.00033%) was obtained as colorless prisms: mp 200–201°; $[\alpha]^{20}D$ −40.0 (c = 0.010, MeOH); uv λ max (MeOH) 234 nm (ε = 12800); ir (KBr) 1706, 1698, 1425, 1312, 1118 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; ms *m/z* (rel. int.) $[M]^+$ 316 (15), 288 (10), 273 (8), 247 (14), 229 (13), 218 (19), 203 (26), 187 (37), 175 (18), 163 (22), 162 (24), 148 (31), 135 (44), 119 (16), 107 (21), 91 (44), 79 (34), 69 (100), 55 (28).

Fraction 6 from the Al_2O_3 column eluted with Me_2CO-petroleum ether (2:5) gave **6** as reported previously (1), and **2** was obtained as white plates. Compound **2** (100 mg, 0.0033%): mp 143.5–145.5° (Me_2CO/petroleum ether); $[\alpha]^{26.5}D$ −392.7 (c = 0.085, MeOH); uv λ max (MeOH) 228 nm (ε = 10600); ir (KBr) 1706, 1693, 1431, 1387, 1300, 1125 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; ms *m/z* (rel. int.) $[M]^+$ 316 (13), 288 (13), 247 (8), 229 (16), 218 (4), 203 (19), 187 (39), 175 (23), 163 (19), 161 (22), 148 (37), 135 (49), 119 (17), 107 (22), 91 (43), 79 (35), 69 (100), 55 (29).

Fraction 10 from the Si gel column, eluted with 25% EtOAc, contained two major components (by tlc). One was identical by its mp, ir, and ^1H and ^{13}C nmr to batyl alcohol, widespread in soft corals. After separation of the batyl alcohol, the residue was further subjected to preparative tlc performed on $AgNO_3$-Si gel F_{254} to give pure clavirolide D [**4**] as colorless syrup. Compound **4** (80 mg, 0.002%): $[\alpha]^{26.5}D$ −118.5 (c = 0.080, MeOH); uv λ max (MeOH) 234 nm (ε = 13900); ir (KBr) 1693, 1668, 1637, 1443, 1381, 1300, 1131 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; ms *m/z* (rel. int.) $[M]^+$ 316 (25), 298 (12), 288 (6), 247 (8), 229 (11), 218 (11), 215 (16), 203 (21), 187 (18), 177 (19), 163 (35), 149 (20), 148 (20), 135 (86), 119 (27), 107 (35), 105 (46), 95 (51), 91 (100), 79 (77), 69 (69), 55 (79).

CATALYTIC HYDROGENATION OF **1**.—To a solution of **1** (4 mg) in EtOAc (0.5 ml) was added prereduced PtO_2 (8 mg) suspended in EtOAc. The reaction medium was stirred overnight under an atmosphere of H_2 and treated as usual to give a mixture of **3** and **7**: ^1H nmr see Table 3. The gc-ms analysis of the product showed two components in the ratio of 64.7:35.3 with the retention time of 52:22 and 52:50 respectively. The predominant product **7**: ms *m/z* (rel. int.) $[M]^+$ 317.8 (32), 299.8 (30), 284 (8), 271.1 (16), 254.3 (6), 218.4 (7), 205.3 (14), 200.9 (25), 187.2 (16), 177.2 (14), 163.2 (31), 161.2 (40), 159.0 (31), 134.9 (100), 118.9 (32), 94.9 (43.3), 91.0 (30), 78.9 (21), 69.0 (32), 55 (23). The minor product **3**: ms *m/z* (rel. int.) $[M]^+$ 317.8 (44), 299.8 (32), 284.2 (3), 271.1 (20), 254.6 (6), 243.2 (5), 218.4 (11), 205.3 (23), 200.9 (36), 187.2 (23), 172.2 (20), 163.2 (35), 161.3 (59), 159.0 (46), 134.9 (100), 120.9 (65), 107.1 (43), 94.5 (34), 91.0 (45), 78.9 (29), 69.0 (45), 55.1 (32).

CATALYTIC HYDROGENATION OF **2**.—Hydrogenation of **2** (5 mg) was carried out under the abovementioned conditions. The catalyst was filtered off, and the solution was evaporated to dryness. The residue **7** exhibited in hrms a molecular ion at 318.2192 ($C_{20}H_{30}O_3$); $[\alpha]^{20}D$ −30.8 (c = 0.046, MeOH); ^1H nmr see Table 3; gc-ms retention time 52:22 min; ms *m/z* (rel. int.) $[M]^+$ 317.8 (42), 299.8 (40), 284.8 (19), 271.1 (28), 254.3 (16), 218.4 (12), 205.3 (25), 200.9 (35), 187.4 (26), 177.2 (25), 163.2 (41), 161.2 (51), 159.0 (40), 134.9 (100), 118.9 (42), 94.9 (55), 91.0 (41), 78.9 (32), 69.0 (43), 55.0 (31).

CATALYTIC HYDROGENATION OF **4**.—Hydrogenation of **4** (5 mg) was carried out under the abovementioned conditions to afford a mixture of **3** and **7** in the ratio of 60.3:39.7 by gc-ms. The predominant

product **3**: retention time 52:50 min; ms *m/z* (rel. int.) $[M]^+$ 318.2 (23), 300.1 (16), 285.1 (5), 271.4 (8), 255.2 (8), 243.2 (7), 218.2 (10), 205.1 (22), 201.0 (26), 187.1 (21), 177.2 (20), 163.4 (24), 161.1 (53), 159.1 (25), 135.1 (100), 121.2 (45), 107.1 (24), 95.1 (64), 91.1 (48), 79.1 (38), 69.1 (65), 55.1 (60). The minor product **7**: retention time 52:22 min; ms *m/z* (rel. int.) $[M]^+$ 318.2 (29), 300.1 (21), 285.1 (8), 271.4 (7), 255.2 (11), 218.2 (20), 205.1 (48), 201.1 (50), 187.2 (36), 177.2 (36), 163.2 (38), 161.1 (95), 159.2 (42), 135.1 (100), 120.7 (52), 118.9 (42), 95.1 (70), 91.1 (50), 79.1 (36), 69.1 (75), 55.1 (52).

BIOLOGICAL ACTIVITIES.—The tumor cells were obtained from the Cancer Institute, Sun Yat-sen University of Medical Sciences. The in vitro cytotoxicity assay was carried out according to procedures described in Donelli *et al.* (10) and Rang and Date (11). Experiments with smooth muscles and atria muscles were based on methods described in a textbook (12).

ACKNOWLEDGMENTS

The authors are grateful to the National Science Foundation of the Peoples Republic of China for financial support of this work. We thank Dr. C.E. Costello and Mr. Chen-hui Zeng of MIT for hrms measurements; Li Zupu of the South China Sea Institute of Oceanology, Academia Sinica for identification of the coral species; Wu Yulin (3) for an authentic sample of clavulactone; and Xiang Hui of Zhongshan University for the bioassays.

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Received 24 April 1990