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Calyciphyllines A and B, Two Novel Hexacyclic Alkaloids from *Daphniphyllum calycinum*

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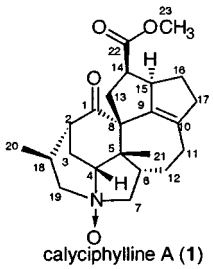
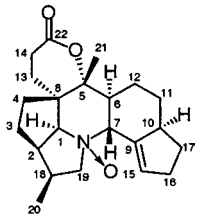
ABSTRACT



calyciphylline A (**1**)



calyciphylline B (**2**)

Two types of *Daphniphyllum* alkaloids with unprecedented fused-hexacyclic ring systems, calyciphyllines A (1) and B (2), have been isolated from the leaves of *Daphniphyllum calycinum* (Daphniphyllaceae), and the structures and relative stereochemistry were elucidated on the basis of spectroscopic data.

Plants of the genus *Daphniphyllum* are known to produce structurally diverse alkaloids with unusual polycyclic skeletons.[1,2] These unique ring systems have attracted great interest as challenging targets for total synthesis as well as biosynthetic studies.[3] Heathcock and co-workers have proposed a biogenetic pathway for *Daphniphyllum* alkaloids and demonstrated a biomimetic total synthesis of several *Daphniphyllum* alkaloids.[3,4]

Recently, we have isolated some novel types of *Daphniphyllum* alkaloids[5−10] such as daphnezomines A and B[5] with a unique aza-adamantane core, daphnezomines F and

(1) For reviews of *Daphniphyllum* alkaloids, see: (a) Yamamura, S.; Hirata, Y. In *The Alkaloids*; Manske, R. H. F., Ed.; Academic Press: New York, 1975; Vol. 15, p 41. (b) Yamamura, S. In *The Alkaloids*; Brossi, A., Ed.; Academic Press: New York, 1986; Vol. 29, p 265. (c) Kobayashi, J.; Morita, H. In *The Alkaloids*; Cordell, G. A., Ed.; Academic Press: New York, 2003; in press.

(2) (a) Jossang, A.; Bitar, H. E.; Pham, V. C.; Sevenet, T. *J. Org. Chem.* **2003**, *68*, 300−304. (b) Hao, X.; Zhou, J.; Node, M.; Fuji, K. *Yunnan Zhiwu Yanjiu* **1993**, *15*, 205−207. (c) Arbain, D.; Byrne, L. T.; Cannon, J. R.; Patrick, V. A.; White, A. H. *Aust. J. Chem.* **1990**, *43*, 185−190. (d) Yamamura, S.; Lamberton, J. A.; Niwa, M.; Endo, K.; Hirata, Y. *Chem. Lett.* **1980**, 393−396. (e) Yamamura, S.; Lamberton, J. A.; Irikawa, H.; Okumura, Y.; Hirata, Y. *Chem. Lett.* **1975**, 923−926. (f) Yamamura, S.; Irikawa, H.; Okumura, Y.; Hirata, Y. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 2120−2123. (g) Yamamura, S.; Hirata, Y. *Tetrahedron Lett.* **1974**, *42*, 3673−3676. (h) Yamamura, S.; Sasaki, K.; Toka, M.; Hirata, Y. *Tetrahedron Lett.* **1974**, 2023−2026 and references therein.

(3) (a) Wallace, G. A.; Heathcock, C. H. *J. Org. Chem.* **2001**, *66*, 450−454. (b) Heathcock, C. H. *Proc. Natl. Acad. Sci., U.S.A.* **1996**, *93*, 14323−14327. (c) Heathcock, C. H.; Joe, D. *J. Org. Chem.* **1995**, *60*, 1131−1142. (d) Heathcock, C. H.; Kath, J. C.; Ruggeri, R. B. *J. Org. Chem.* **1995**, *60*, 1120−1130. (e) Heathcock, C. H. *Angew. Chem.* **1992**, *104*, 675−691. (f) Heathcock, C. H. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 665−681 and references therein.

(4) Ruggeri, R. B.; Hansen, M. M.; Heathcock, C. H. *J. Am. Chem. Soc.* **1988**, *110*, 8734−8736.

(5) Morita, H.; Yoshida, N.; Kobayashi, J. *J. Org. Chem.* **1999**, *64*, 7208−7212.

(6) Morita, H.; Yoshida, N.; Kobayashi, J. *J. Org. Chem.* **2000**, *65*, 3558−3562.

(7) (a) Morita, H.; Yoshida, N.; Kobayashi, J. *Tetrahedron* **1999**, *55*, 12549−12556. (b) Morita, H.; Yoshida, N.; Kobayashi, J. *Tetrahedron* **2000**, *56*, 2641−2646. (c) Morita, H.; Kobayashi, J. *Tetrahedron* **2002**, *58*, 6637−6641. (d) Morita, H.; Takatsu, H.; Kobayashi, J. *Tetrahedron* **2003**, *59*, 3575−3579.

(8) Kobayashi, J.; Inaba, Y.; Shiro, M.; Yoshida, N.; Morita, H. *J. Am. Chem. Soc.* **2001**, *123*, 11402−11408.

(9) Morita, H.; Yoshida, N.; Kobayashi, J. *J. Org. Chem.* **2002**, *67*, 2278−2282.

(10) Kobayashi, J.; Ueno, S.; Morita, H. *J. Org. Chem.* **2002**, *67*, 6546−6549.

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Table 1. ^{1}H and ^{13}C NMR Data of Calyciphyllines A (**1**) and B (**2**) in CD_3OD at 300 K

	1		**2**	
	δ_H	δ_C	δ_H	δ_C
1		215.9	4.08 (1H, d, 5.7)	94.4
2	2.45 (1H, brs)	43.6	3.05 (1H, m)	47.0
3	2.44 (2H, m)	19.5	1.83 (2H, m)	20.7
4a	3.80 (1H, brs)	89.6	1.91 (1H, m)	36.4
4b			1.95 (1H, m)	
5		62.7		87.5
6	2.88 (1H, m)	49.6	2.59 (1H, ddd, 2.4, 11.3, 13.0)	45.2
7a	3.21 (1H, dd, 11.1, 12.3)	74.4	4.09 (1H, d, 13.0)	68.8
7b	3.35 (1H, dd, 7.6, 12.3)			
8		52.8		51.9
9		141.1		139.8
10		140.9	3.15 (1H, m)	42.0
11a	2.05 (1H, brdd, 6.2, 17.3)	27.1	1.46 (1H, brddd, 13.6, 4.5, 4.5)	31.3
11b	2.18 (1H, brdd, 12.1, 16.4)		2.05 (1H, m)	
12a	1.59 (1H, m)	30.0	1.87 (1H, m)	20.8
12b	1.91 (1H, m)		1.15 (1H, m)	
13a	2.80 (1H, m)	41.5	1.65 (1H, ddd, 3.8, 9.3, 13.5)	31.2
13b	2.41 (1H, dd, 10.2, 16.2)		1.85 (1H, m)	
14a	2.82 (1H, m)	42.7	2.52 (1H, ddd, 3.8, 8.3, 17.9)	28.1
14b			2.63 (1H, ddd, 4.0, 9.3, 17.9)	
15	3.40 (1H, m)	54.3	6.03 (1H, dd, 2.5, 5.0)	135.7
16a	1.34 (1H, m)	28.2	2.39 (2H, m)	32.9
16b	1.93 (1H, m)			
17a	2.41 (1H, dd, 10.2, 16.2)	41.6	1.39 (1H, ddd, 7.2, 11.7, 16.9)	35.7
17b	2.65 (1H, m)		2.33 (1H, m)	
18	2.51 (1H, m)	32.8	3.01 (1H, m)	34.2
19a	3.56 (1H, dd, 6.8, 13.5)	67.5	3.54 (1H, dd, 5.2, 11.0)	73.7
19b	3.03 (1H, dd, 8.8, 13.5)		3.13 (1H, dd, 11.0, 13.2)	
20	1.22 (3H, d, 6.9)	19.3	1.13 (3H, d, 6.7)	11.9
21	1.57 (3H, s)	27.8	1.36 (3H, s)	20.7
22		176.1		174.6
23	3.63 (3H, s)	51.8		

G[6] with a 1-azabicyclo[5.2.2]undecane ring system, daphnicyclidins A−H (see Scheme 1),[8] J, and K[9] with a unique hexa- or pentacyclic ring system, daphmanidin A[10] with an unprecedented fused-hexacyclic skeleton from the leaves and stems of *D. teijismanni* and/or *D. humile*, and daphniglaucin A[11] with a fused-polycyclic skeleton containing a 1-azoniatetracyclo[5.2.2.0.^{1,6}0.^{4,9}] undecane ring and a quaternary nitrogen from the leaves of *D. glaucescens*. In our continuing search for structurally unique and biogenetically interesting *Daphniphyllum* alkaloids, calyciphyllines A (**1**) and B (**2**), two types of alkaloids with unprecedented fused-hexacyclic ring systems, were isolated from the leaves of *Daphniphyllum calycinum* (Daphniphyllaceae). This paper describes the isolation and structural elucidation of **1** and **2**.

The leaves of *D. calycinum* were extracted with MeOH, and the extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted at pH 9 with saturated Na_2CO_3, were extracted with $CHCl_3$. $CHCl_3$-soluble materials were subjected to an amino silica gel column (hexane/EtOAc, 9:1 → 1:1 and then $CHCl_3$/MeOH, 1:0 → 0:1), from which a fraction eluted with $CHCl_3$/MeOH (7:3) was purified by an ODS column (MeOH/H_2O, 2:3 → 1:0) followed by LH-20 ($CHCl_3$/MeOH 1:1) and silica gel columns ($CHCl_3$/MeOH/EtOAc, 8:1:1) to afford calyciphyllines A[12] (**1**, 0.001% yield) and B[13] (**2**, 0.0001%).

Calyciphylline A (**1**) showed the pseudomolecular ion peak at *m*/*z* 386 $(M + H)^+$ in the FABMS, and the molecular formula, $C_{23}H_{31}NO_4$, was established by HRFABMS [*m*/*z* 386.2346, $(M + H)^+$, Δ +1.4 mmu]. IR absorptions implied the presence of ester carbonyl and ketone (1735 and 1705 cm^{-1}, respectively) functionalities. ^{13}C NMR data (Table 1) revealed 23 carbon signals due to 1 tetrasubstituted olefin, 2 carbonyls, 2 sp^3 quaternary carbons, 6 sp^3 methines, 8 sp^3 methylenes, 2 methyls, and 1 methoxy. Among them, 2 methylenes (δ_C 74.4, δ_H 3.21 and 3.35; δ_C 67.5, δ_H 3.03 and 3.56) and 1 methine (δ_C 89.6; δ_H 3.80) were ascribed to those bearing an oxidative nitrogen.[14]

(11) Kobayashi, J.; Takatsu, H.; Shen, Y.-C.; Morita, H. *Org. Lett.* **2003**, *5*, 1733−1736.

(12) Calyciphylline A (**1**): colorless solid; $[\alpha]_D$ −51° (*c* 1.0, CH_3OH); IR (neat) ν_{max} 3390, 2930, 1735, 1705, 1650, 1455, and 1060 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); FABMS *m*/*z* 386 $(M + H)^+$; HRFABMS *m*/*z* 386.2346 (M + H; calcd for $C_{23}H_{32}NO_4$, 386.2332).

(13) Calyciphylline B (**2**): colorless solid; $[\alpha]_D$ −58° (*c* 0.4, CH_3OH); IR (KBr) ν_{max} 3280, 2930, 1740, 1460, 1270, and 750 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); FABMS *m*/*z* 358 $(M + H)^+$; HRFABMS *m*/*z* 358.2392 (M + H; calcd for $C_{22}H_{32}NO_3$, 358.2382).

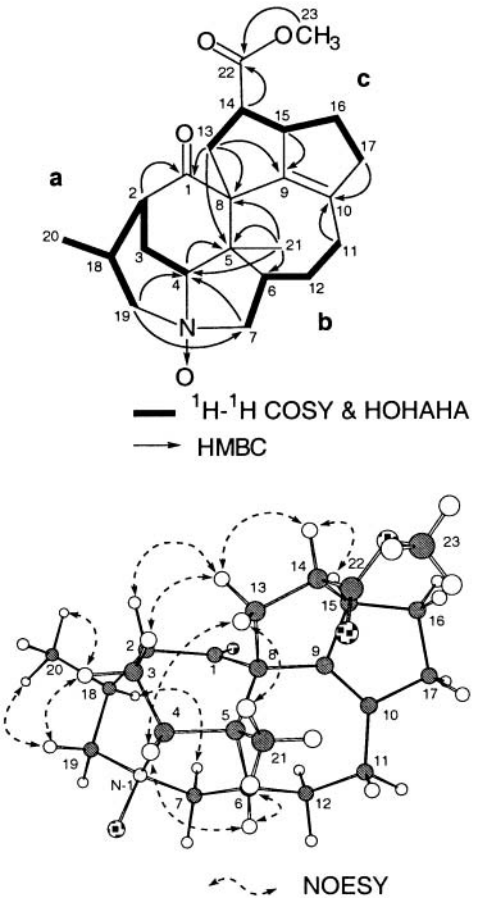


Figure 1. Selected two-dimensional NMR correlations for calyciphylline A (**1**).

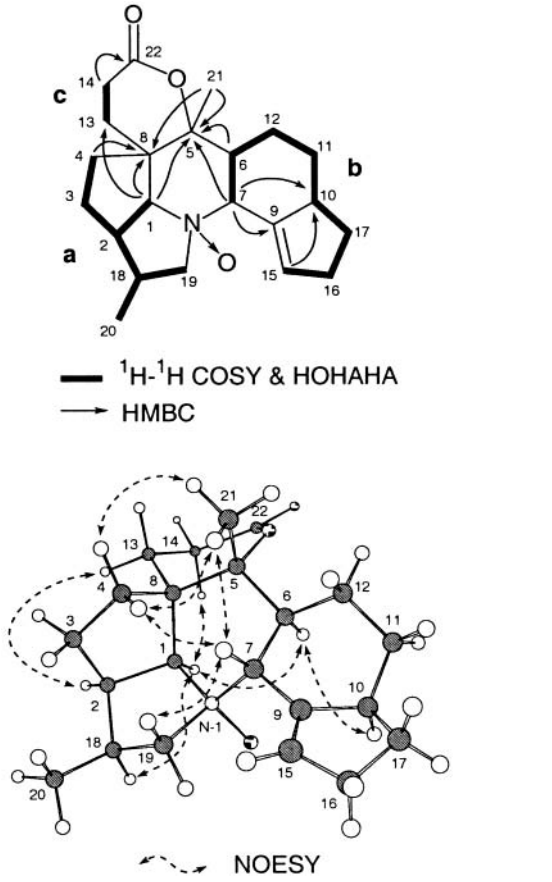


Figure 2. Selected two-dimensional NMR correlations for calyciphylline B (**2**).

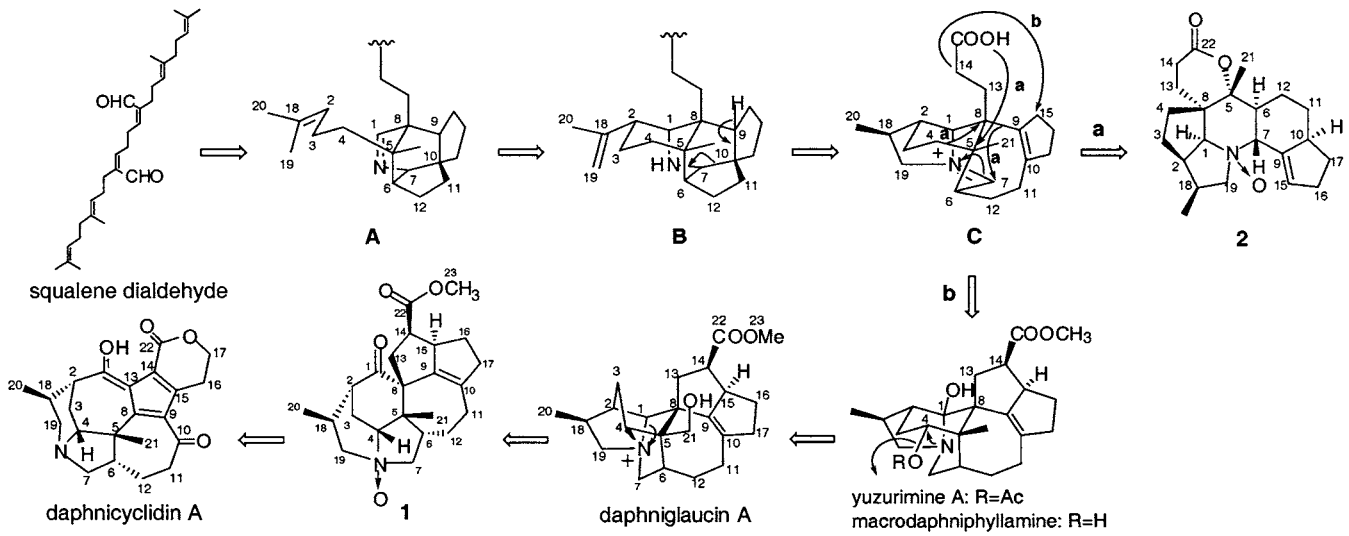
The ^{1}H−^{1}H COSY and HOHAHA spectra revealed connectivities of three partial structures **a** (C-2 to C-4, C-2 to C-18, and C-18 to C-19 and C-20), **b** (C-6 to C-7 and C-12, and C-11 to C-12), and **c** (C-13−C-17) as shown in Figure 1. HMBC correlations were observed for H-19b to C-7 (δ_C 74.4) and H-7b and H-19a to C-4 (δ_C 89.6), suggesting that C-4, C-7, and C-19 were connected to each other through a nitrogen atom. The chemical shifts of C-4, C-7, and C-19 (δ_C 67.5), which resonated at a lower field than those of carbons bearing a nitrogen,[7,14] and a fragment ion at *m*/*z* 370 $(M - 16)^+$ in the FABMS spectrum indicated the presence of an *N*-oxide function. The connectivity of C-21 to C-4, C-6, and C-8 through C-5 was implied by HMBC correlations for H_3-21 to C-4, C-5 (δ_C 62.7), C-6 (δ_C 49.6), and C-8 (δ_C 52.8). HMBC cross-peaks for H_2-11 and H_2-17 to C-10 (δ_C 140.9) indicated connectivities of units **b** and **c** through C-10. The presence of a ketone at C-1 was suggested by the HMBC correlation for H-2 to C-1 (δ_C 215.9). The connectivity of C-1 and C-13 to C-9 through C-8 was implied by HMBC correlations for H_2-13 to C-1, C-8, and C-9 (δ_C 141.1). In addition, the HMBC correlation for H-15 to C-9 indicated the connectivity of C-9 to C-15. A methoxy group was attached to C-22 by HMBC correlations for H_3-23 and H-14 to C-22 (δ_C 176.1). Thus, the gross structure of calyciphylline A was assigned as **1** having an unprecedented fused-hexacyclic ring system (three five-, two six-, and one seven-membered rings) containing an *N*-oxide group as shown in Figure 1.

The relative stereochemistry of **1** was deduced from NOESY correlations as shown in Figure 1. The NOESY correlation of H-3b/H-13a suggested that the cyclohexane ring (C-1∼C-5 and C-8) took a boat form.

Calyciphylline B (**2**) was shown to have the molecular formula of $C_{22}H_{31}NO_3$ by HRFABMS [*m*/*z* 358.2392, $(M + H)^+$, Δ +1.0 mmu], which was smaller than that of **1** by a CO unit. The IR absorption implied the presence of a δ-lactone (1740 cm^{-1}) functionality. ^{13}C NMR data (Table 1) revealed 22 carbon signals due to 1 trisubstituted olefin, 1 ester carbonyl, 2 sp^3 quaternary carbons, 6 sp^3 methines, 9 sp^3 methylenes, and 2 methyls. Among them, 1 methylene (δ_C 73.7; δ_H 3.13 and 3.54) and 2 methines (δ_C 94.4; δ_H 4.08; δ_C 68.8; δ_H 4.09) were ascribed to those bearing an *N*-oxide group,[14] while 1 sp^3 quaternary carbon (δ_C 87.5) corresponded to that bearing an oxygen. Since 2 out of 8 unsaturations were accounted for, **2** was inferred to possess

(14) ^{13}C NMR signals for sp^3 carbons bearing an *N*-oxide are shifted about 10 ppm downfield as compared with those bearing secondary and tertiary amines: Kashiwaba, N.; Ono, M.; Toda, J.; Suzuki, H.; Sano, T. *J. Nat. Prod.* **1998**, *61*, 253−255.

Scheme 1. Plausible Biogenetic Path for Calyciphyllines A (**1**) and B (**2**)



6 rings. The gross structure of **2** was elucidated by analyses of two-dimensional NMR data, including ^{1}H−^{1}H COSY, HOHAHA, HMQC, and HMBC spectra in CD_3OD (Figure 2).

Connectivities of C-1−C-4, and C-2 to C-18, and C-18 to C-19 and C-20 (unit **a**), C-6 to C-7 and C-12, C-10−C-12 and C-10 to C-17, and C-15−C-17 (unit **b**), and C-13 to C-14 (unit **c**) were revealed by the ^{1}H−^{1}H COSY and HOHAHA spectra. The presence of a hexahydroindene moiety (unit **b**) was deduced from HMBC correlations for H-7 to C-9 and C-10, and H-15 to C-10. HMBC cross-peaks for H-1 and H_3-21 to C-5 and C-8, H-6 and H-7 to C-5, and H_2-4 to C-8 indicated connections of C-1 and C-4 to C-8, and C-5 to C-7 and C-8, constructing an octahydroindolizine ring system fused to a cyclopentane ring. On the other hand, the C-14 methylene (δ_c 28.1; δ_H 2.52 and 2.63) was attached to a carbonyl group at C-22 (δ_c 174.6), which was connected to C-5 (δ_c 87.5) through an ester linkage to form a δ-lactone ring, since the HMBC correlation of H-1 to C-13 was observed. These results indicated that the δ-lactone ring was connected to the octahydroindolizine ring at C-5 and C-8. Thus, the gross structure of calyciphylline B was assigned as **2** having an unprecedented hexacyclic ring system consisting of a hexahydroindene ring and an octahydroindolizine ring fused to a cyclopentane ring with a δ-lactone ring at C-5 and C-8. The relative stereostructure of **1** was deduced from cross-peaks observed in the phase-sensitive NOESY spectrum as shown in computer-generated three-dimensional drawing (Figure 2).

A plausible biogenetic pathway for calyciphyllines A (**1**) and B (**2**) is proposed as shown in Scheme 1. Calyciphylline A (**1**) might be generated from the yuzurimine-type alkaloids such as yuzurimine A[15] and macrodaphniphyllamine[16] through daphniglaucin A as follows.[11] Cleavage of the C-1−N-1 bond of daphniglaucin A will give the skeleton of calyciphylline A (**1**), from which daphnicyclidin A may be generated through an intermediate as proposed previously.[8] On the other hand, the biogenetic origin of calyciphylline B (**2**) seems to be imine intermediate **C**, which might be produced through fragmentation reaction of the secodaphniphylline-type skeleton (**B**) derived from imine intermediate **A** proposed by Heathcock et al.[3] Calyciphylline B (**2**) might be generated from attack of the carbonyl group to C-5 of the intermediate **C** and cleavage of C-4−C-5 and C-8−C-9 bonds followed by C-7−C-9 bond formation. Stereochemistry at C-6 was believed to epimerize through enamine formation during these backbone rearrangements.

Calyciphyllines A (**1**) and B (**2**) exhibited cytotoxicity against murine lymphoma L1210 cells (IC_{50}, 2.1 and 4.2 μg/mL, respectively) in vitro.

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Supporting Information Available: One- and two-dimensional NMR spectra for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) Sakurai, H.; Irikawa, H.; Yamamura, S.; Hirata, Y. *Tetrahedron Lett.* **1967**, 2883−2888. Irikawa, H.; Yamamura, S.; Hirata, Y. *Tetrahedron* **1972**, *28*, 3727−3738.

(16) Nakano, T.; Saeki, Y. *Tetrahedron Lett.* **1967**, 4791−4797.