Tetrahedron Vol. 47, No. 18/19, pp. 2999-3006, 1991
Printed in Great Britain

0040-4020/91 $3.00+.00

CALYCULINS E, F, G, AND H, ADDITIONAL INHIBITORS OF PROTEIN PHOSPHATASES 1 AND 2A, FROM THE MARINE SPONGE *DISCODERMIA CALYX*

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(*Received in Japan* 24 *November* 1990)

Abstract: Calyculins E (**5**), F (**6**), G (**7**), and H (**8**) were isolated from the marine sponge *Discodermia calyx*. The structures for **5-8** were assigned on the basis of the interpretation of spectral data. These novel calyculins were potent inhibitors of protein phosphatases 1 and 2A: effective doses for 50% inhibition of protein phosphatases 2A activity for these calyculins were 2.7-6.0 nM.

Calyculin A (**1**) from the marine sponge *Discodermia calyx* was found to be a potent antitumor agent.[2,3] Besides cytotoxic activity, calyculin A induced contraction in smooth muscle fibers, which resulted from its specific inhibition of protein phosphatases 1 and 2A.[4] Calyculin A was a potent tumor promoter of the okadaic acid class on mouse skin.[5]

Along with calyculin A, calyculins B (**2**), C (**3**), and D (**4**) were isolated from the same sponge.[2] During the course of our effort to reisolate calyculin A for further biochemical study, we found other UV-absorbing HPLC peaks, which revealed TLC spots characteristic of the compounds of the calyculin family.[6] We now describe the isolation and structure determination of four more calyculins.

The CH_2Cl_2 soluble portion of the MeOH extract of *D. calyx* was fractionated on open columns of silica gel and C_{18}-silica gel. The calyculin fractions were finally purified by reverse-phase HPLC to obtain calyculins E (**5**, 1.8 x 10^{-3}% yield, wet weight), F (**6**, 1.3 x 10^{-3} %), G (**7**, 9 x 10^{-4} %), and H (**8**, 9 x 10^{-4} %).

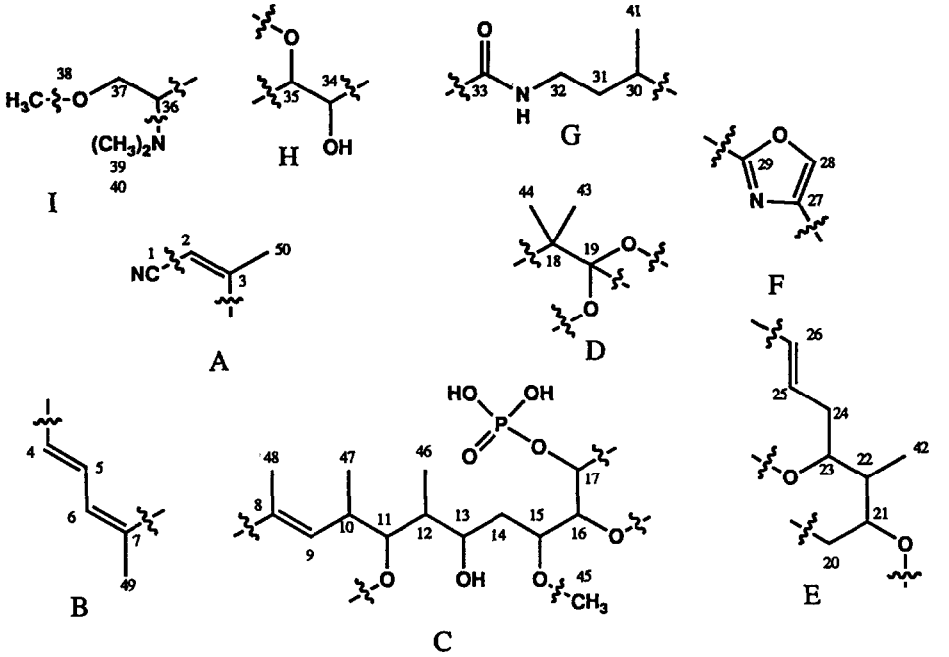
Calyculin E had the molecular formula of $C_{50}H_{81}N_4O_{15}P$ as determined by FAB mass spectroscopy and NMR data. The UV spectrum for **5** (λ_{max} 321, 227 nm) suggested that its chromophore was slightly different from that of calyculins A-D (λ_{max} 342, 230 nm). The COSY spectrum combined with the HMQC spectrum[7] allowed us to deduce structural units A-I.

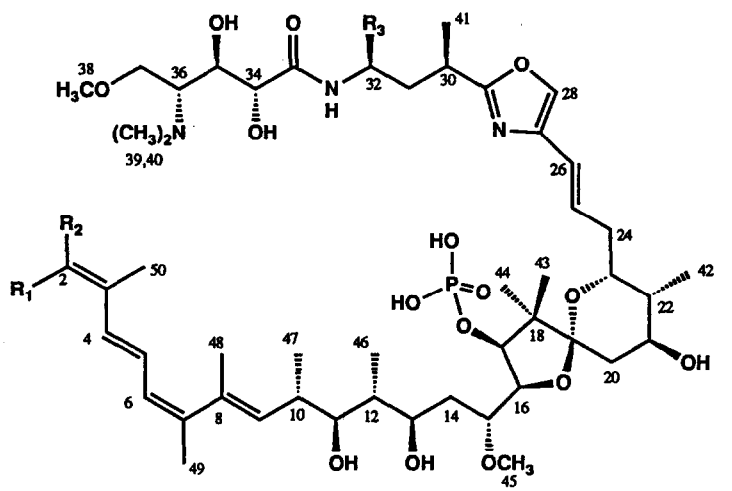
There was an olefinic proton at δ 4.53 (H2) which was allylically coupled to the C50 methyl protons at δ 1.73, giving rise to unit A. In unit B, H4 (δ 6.97, d, J=15.4 Hz) showed *trans* coupling to H5 (δ 7.08), which in turn was coupled to H6 at δ 5.92 by 10.9 Hz. H6 was further coupled to H349 at δ 1.83. Analysis of the COSY spectrum gave rise to connectivities all the way from H9 to H17, making up unit C, in which an olefinic methyl protons at δ 1.63 (H348) was coupled to H9 at δ 6.22. The position of a free hydroxyl group was readily implied by a coupling of a hydroxyl proton at δ

6.84 to H13 at δ 3.78 by 9.7 Hz. H17 appeared as a doublet of doublets, though it had only one coupling partner (H16) in the COSY spectrum, thus the phosphate ester was placed on this carbon ($^3J_{P\text{-}H}$ 10.3 Hz). There were one proton and two carbon signals which showed long-range coupling to phosphorus: H16 (1.0 Hz), C17 (5.8 Hz), and C16 (5.3 Hz). Unit E was also constructed by interpretation of the COSY spectrum starting from the H26 olefinic proton (δ 5.96) to the $H_2$20 methylene protons (δ 1.64 and 1.72) which could be placed adjacent to an sp^3 quaternary carbon judging by their chemical shifts. Starting from the amide proton at δ 8.65, the presence of unit G was easily inferred. Chemical shift of C30 (δ_C 29.1; δ_H 3.57) indicated that this unit was connected to an sp^2 non-protonated carbon. In unit H, a doublet at δ 4.62 (H35) was coupled to a carbinol methine at δ 3.69 (H34) which in turn was coupled to a hydroxyl proton at δ 7.82 by 10 Hz. Oxygenated methylene protons in unit I at δ 3.69 and 3.59 ($H_2$37) were coupled to an amine methine (δ_H 3.91, δ_C 64.3). There were as yet these unassigned ^1H signals: an aromatic proton at δ 6.77 (s, H28) in unit F; two methyl singlets which were W-coupled to each other in unit D; two each of *N*-methyl and *O*-methyl groups; and three hydroxyl protons.

Connection of these 9 units and assignment of functional groups were accomplished by interpretation of the HMBC spectrum.[7] A cyano group was placed at C2 on the basis of the HMBC cross peak between H2 and C1 at δ 117.4, which was supported by the high field chemical shift for C2 (δ 95.2), as well as the low field chemical shift for C3 (δ 156.2). The other end of unit A was linked to unit B on the basis of the HMBC cross peaks between H3$_2$50 and C4; H2 and C4; H4 and C2. Likewise, HMBC cross peaks between H3$_2$49 and C6, C7, and C8, as well as between H3$_2$48 and C7, C8, and C9, allowed us to connect units B and C through the C7-C8 bond. In unit C there were five oxygenated methines, two of which were already assigned as a hydroxyl and a phosphate ester. An exchangeable proton at δ 7.39 (s) was correlated with C10 and C11 in the HMBC spectrum, indicating that C11 was a secondary alcohol. C15 showed 3-bond coupling to the methoxyl protons at δ 3.78 to place a methoxyl function on C15. The low-field chemical shift of C16 (δ 85.6) suggested that this carbon formed a five-membered cyclic ether. C17 had HMBC cross peaks with H3$_2$43 and H3$_2$44, both of which were also correlated with an sp^3 quaternary carbon at δ 50.4 (C18) and with a ketal at δ 109.2 (C19). Now it became clear that units C and D were connected by C17-C18 and C16-O-C19 bonds.

Units D and E were connected through the C19-C20 bond, which was indicated by an HMBC cross peak between H21 and C19. Either oxygen on C21 or C23 must be linked to C19 to form a ketal; the C23 oxygen was more likely to form a six-membered ring rather than a four-membered by participation of oxygen on C21. Thus, a free hydroxyl group was placed on C21. The aromatic proton on C28 (δ_H 6.77 and δ_C 134.0) showed coupling to C27 (δ 140.1) and C29 (δ 172.0). The chemical shifts for the three carbons as well as the $^1J_{CH}$ value between H28 and C28 (205 Hz) as revealed by the residual coupling in the HMBC spectrum were reminiscent of an oxazole ring (unit F). HMBC cross peaks between C27 and both H25 and H26 led us connect unit D and E through a C26-C27 bond, while HMBC correlations between C29 and both H3$_1$41 and H30 provided evidence for the C29-C30 connection. The linkage between units G and H was proven by the HMBC peaks from the C33 amide carbonyl (δ 177.0) to both the amide proton (δ 8.65) and H35 (δ 4.62). There was an exchangeable proton at δ 6.89 (s), which gave HMBC correlations with C34, C35, and C36,





	R_1	R_2	R_3		
5:	CN	H	H	**1**:	6*E* isomer of **5**
6:	H	CN	H	**2**:	6*E* isomer of **6**
7:	CN	H	CH_3	**3**:	6*E* isomer of **7**
8:	H	CN	CH_3	**4**:	6*E* isomer of **8**

allowing us to place a hydroxyl group on C35 and to form a bond between C35 and C36, which was supported by the HMBC correlations from H35 to C34, C36, and C37. Two *N*-methyl protons appeared as broad singlets at δ 2.02 and 2.12, thereby suggesting that the amine was present as the free base.[8] Though no HMBC correlations were observed for these protons due to their broad nature, correlations between H36 (δ 3.91) and the *N*-methyl carbons (δ 36.7 and 43.4) led us to place the *N*-dimethyl function on C36. Finally, an HMBC cross peak between the remaining *O*-methyl protons and C37 (δ 65.9) implied that C37 was methoxylated.

Geometries of the tetraene portion were determined as 2*Z*, 4*E*, 6*Z*, 8*E* on the basis of the ^{1}H NMR data. A strong NOE was observed between H2 and H350, revealing 2*Z* geometry. A vicinal coupling constant of 15.4 Hz between H4 and H5 indicated 4*E* geometry and the NOESY[9] cross peak between H6 and H349 suggested 6*Z* geometry. Although H9 showed NOESY correlations with neither H348 nor H347, it was correlated with H5, suggesting 8*E* geometry with *s*-cis conformation for the C7-C8 bond.[10] The ^{13}C NMR shifts for C48 (δ 15.1) and C49 (δ 23.7) supported 6*Z*, 8*E* geometry.

When allowed to remain in MeOH at room temperature, calyculin E was converted to a mixture of calyculins A, B, E, and F within 1 h, as revealed by analytical HPLC. The same was true for calyculins A, B, and F. However no interconversion took place in the dark, thus indicating that isomerization of the double bonds was due to photochemical rearrangement. Thus, the difference between calyculins E and A should be only in the geometry of the C6-C7 double bond. NOESY and ROESY[11] spectra measured for calyculin A revealed that its conformation in solution was identical with that in the solid state, which was disclosed by X-ray crystallography.[2] NOESY and ROESY spectra for calyculin E gave essentially the same correlations as in case of calyculin A, except for the tetraene portion, thereby suggesting that **5** has the same conformation in solution as **1**. Interestingly, ROESY correlations were observed between NH and 11-OH; NH and H9; H31a and 11-OH, supporting the bent structure of calyculin E. It should be noted that the chemical shift for the 37-*O*-methyl proton signal was affected by the geometry of the tetraene portion.

Calyculin F has the same molecular formula as **5**. The major difference in the ^{1}H NMR spectra between **5** and **6** lay in the tetraene portion and the 37-*O*-methyl protons. There was a strong ROESY peak between H2 and H4, instead of one between H2 and H350 in **5**; otherwise the ROESY correlations were identical with those of calyculin E. Thus calyculin F was a geometrical isomer of **5** at the C2-C3 double bond, which was supported by the HMBC, ROESY, and NOESY spectra.

Calyculin G had a molecular weight 14 units larger than calyculins E and F. The molecular formula of $C_{51}H_{83}N_4O_{15}P$ was deduced by a combination of FAB mass [*m/z* 1023 $(MH)^+$] and NMR spectral data. Comparison of the COSY spectrum with that of **5** readily revealed the presence of a methyl group on C32, as the cross peaks attributable to other portions of the molecule were identical. Though the stereochemistry at C32 was not elucidated for **7** due to the degeneracy of the chemical shifts for the methyls on C32 and C30, it should be identical with that of calyculin C, because calyculins C, D, G and H were interconvertible as was the case of calyculins A, B, E, and F. The proposed structure was supported by the HMQC, HMBC, and ROESY spectra.

Calyculin H has the same molecular formula as **7**. The NMR data of this compound were identical with those of calyculin G except for the tetraene portion of the latter, which was almost

Table 1. ^{1}H and ^{13}C NMR Data of Calyculin E in C_6D_6[12]

atom	^{13}C mult	^{1}H mult	J (Hz)	HMBC connectivities (#C)
1	117.4 s			
2	95.2 d	4.53 q	1.3	1, 3, 4, 50
3	156.2 s			
4	126.9 d	6.97 d	15.4	2, 3, 5, 6, 50
5	135.7 d	7.08 dd	15.4, 10.9	3, 4, 7
6	125.3 d	5.92 dq	10.9, 0.7	4, 8, 49
7	150.3 s			
8	134.0 s			
9	131.9 d	6.22 dq	9.6, 1.1	7, 11, 48
10	35.6 d	2.63 dqd	9.6, 6.9, 2.6	8, 9, 47
11	80.5 d	3.69 dd	9.6, 2.6	9
12	42.4 d	1.79 ddq	10, 9.6, 6.7	11
13	75.1 d	3.78 dddd	11.1, 10, 9.7, 2	
14a	39.3 t	1.99 dd	13.7, 11.1	13
14b		1.75 ddd	13.7, 9.6, 2	
15	77.5 d	4.16 dd	9.7, 9.6	14, 16, 45
16	85.6 d	4.10 ddd	9.7, 3.7, 1.0	15
17	82.5 d	4.23 dd	10.3, 3.7	19
18	50.4 s			
19	109.2 s			
20a	30.4 t	1.71 dd	13.5, 3.4	
20b		1.64 dd	13.5, 2.8	
21	71.6 d	4.01 ddd	3.4, 3, 2.8	19
22	38.5 d	1.90 qdd	7.1, 3, 2.4	
23	67.7 d	4.68 dt	12.1, 2.4	42
24a	36.5 t	2.49 dddd	13.3, 12.1, 3.7, 1.4	23, 25, 26
24b		1.86 ddd	13.3, 10.8, 2.4	25, 26
25	133.1 d	7.38 ddd	15.9, 10.8, 3.7	27
26	116.7 d	5.96 dd	15.9, 1.4	24, 25, 27
27	138.0 s			
28	134.0 d	6.77 s		27, 29
29	170.4 s			
30	29.1 d	3.57 dqd	12.2, 6.9, 1.4	29, 31, 41
31a	34.2 t	2.24 dddd	13, 11, 3, 1.4	32, 41
31b		1.73 dddd	13, 12.2, 3, 3	
32a	34.5 t	4.17 dddd	13, 11, 10.2, 3	
32b		3.00 dddd	13, 3, 3, 2	30
33	177.0 s			
34	69.3 d	3.69 dd	10.2, 10	35
35	74.2 d	4.62 d	10.2	33, 34, 36, 37
36	64.3 d	3.91 dd	8.7, 2.3	35, 37, 39, 40
37a	65.9 t	3.69 dd	12.8, 2.3	38
37b		3.59 dd	12.8, 8.7	36, 38
38	58.8 q	3.19 s		37
39	36.7 q	2.02 s		
40	42.4 q	2.12 s		
41	17.7 q	1.35 d	6.9	29, 30, 31
42	11.1 q	0.83 d	7.1	21, 22, 23
43	22.6 q	0.88 s		17, 18, 19, 44
44	18.2 q*	1.56 s		17, 18, 19, 43
45	61.1 q	3.78 s		15
46	12.9 q	0.65 d	6.7	11, 12, 13
47	18.3 q*	1.32 d	6.9	9, 10, 11
48	15.1 q	1.63 d	1.1	7, 8, 9
49	23.7 q	1.83 d	0.7	6, 7, 8
50	19.1 q	1.73 d	1.3	2, 3, 4
11 OH		7.39 s		10, 11
13 OH		6.84 d	9.7	13
21 OH		4.45 br s		
34 OH		7.82 d	10	
35 OH		6.89 s		34, 35, 36
NH		8.65 dd	10.2, 2	33
POH		11.96 br s		
POH		13.38 br s		

* assignment may be interchanged.

superimposable on that of calyculin F. Therefore, calyculin H was assigned as the geometrical isomer of calyculin G at the C2-C3 double bond.

The effective doses for 50% inhibition of the calyculins on protein phosphatase 2A activity were 0.9, 6.0, 1.0, 5.2, 2.7, 3.2, 5.6, and 6.0 nM for **1**-**8**, respectively. It is interesting to note that all calyculins exhibit similar potent inhibitory activity on protein phosphatases 1 and 2A, thus indicating that the contribution of the tetraene portion and the presence or absence of a methyl group on C31 is marginal for their biological activity.[13]

Acknowledgment. We thank Professor Paul J. Scheuer of the University of Hawaii for critical reading of the manuscript. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

Experimental Section

General UV spectra were recorded on a Hitachi 330 spectrophotometer. FAB mass spectra were measured on a JEOL SX-102 mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM600 NMR spectrometer. Optical rotations were determined on a JASCO DIP-4 plolarimeter.

Isolation The frozen sponge (800 g) was homogenized and extracted in MeOH (3 x 3 L). The extract was evaporated and partitioned between water and CH_2Cl_2. The organic phase was evaporated to yield 3.8 g of a brownish gum, which was applied to a silica gel column (4 x 30 cm) and eluted with CH_2Cl_2 (1 L), CH_2Cl_2-MeOH (99:1, 1 L), and CH_2Cl_2-MeOH (98:2, 5.4 L). The 2.4-4.0 L fractions of the last eluant were collected (yield 630 mg) and subjected to C_{18}-silica gel column chromatography (4 x 20 cm) with 80 % MeOH. The 400-1200 mL fractions were collected and further purified by reverse phase HPLC on ODS-120T (TOSOH) with 78% aq. MeOH to yield **1** (123 mg), **2** (72 mg), a mixture composed of **5** and **6**, **3** (31 mg), a mixture composed of **7** and **8**, and **4** (13 mg), in the order of elution. Mixtures of calyculins E plus F and G plus H were further purified by using 70% aq. MeCN on the same column to yield **5** (14 mg), **6** (10 mg), **7** (7 mg), and **8** (7 mg). All operations following silica gel column chromatography were carried out in glass covered with aluminum foil.

Calyculin E (**5**): $[\alpha]^{23}_{D}$ -83° (*c* 0.2, EtOH); UV (EtOH) 321 (ε16400), 227 nm (13800); FABMS (glycerol), *m/z* 1009 $(MH)^+$, 808, 750, 506, 456, 398; ^{1}H and ^{13}C NMR (see Table 1).

Calyculin F (**6**): $[\alpha]^{23}_{D}$ -33° (*c* 0.2, EtOH); UV (EtOH) 321 (ε18400), 227 nm (15200); FABMS (glycerol), *m/z* 1009 $(MH)^+$, 808, 750, 506, 456, 398; ^{1}H and ^{13}C NMR (see Table 2).

Calyculin G (**7**): $[\alpha]^{23}_{D}$ -81° (*c* 0.1, EtOH); UV (EtOH) 321 (ε 20800), 227 nm (19000); FABMS (glycerol), *m/z* 1023 $(MH)^+$, 822, 764, 520, 470, 412; ^{1}H and ^{13}C NMR (see Table 2).

Calyculin H (**8**): $[\alpha]^{23}_{D}$ -36° (*c* 0.05, EtOH); UV (EtOH) 321 (ε 28000), 227 nm (22800); FABMS (glycerol), *m/z* 1023 $(MH)^+$, 822, 764, 520, 470, 412; ^{1}H and ^{13}C NMR (see Table 2).

Isomerization of calyculin A A 1 mg portion of calyculin A in MeOH (1 mL) was kept in a test tube with a glass stopper under fluorescent lamp. After 1 hour, a portion of the solution was analyzed by reverse phase HPLC system mentioned above: the ratio of calyculins A, B, E, and F was 3:1:0.5:0.5. There was an additional peak for an unnatural isomer which eluted before calyculin A; we have not attempted to elucidate its structure.

Table 2. ^{1}H and ^{13}C NMR Data of **6**-**8** in C_6D_6[14]

	^{13}C			^{1}H		
atom	6	7	8	6	7	8
1	118.1	117.3	118.1			
2	96.2	95.1	96.2	4.85	4.50	4.82
3	157.2	156.3	157.2			
4	129.1	126.6	129.2	5.93	6.95	5.90
5	135.3	135.9	135.2	7.02	7.04	6.98
6	124.9	125.1	124.8	5.85	5.90	5.83
7	150.3	150.5	150.7			
8	133.9	133.8	133.6			
9	131.5	131.7	131.6	6.10	6.17	6.03
10	35.5	35.6	35.5	2.66	2.62	2.64
11	80.8	80.8	81.1	3.67	3.66	3.64
12	42.6	42.3	42.4	1.82	1.75	1.80
13	75.3	75.1	75.2	3.78	3.75	3.77
14a	39.0	39.5	39.1	2.06	2.00	2.06
14b				1.78	1.74	1.78
15	77.6	77.5	77.7	4.19	4.13	4.15
16	85.6	85.5	85.5	4.21	4.13	4.21
17	82.7	82.5	82.7	4.39	4.19	4.35
18	50.5	50.5	50.6			
19	109.3	109.3	109.3			
20a	30.3	30.4	30.3	1.68	1.71	1.69
20b				1.62	1.64	1.62
21	71.6	71.7	71.6	4.00	4.01	3.99
22	38.6	38.6	38.5	1.89	1.90	1.89
23	67.7	67.9	67.7	4.69	4.70	4.71
24a	36.5	36.3	36.3	2.50	2.50	2.50
24b				1.86	1.85	1.86
25	133.2	133.4	133.5	7.40	7.38	7.40
26	116.6	116.7	116.6	5.95	5.97	5.97
27	138.0	138.2	138.1			
28	134.0	133.8	133.9	6.76	6.79	6.81
29	170.4	170.4	170.3			
30	29.0	30.0	30.0	3.59	3.63	3.66
31a	34.0	40.2	39.9	2.23	2.14	2.15
31b				1.68	1.79	1.76
32a	34.6	41.3	41.5	4.19	4.62	4.60
32b				3.00		
32-Me		20.7	20.6		1.36	1.32
33	176.9	176.4	176.3			
34	69.3	69.3	69.4	3.61	3.66	3.62
35	74.2	74.2	74.2	4.60	4.62	4.62
36	63.8	64.2	63.6	3.81	3.87	3.79
37a	66.1	66.0	66.1	3.47	3.66	3.47
37b				3.45	3.60	3.45
38	58.4	58.7	58.4	2.89	3.21	2.88
39	36.6	36.7	36.6	1.99	2.02	2.01
40	43.7	43.8	43.7	2.20	2.12	2.19
41	17.7	19.1	19.0	1.32	1.36	1.34
42	11.1	11.1	11.1	0.82	0.84	0.84
43	22.6	22.7	22.6	1.03	0.89	1.01
44	18.5	18.1	18.7	1.62	1.57	1.61
45	61.0	61.1	61.0	3.80	3.79	3.80
46	12.7	13.0	12.8	0.68	0.66	0.68
47	18.2	18.4	18.1	1.32	1.30	1.29
48	15.2	15.2	15.2	1.64	1.63	1.64
49	23.6	23.8	23.6	1.87	1.82	1.88
50	17.1	19.2	17.1	2.14	1.72	2.10
11 OH				7.24	7.29	7.10
13 OH				6.74	6.70	6.59
21 OH				4.40	4.40	4.42
34 OH				7.98	7.66	7.82
35 OH				6.95	6.92	6.98
NH				8.52	8.39	8.22
POH				12.14	11.99	12.12
POH				13.62	13.48	13.74

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6. Calyculins gave intense UV absorbing spots, which were stained with I_2 vapor, on TLC plates; they turned yellow, when sprayed with H_2SO_4 followed by brief heating. It is possible that the new calyculins are formed during isolation procedure. However, there is one apparent unnatural HPLC peak which elutes before calyculin A. The amount of this peak, which was almost undetectable in the beginning, increased as time passed, whereas peaks for calyculins E, F, G, and H were present in the crude calyculin fraction in which the above-mentioned unnatural calyculin was absent. Therefore, we concluded that calyculins E, F, G, and H were natural products.
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8. Several NMR misassignments were found in calyculins A, B, C, and D.[3] (1) Chemical shifts for C34 and C35 as well as H34/OH34 and H35/OH35 should be exchanged. (2) The ^1H and ^{13}C chemical shifts assigned for the *N*-methyl groups in C_6D_6 should be assigned to the *O*-methyl group on C37. (3) *N*-methyl protons and carbons appeared at δ_H 1.98 (3H br s) and 2.03 (3H br s) and δ_C 36.7 q and 46.3 q in calyculin A; their chemical shifts in calyculins B-C are essentially identical.
9. Bodenhausen, G.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 304-9.
10. The assignment of the geometry of the C8-C9 double bond needs a comment. The ROESY spectrum revealed an intense positive cross peak between H9 and H348, which initially led us to assign 8*Z* geometry. However, the ^{13}C chemical shift for C48 (δ 15.1) strongly suggested 8*E* geometry. A difference NOE experiment upon irradiation of H9 gave peaks for H5 and the amide proton, but not for H348 and H349. A NOESY spectrum with a mixing time of 700 msec supported the difference NOE data. It is now clear that the ROESY cross peak between H9 and H348 was an artifact as a result of a COSY or HOHAHA correlation. A ROESY experiment for calyculin A worked well to give an intense negative cross peak between H9 and H348. Comparison between the entire NOESY and ROESY specta revealed that all cross peaks except for the peaks between some geminal methylene protons, and H342 and H15, as well as between H9 and H348, were identical.
11. Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811-13.
12. ^{13}C and ^1H NMR internal standards were C_6D_6 (128 ppm) and the residual C_6HD_5 (7.20 ppm), respectively.
13. Absolute stereochemistry of the calyculins was not determined yet. Full biological activities for **2**-**8** will be published elsewhere. While we were preparing this manuscript, structures of calyculins E and F appeared in Japan Patent (JP 2-243696, 1990).
14. ^{13}C NMR signal assignments for **6** and **7** were carried out by interpretation of HMBC spectra, while that for **8** was done by comparison with the data for **1**-**7**. ^1H NMR signals were assigned on the basis of the COSY, ROESY, and NOESY spectra.