New Alkaloids from *Daphniphyllum calycinum*

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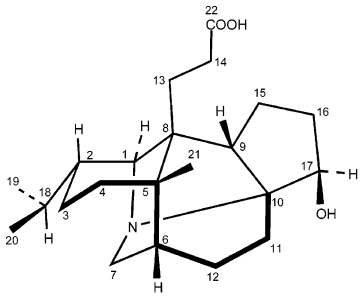
Four new alkaloids, 17-hydroxyhomodaphniphyllic acid (**1**), daphcalycinosidine C (**2**, a new iridoid alkaloid), yuzurimine E (**3**), and yuzurimic acid B (**4**), were isolated from the seeds of *Daphniphyllum calycinum*. The structures of these *Daphniphyllum* alkaloids were determined by spectroscopic analysis including mass spectrometry and 2D NMR.

The *Daphniphyllum* genus, the only one of the Daphniphyllaceae family, is composed of 10 species and is remarkable for its ability to biosynthesize various alkaloid classes with highly complex and unique polycyclic structures.[1,2] Recently, various novel types of *Daphniphyllum* alkaloids were characterized, and more than 60 compounds are now known.[3−16] Heathcock and co-workers developed biomimetic total syntheses of several of these alkaloids.[17] *Daphniphyllum* species are used in folk medicines in various Asian countries and especially *D. calycinum* Benth., a shrub native to North Vietnam and China, which is used for wound healing and as an antiinflammatory remedy.[18] Recent studies on different parts of *D. calycinum* reported the isolation of new structural types of alkaloids such as daphcalycine,[3] daphcalycic acid, daphcalycinosidines A and B,[4] and calyciphyllines A and B.[14] Further investigation of the seeds of *D. calycinum* resulted in the isolation of four new alkaloids: 17-hydroxyhomodaphniphyllic acid (**1**), daphcalycinosidine C (**2**), yuzurimine E (**3**), and yuzurimic acid B (**4**). This paper describes their isolation and structural elucidation.



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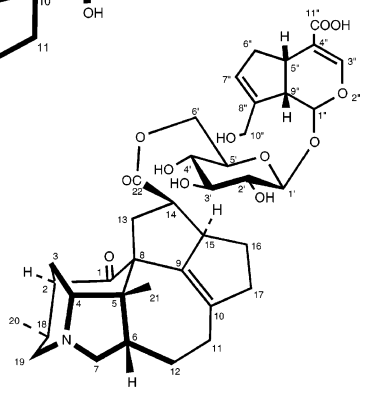

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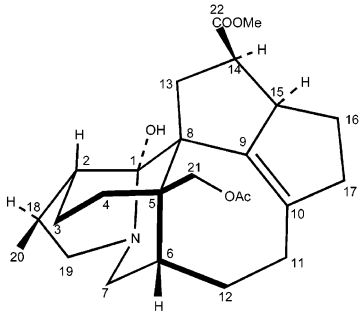

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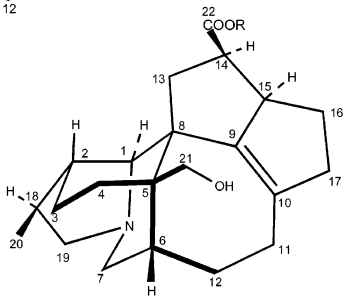

4 : R = H
5 : R = CH_3

Results and Discussion

The seeds of *D. calycinum* Benth. collected in North Vietnam were ground, defatted with cyclohexane, and then extracted with MeOH. The methanolic extract was chromatographed over reversed-phase silica gel column to yield fractions, which were further purified by combination of column chromatography and TLC procedures to afford four new compounds: 17-hydroxyhomodaphniphyllic acid (**1**, 0.0001%), daphcalycinosidine C (**2**, 0.0004%), yuzurimine E (**3**, 0.0004%), and yuzurimic acid B (**4**, 0.001%).

Compound **1** is an optically active solid, $[\alpha]^{22}_D$ −17° (*c* 0.2, MeOH), the molecular formula of which was determined as $C_{22}H_{35}NO_3$ by HRFABMS (*m/z* 362.2638, $[M + H]^+$, calc 362.2696), involving six sites of unsaturation. Its IR absorption spectrum showed the presence of hydroxyl (3302 cm^{-1}) and carboxylic (1715 cm^{-1}) functionalities. ^1H and ^{13}C NMR spectra (Pyr-d_5, Table 1) showed signals due to four quaternary carbons (1 sp^2, 3 sp^3), six methines, nine methylenes, and three methyls.

The ^{13}C chemical shifts of the methylene at δ_C 45.4 (C-7), the methine at δ_C 64.6 (C-1), and the quaternary carbon at δ_C 79.4 (C-10) suggested they were linked to a nitrogen atom, whereas that of the methine at δ_C 78.0 (C-17) indicated it bore an oxygen atom. The $^1H-^1H$ COSY spectrum revealed connectivities of partial structures **a**

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Table 1. ^{1}H (400.13 MHz) and ^{13}C (75.47 MHz) NMR Data for Compounds **1**, **3**, and **4**

	1 in pyridine-d_5		**3** in CD_3OD		**4** in CD_3OD	
C no.	δ_H *J* (Hz)	δ_C	δ_H *J* (Hz)	δ_C	δ_H *J* (Hz)	δ_C
1	3.50 d 3.5	64.6		101.5	3.30 d 4.2	70.0
2	1.46 m	37.5	2.00 ddd 8.7/7.7/5.1	48.1	2.52 m	37.9
3a	1.68 m	26.5	1.54 m	21.9	1.81 m	22.2
3b	1.41 m		1.48 m		1.76 m	
4a	1.93 m	35.9	1.66 m	34.4	1.98 ddd 12.5/12.5/6.0	33.1
4b	1.34 m		1.64 m		1.62 ddd 12.5/5.0/5.0	
5		37.1		43.8		39.1
6	1.50 m	39.2	2.29 m	40.0	2.25 brddd 9.5/6.5/3.0	41.4
7a	3.52 brd 13.6	45.4	3.52 dd 13.1/4.0	56.3	3.59 dd 14.0/3.0	58.6
7b	3.40 brd 13.6		3.23 dd 13.1/9.6		3.53 dd 14.0/9.5	
8		47.2		51.6		45.0
9	2.48 dd 10.4/7.7	49.3		144.6		145.8
10		79.4		137.0		135.5
11a	2.28 m	22.6	2.45 m	26.0	2.32 ddd 15.0/7.5/7.5	25.8
11b	1.60 m		2.08 m		2.16 ddd 15.0/4.0/4.0	
12a	1.70 m	20.4	1.90 m	28.1	1.48 m	28.3
12b	1.59 m		1.45 m		2.28 m	
13a	2.32 ddd 15.0/10.0/6.5	26.8	2.62 dd 14.7/4.2	39.8	3.02 dd 15.0/2.5	40.6
13b	1.68 m		2.55 dd 14.7/9.3		1.99 dd 15.0/8.9	
14a	2.75 ddd 16.8/10.5/6.5	32.2	2.99 ddd 13.4/9.3/4.2	44.5	2.82 ddd 11.6/8.9/2.5	47.8
14b	2.59 ddd 16.8/10.0/4.8					
15a	1.68 m	22.7	3.48 m	57.5	3.52 m	55.7
15b	1.52 m					
16a	1.97 m	31.2	1.83 dddd 11.5/7.0/7.0/1.0	29.1	1.93 ddd 12.5/7.0/7.0	29.2
16b	1.63 m		1.42 dddd 11.5/11.5/11.5/11.5		1.63 dddd 12.5/11.0/11.0/9.2	
17a	4.60 dd 11.4/6.0	78.0	2.54 m	44.0	2.68 brdd 14.5/11.0	44.1
17b			2.34 brdd 14.2/7.6		2.37 brdd 14.5/7.0	
18	1.84 m	30.3	2.46 m	35.0	2.65 m	36.9
19a	1.20 d 6.4	21.9	3.34 m	66.2	3.81 dd 12.4/11.0	65.5
19b			2.50 m		2.79 m	
20	0.75 d 6.5	20.7	1.02 d 6.8	13.8	1.15 d 7.1	14.4
21a	0.95 s	24.6	4.32 d 11.6	71.0	4.01 brd 11.7	68.7
21b			4.30 d 11.6		3.53 d 11.7	
22		175.8		177.2		182.8
23			3.67 s	51.7		
24				173.0		
25			2.04 s	20.9		

(C-2 to C-1 and C-18, and C-18 to C-19 and C-20), **b** (C-3 to C-4), **c** (C-6 to C-7 and C-12, and C-12 to C-11), **d** (C-15 to C-9 and C-16, and C-16 to C-17), and **e** (C-13 to C-14) (Figure 1). Connections between **a** and **b** units were depicted by HMBC cross-peaks between H-1 and both C-2 (δ_C 37.5) and C-3 (δ_C 26.5) and between H-4 and C-2. Connections among C-1, C-4, C-6, C-13, and C-21 via two consecutive quaternary carbons C-5 (δ_C 37.1) and C-8 (δ_C 47.2) were deduced by HMBC cross-peaks for H-6 and H_2-7 to C-5, H_2-4 to C-6 (δ_C 39.2) and C-8, H_2-13 to C-1 (δ_C 64.6), C-5, and C-8, and H_3-21 to C-4, C-5, C-6, and C-8, indicating the linkage of the methyl group (δ_C 24.6) at C-5. The connectivities between **c** and **d** through C-10 (δ_C 79.4) were deduced from HMBC correlations of H-9 to C-11 (δ_C 22.6), and of H_2-11, H_2-16, and H-17 to C-10, with a hydroxyl group at C-17 (δ_C 78.0). Long-range couplings for H_2-13 to C-9 (δ_C 49.3) indicated the connection between **d** and **e** units via C-8. The HMBC correlations of H_2-14 and H_2-13 to C-22 (δ_C 175.8) revealed that the carboxyl group was attached to C-14. Thus, **1** has the daphnane skeleton and its structure is related to that of methyl homodaphniphyllate[19] and was named 17-hydroxyhomodaphniphyllic acid.

NOESY correlations for **1** indicated the same relative configuration as that of methyl homodaphniphyllate (Figure 1). The protons of the methyl H_3-21 showed NOE correlations with H-6, H-9, and H-12a. The hydroxyl group at C-17 was located on the same side of the cyclopentane ring as H_2-11, because a strong NOE cross-peak was observed between its geminal proton (H-17) and H-1 and not with H_2-11; the relative configuration at C-17 is thus *R**. A chair conformation for the cyclohexane bearing (C-1−C-5 and C-8) was suggested by NOESY cross-peaks of H-3b with H-7a and of H-4a with H-13a.

A plausible pathway for the biosynthesis of **1** from methyl homosecodaphniphyllate is proposed in Figure 2: an elimination−fragmentation process takes place through an oxidation of the nitrogen atom of methyl homosecodaphniphyllate(I), providing the intermediates II and III, and the reduction of the latter yields IV.[17c] Then, the C10−C17 double bond of IV is oxidized to form the intermediate epoxide V. Finally, 17-hydroxyhomodaphniphyllic acid is suggested to result from a rearrangement due to the nucleophilic attack of the nitrogen atom at C-10.

Daphcalycinosidine C (**2**) was isolated as an optically active solid, $[\alpha]^{22}_D$ −15° (*c* 0.6, MeOH). Its HRFABMS showed the quasimolecular ion $[M + H]^+$ at *m/z* 712.3349 (calc 712.3334), corresponding to the molecular formula $C_{38}H_{49}NO_{12}$, which indicated 15 degrees of unsaturation. The IR absorption spectrum showed the presence of hydroxyls (3307 cm^{-1}) and carbonyls (1735, 1720, and 1705 cm^{-1}).

The ^{13}C NMR spectrum (DMSO-d_6, Table 2) had 38 carbon signals due to two methyls, 11 methylenes, 16 methines (2 sp^2, 14 sp^3), and nine quaternary carbons (7 sp^2, 2 sp^3).

The carbonyl signal at δ_C 215.5 was assigned to a ketone and those at 173.8 and 169.3 to carboxyls, with the latter being conjugated. The ^{13}C NMR data (Table 2) suggested that the methine at 65.8 (C-4) and the methylenes at 55.6 (C-7) and 48.8 (C-19) were linked to the nitrogen atom. The chemical shifts of the methine carbons at δ_C 98.5 (C-1′) and

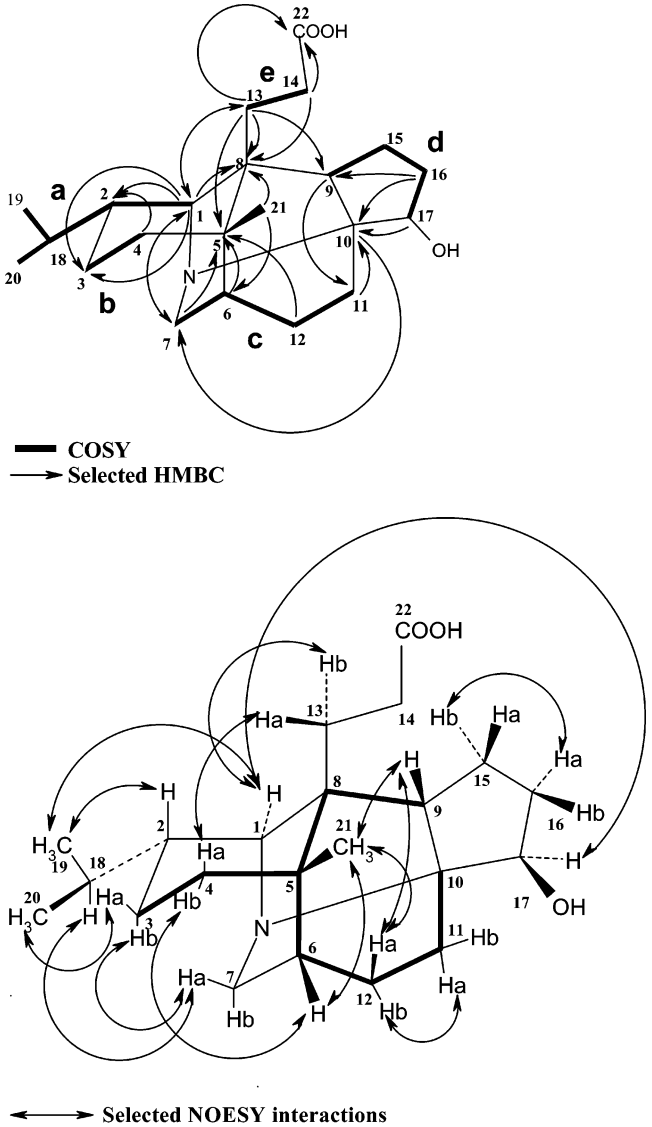

Figure 1. Selected 2D NMR correlations for 17-hydroxyhomodaphniphyllic acid (**1**).

95.8 (C-1″) revealed they were hemiketal carbons. The methylenes at δ_C 63.6 (C-6′) and 59.4 (C-10″) were linked to oxygen atoms. Of the 15 degrees of unsaturation, six were assigned on the basis of this spectrum to the three carbonyls and three carbon−carbon double bonds (Table 2), and thus **2** has nine rings. Analysis of the ^{1}H−^{1}H COSY spectrum showed spin systems of the six partial structures: **a** (C-2 to C-4), **b** (C-18 to C-20), **c** (C-6 to C-7 and C-12, and C-12 to C-11), **d** (C-13 to C-17), **e** (C-1′ to C-6′), and **f** (C-6″ to C-5″ and C-7″, and C-9″ to C-1″ and C-5″) (Figure 3). The carbon signals at δ_C 98.5, 76.3, 74.0, 73.1, 70.0, and 63.6 (substructure **e**) together with 2D NMR data indicated the presence of a glucopyranose moiety. The large coupling (J = 8.0 Hz) of the anomeric proton at δ_H 4.52 indicated a β-glucose. HMBC correlations characterized substructure **f** as a de-*O*-methylgenipin unit (Figure 3). The HMBC connectivities between H-1″ and C-1′ (δ_C 98.5) and between H-1′ and C-1″ (δ_C 95.8) supported the C-1′ to C-1″ link through an oxygen, and this linkage formed a geniposidic acid substructure. HMBC correlations were observed for H-4 and H_2-19 to C-7, and for H_2-7 and H_2-19 to C-4, suggesting that C-4, C-7, and C-19 were connected to each other through the nitrogen atom. Connections among the **a** and **b** units were depicted by HMBC cross-peaks between H_2-19 and H_3-20 and C-2 (δ_C 43.3) and between H-18 and C-3 (δ_C 19.8). The long-range correlations of both H-2 and H_2-3 to C-1 (δ_C 215.5) linked the ketone carbonyl C-1 to C-2. HMBC cross-peaks for H_2-13 to C-1, C-5 (δ_C 51.3), and C-8 (δ_C 61.0) and for H-4 to C-8 indicated connectivities of C-1 to C-13 through C-8 and also of C-4 to C-8 through C-5. The linkage of C-21 (δ_C 23.9) to C-5 was suggested by HMBC correlations for H_3-21 to C-4, C-5, C-6 (δ_C 50.6), and C-8. Connections among **c** and **d** units and the tetrasubstituted olefin C-9 (δ_C 141.8) and C-10 (δ_C 136.8) were provided by HMBC correlations of H_2-13, H-14, H-15, H_2-16, and H_2-17 to C-9 and of H_2-11, H_2-12, and H_2-17 to C-10. Furthermore, HMBC correlations of H_2-13, H-14, and H_2-6′ to C-22 (δ_C 173.8) showed that C-22 was linked to C-14 and attached to C-6′ by an ester bond. Saponification of **2** with KOH (2%) afforded, after acidification and purification by TLC, de-*N*-oxide-calyciphylline A[14] and geniposidic acid; spectral data and $[\alpha]_D$ of the latter were identical with those of the natural product, $[\alpha]^{22}_D$ +3°

Table 2. ^{1}H (400.13 MHz) and ^{13}C (75.47 MHz) NMR Data for Compound **2** (in DMSO-d_6)

C no.	δ_H J (Hz)	δ_C
1		215.5
2	2.09 m	43.3
3a	1.97 brdd 14.3/6.0	19.8
3b	1.82 brdd 14.3/4.6	
4a	3.15 brd 4.6	65.8
4b		
5		51.3
6	2.12 m	50.6
7a	2.79 dd 11.8/6.9	55.6
7b	2.49 dd 11.8/8.2	
8		61.0
9		141.8
10		136.8
11a	1.97 m	25.1
11b	1.87 m	
12a	1.82 m	26.3
12b	1.52 m	
13a	2.63 dd 13.5/7.5	39.2
13b	2.22 dd 13.5/10.0	
14a	2.65 m	41.6
14b		
15a	3.19 m	53.2
15b		
16a	1.71 m	27.1
16b	1.19 m	
17a	2.53 m	41.5
17b	2.28 m	
18	2.64 m	33.1
19a	2.63 m	48.8
19b	2.35 m	
20	0.87 d 6.3	18.2
21a	1.20 s	23.9
21b		
22		173.8
1′	4.52 d 8.0	98.5
2′	2.99 dd 8.8/8.0	73.1
3′	3.19 dd 9.0/8.8	76.3
4′	3.04 dd 9.5/9.0	70.0
5′	3.30 ddd 9.5/6.9/1.9	74.0
6′a	4.34 dd 11.9/1.9	63.6
6′b	3.92 dd 11.9/6.9	
1″	4.87 d 7.3	95.8
3″	7.20 d 1.3	148.5
4″		114.4
5″	3.00 brddd 8.0/7.3/7.0	35.6
6″a	2.70 dd 17.0/7.0	38.7
6″b	1.95 dd 17.0/8.0	
7″	5.63 s	125.6
8″		144.6
9″	2.52 dd 8.0/7.3	45.8
10″a	4.04 brd 14.1	59.4
10″b	3.96 dd 14.1/2.0	
11″		169.3

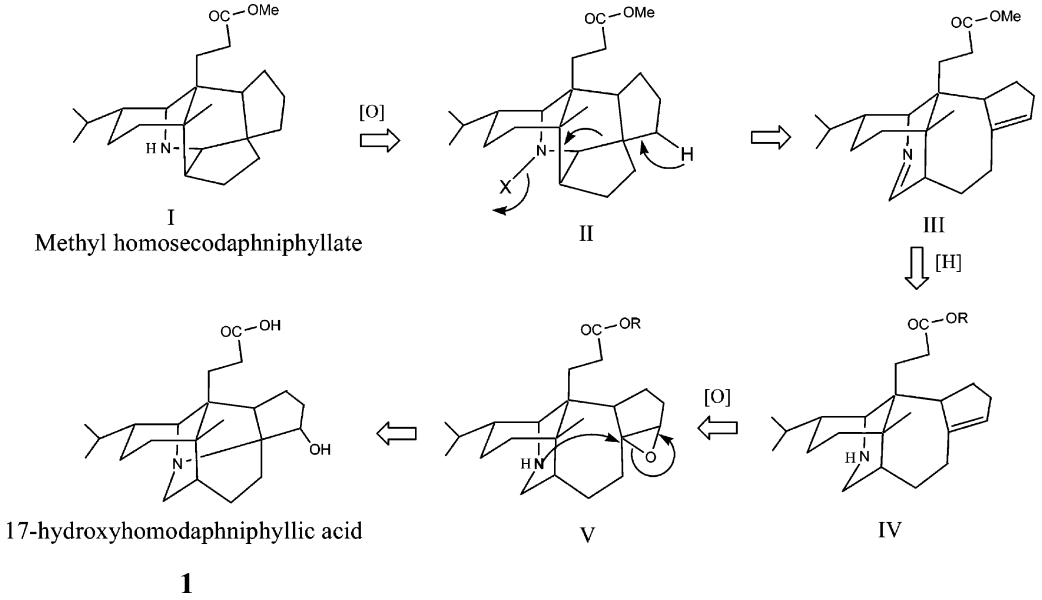

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Figure 2. Biogenetic pathway proposed for 17-hydroxyhomodaphniphyllic acid (**1**) formation from methylhomosecodaphniphyllate.

(*c* 0.1, MeOH).[20] Thus, **2** is a new *Daphniphyllum* iridoid alkaloid, for which we propose the name daphcalycinosidine C.

Analysis of the fragmentation pattern of the quasi-molecular ion $[M + H]^+$ of daphcalycinosidine C (*m*/*z* 712) by positive ion ESI-TOF MS/MS showed fragment ions supporting the proposed structure: the ions at *m*/*z* 518 and 500 corresponded to the cleavage of the ether bond between the de-*O*-methylgenipin and the glucose moieties at the O/C-1″ and O/C-1′ level, respectively. Other major fragments at *m*/*z* 356 and 338 corresponded to the cleavage of the ester bond between the glucose and the alkaloidal moieties at the O/C-6′ and O/C-22 level, respectively.

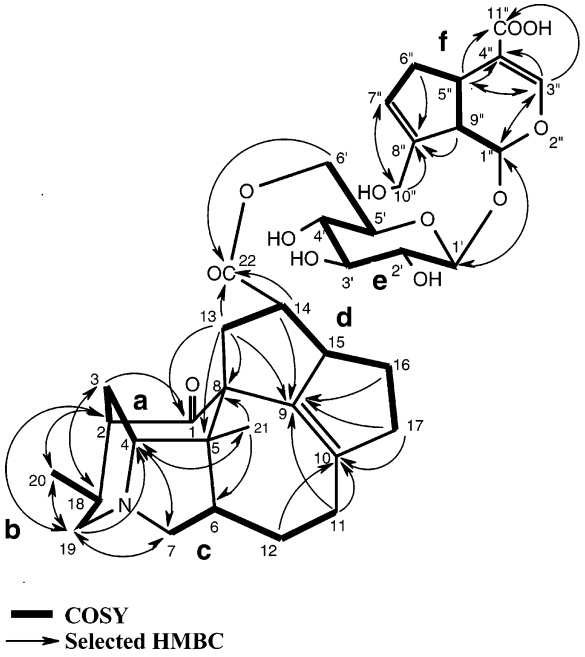
The relative configuration of daphcalycinosidine C was deduced from NOESY correlations (Figure 3). Strong NOEs for the alkaloidal moiety were observed between H-3a and the two protons of the methylene H_2-13, suggesting a boat conformation for the cyclohexanone ring bearing C-3. Furthermore, the NOESY correlations between H-3b and H-19b on one hand, and between H-3b and H_3-20 on the other hand, suggested an equilibrium between the boat and chair conformations for the piperidine ring involving C-18 and C-19. The strong NOE correlations of H-5″ with H-9″ and the absence of cross-peaks between H-1″ and both H-5″ and H-9″ indicated the same relative configuration as geniposidic acid.[20] In addition, H-1″ (δ_H 4.87) gave a large coupling with H-9″ ($J = 7.3$ Hz), in agreement with a *trans* relative disposition. Another strong NOE correlation was depicted between H-1″ and H-16a, suggesting a preferred conformation of the two substituents at C-1′ and C-6′ of the glucose moiety.

The molecular formula of yuzurimine E (**3**), $[\alpha]^{22}_D$ −33° (*c* 0.3, MeOH), was determined as $C_{25}H_{35}NO_5$ by HR-FABMS (*m*/*z* 430.2590, $[M + H]^+$, calc 430.2594), corresponding to nine degrees of unsaturation. Its ^{13}C NMR spectrum (CD_3OD, Table 1) showed signals due to seven quaternary carbons (four sp^2 and three sp^3), five methines, 10 methylenes, and three methyls including one methoxyl and one acetoxyl group. The IR absorption spectrum suggested the presence of hydroxyl (3425 cm^{-1}), ester carbonyl (1734 cm^{-1}), and olefinic (1639 cm^{-1}) functionalities. Of the nine levels of unsaturation, three corresponded to one tetrasubstituted double bond (Table 1) and two ester carbonyls (δ_C 173.0 and 177.2), and thus six rings were assigned to **3**.

The ^{13}C NMR spectrum depicted signals for two methylenes at δ_C 56.3 (C-7) and 66.2 (C-19), bearing a nitrogen, and one quaternary carbon δ_C 101.5 (C-1) linked to both a nitrogen and an oxygen atom. The chemical shift of the methylene at δ_C 71.0 (C-21) suggested it is linked to an oxygen atom. The presence of four partial structures (Figure 4), **a** (C-2 to C-4, and C-18 to C-2, C-19, and C-20), **b** (C-6 to C-7 and C-12, and C-12 to C-11), **c** (C-13 to C-14), and **d** (C-15 to C-17), was demonstrated by detailed analyses of $^1H-^1H$ COSY and HSQC data. The **a**−**d** units were connected to one another on the basis of the following HMBC correlations: H-2 and H_2-3 to C-1 (δ_C 101.5), H_2-7 to C-1 and C-19 (δ_C 66.2), H_2-21 to C-4 (δ_C 34.4), C-6 (δ_C 40.0), and C-8 (δ_C 51.6), H_2-13 to C-1, C-5, C-8, C-9 (δ_C 144.6), and C-15 (δ_C 57.5), and H_2-11, H_2-16, and H_2-17 to C-9 and C-10 (δ_C 137.0). The linkage of the methoxycarbonyl at C-14 was established by HMBC correlations of H_2-13, H-14, and H_3-23 to C-22 (δ_C 177.2). HMBC cross-peaks of H_2-21 and H_3-25 with carbonyl at C-24 (δ_C 173.0) indicated an acetoxyl group to be at C-21 (δ_C 71.0). Compound **3** was thus assigned as the C-4 deacetoxy form of yuzurimine[21] and named yuzurimine E. The NOESY correlations in **3** indicated the same relative configuration as yuzurimine (Figure 4). The conformations of the cyclohexane (C-1−C-5 and C-8) and piperidine (N, C-1, C-8, and C-5−C-8) rings were assigned as chairs from NOE correlations of H-3b with H-7a, of H-4a with H-13a, and of H-2 with H-13b.

Yuzurimic acid B (**4**), $[\alpha]^{22}_D$ +11° (*c* 0.3, MeOH), was shown to have the molecular formula $C_{22}H_{31}NO_3$ by HR-FABMS (*m*/*z* 358.2379, for $[M + H]^+$, calc 358.2383). In its ^{13}C NMR spectrum (CD_3OD, Table 1), 22 carbon signals including five quaternary carbons (3 sp^2, 2 sp^3), six methines, 10 methylenes, and one methyl were observed. The IR spectrum showed the presence of hydroxyl (3429 cm^{-1}), carboxyl (1720 cm^{-1}), and olefinic (1646 cm^{-1}) functionalities. The ^1H and ^{13}C NMR data suggested that **4** was structurally related to yuzurimine E (**3**) (Table 1), but differed from the latter as it lacked the hydroxyl group at C-1, the acetyl group on the C-21 oxygen, and the methyl group at C-22. In fact, detailed analyses of the 2D NMR data indicated that **4** was the acid form of yuzurimine B,[22] and thus named yuzurimic acid B.

The relative configuration was elucidated to be the same as that yuzurimine E (**3**) from NOESY data. The meth-



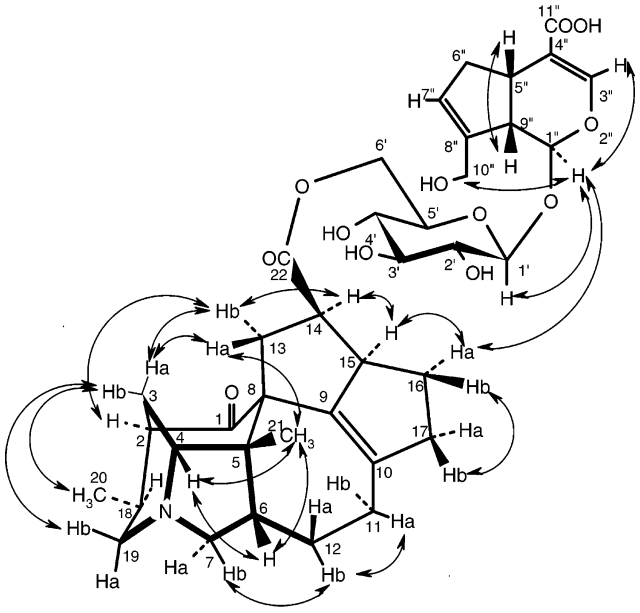


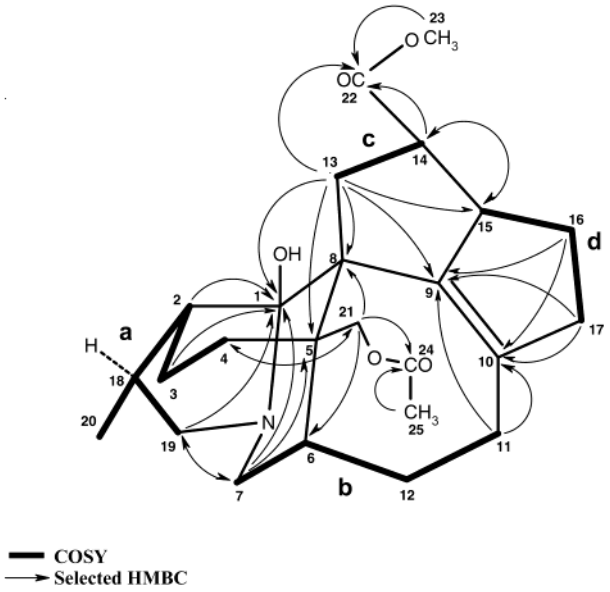
Figure 3. Selected 2D NMR correlations for daphcalycinosidine C (**2**).

ylation of **4** with diazomethane afforded the corresponding methyl ester (**5**), spectral data of which were identical with those of yuzurimine B.[22] Thus, the structures of yuzurimine E and yuzurimic acid B were assigned as **3** and **4**, respectively.

As compounds **1**, **3**, **4**, yuzurimine B, and yuzurimine have a plausible common precursor, such as methyl homosecodaphniphyllate, for which the absolute configuration has been determined by X-ray crystallography, the absolute configuration of the new compounds could be related to that of yuzurimine and methyl homosecodaphniphyllate.[22b]

Experimental Section

General Experimental Procedures. Optical rotations were measured in MeOH at 28 °C on a Perkin-Elmer 241 polarimeter. IR spectra were recorded in KBr disks on a Nicolet Impact 400 D spectrophotometer. Mass spectra were



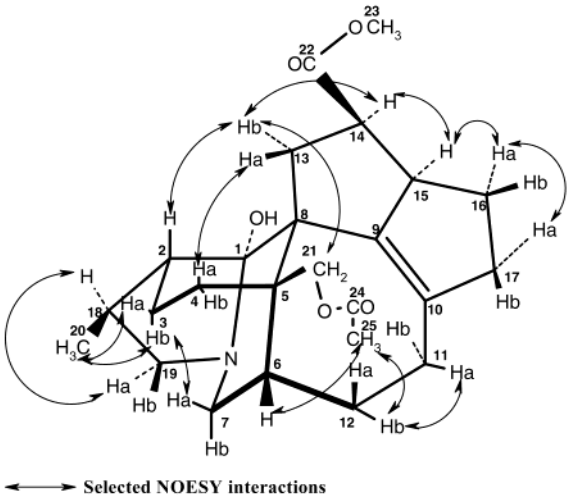


Figure 4. Selected 2D NMR correlations for yuzurimine E (**3**).

obtained either on a ZAB_2-SEQ (VG analytical) for the FABMS or on an API Q-STAR (Applied Biosystem) for the ESI-TOFMS. ^{13}C NMR spectra were recorded on a Bruker AC 300 spectrometer (^{13}C: 75.47 MHz) and ^1H NMR spectra on an Avance 400 Bruker (^1H: 400.13 MHz) with internal reference (^1H: CHD_2OD at δ_H 3.31, $CHCl_3$ at δ_H 7.27, pyridine at δ_H 7.19, DMSO at δ_H 2.49; ^{13}C: CD_3OD at δ_C 49.0, $CDCl_3$ at δ_C 77.0, Pyr-d_5 at δ_C 123.5, and DMSO-d_6 at δ_C 39.5).

Plant Material. The seeds of *D. calycinum* Benth. (Daphniphyllaceae) were harvested in North Vietnam in October 1999. A voucher specimen has been deposited in the herbarium (VN 579) of CNSNT, Hanoi.

Extraction and Isolation. The seeds of *D. calycinum* (1.2 kg) were ground, defatted with cyclohexane, and extracted at room temperature with MeOH for 3 days, and the extract was concentrated under reduced pressure to yield 80 g of crude methanolic extract. The extract was chromatographed on a RP2 silica gel column eluted with a H_2O/MeOH (1:0 to 0:1) gradient and afforded 20 fractions (F1−F20). The less polar fraction F20 (50 mg) was purified by TLC on SiO_2 gel (CH_2Cl_2/MeOH, 90:10) to yield **1** (1.5 mg). Fractions F7−F12 were combined (4 g) and further separated on a RP2 silica gel column eluting with a H_2O/MeOH (1:0 to 0:1) gradient followed by Sephadex LH20 CC (MeOH) to yield **2** (5 mg). Fractions F16−F19 were combined (5 g) and purified on silica gel CC using a CH_2Cl_2/MeOH (1:0 to 0:1) gradient to give **3** (5 mg).

Then, fractions F4−F6 (0.9 g) were chromatographed on a RP2 silica gel column eluted with a H_2O/MeOH (1:0 to 0:1) gradient to yield compound **4** (15 mg).

17-Hydroxyhomodaphniphyllic acid (1): $C_{22}H_{35}NO_3$ (M = 361), microcrystals (MeOH), mp 97−99 °C, $[\alpha]_D$ −17° (*c* 0.2, MeOH); HRFABMS, *m/z* 362.2638, $[M + H]^+$, calc 362.2696; IR (KBr) ν_{max} 3302, 2935, 1715, and 1568 cm^{-1}; ^1H and ^{13}C NMR data (Pyr-d_5, Table 1).

Daphcalycinosidine C (2): $C_{38}H_{49}NO_{12}$ (M = 711), colorless amorphous solid, $[\alpha]^{22}_D$ −15° (*c* 0.6, MeOH); HRFABMS *m/z* 712.3349, $[M + H]^+$, calc 712.3334; IR (KBr) ν_{max} 3307, 2922, 1735, 1720, 1705, and 1588 cm^{-1}; UV (MeOH) λ_{max} 222 nm (ϵ 10.000); ^1H and ^{13}C NMR (DMSO-d_6, Table 2).

Yuzurimine E (3): $C_{25}H_{35}NO_5$ (M = 429), colorless amorphous solid, $[\alpha]^{22}_D$ −33° (*c* 0.3, MeOH); HRFABMS *m/z* 430.2590, $[M + H]^+$, calc 430.2594; IR (KBr) ν_{max} 3425, 2930, 1734, and 1639 cm^{-1}; ^1H and ^{13}C NMR data (CD_3OD, Table 1).

Yuzurimic acid B (4): $C_{22}H_{31}NO_3$ (M = 357), microcrystals (MeOH), mp 253−255 °C, $[\alpha]^{22}_D$ +11° (*c* 0.3, MeOH); HRFABMS *m/z* 358.2379, $[M + H]^+$, calc 358.2383; IR (KBr) ν_{max} 3429, 2930, 1720, and 1646 cm^{-1}; ^1H and ^{13}C NMR data (CD_3OD, Table 1).

Methylation of Yuzurimic Acid B (4). A solution of **4** (2 mg) in methanol (0.5 mL) was treated with diazomethane at 5 °C. The mixture was stirred at room temperature for 30 min and concentrated in vacuo. The residue was purified by TLC (100% MeOH) to give the corresponding methyl derivative (**5**), whose spectral data were identical with those of yuzurimine B. $C_{23}H_{33}NO_3$ (M = 371), microcrystals (MeOH), mp 280−282 °C, $[\alpha]^{22}_D$ −22° (*c* 0.2, MeOH); HRFABMS *m/z* 372.2531, $[M + H]^+$, calc 372.2540; IR (KBr) ν_{max} 3435, 2928, 1732, and 1646 cm^{-1}; ^{13}C NMR data (75 MHz, CD_3OD) δ 69.0 (1), 39.2 (2), 23.1 (3), 33.8 (4), 40.1 (5), 39.5 (6), 59.7 (7), 45.5 (8), 145.0 (9), 136.1 (10), 26.2 (11), 29.5 (12), 40.1 (13), 43.9 (14), 55.7 (15), 29.2 (16), 44.1 (17), 38.6 (18), 66.1 (19), 15.2 (20), 67.6 (21), 177.5 (22), 51.6 (23); ^1H NMR data (400 MHz, CD_3OD) δ 2.89 (m, H-1), 2.30 (m, H-2), 1.66 (m, H-3a), 1.66 (m, H-3b), 2.00 (m, H-4a), 1.63 (m, H-4b), 2.34 (m, H-6), 3.31 (m, H-7a), 3.30 (m, H-7b), 2.34 (m, H-11a), 2.11 (m, H-11b), 2.18 (m, H-12a), 1.50 (m, H-12b), 2.66 (dd 14.9/3.1, H-13a), 2.08 (dd 14.9/9.2, H-13b), 2.94 (ddd 12.4/9.2/3.1, H-14), 3.53 (m, H-15), 1.93 (m, H-16a), 1.63 (m, H-16b), 2.68 (m, H-17a), 2.37 (m, H-17b), 2.45 (m, H-18), 3.50 (m, H-19a), 2.48 (m, H-19b), 1.12 (d 6.9, H-20), 3.99 (d 11.6, H-21a), 3.73 (d 11.6, H-21b), 3.65 (s, H-23).

Hydrolysis of Daphcalycinosidine C (2). A solution of the daphcalycinosidine C (**2**, 4 mg) in 2% KOH (1 mL) was stirred at room temperature for 2 h. The residue was dissolved in methanol (0.3 mL) and neutralized with HCl (2 N). After evaporation, the residue was purified by TLC (50 MeOH, 50 CH_2Cl_2) to afford de-*N*-oxide-calyciphylline A (1.5 mg) and geniposidic acid (1.3 mg).

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