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CHEMICAL CONSTITUENTS OF TWO ORIENTAL ORCHIDS, *CALANTHE DISCOLOR* AND *C. LIUKIUENSIS*: PRECURSOR INDOLE GLYCOSIDE OF TRYPTANTHRIN AND INDIRUBIN†

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Abstract — Two indole glycosides, calanthoside and glucoindican, were isolated from two oriental orchids, *Calanthe discolor* LINDL. and *C. liukiuensis* SCHLTR., together with calaphenanthreol, calaliukiuenoside, and bioactive known alkaloids, tryptanthrin, indirubin, and isatin. The structures of calanthoside, glucoindican, calaphenanthreol, and calaliukiuenoside were determined on the basis of physicochemical and chemical evidence. Furthermore, it was found that enzymatic hydrolysis of calanthoside furnished tryptanthrin as the main product, whereas indirubin and isatin were obtained by acid hydrolysis of calanthoside.

The Orchidaceae plant *Calanthe discolor* LINDL. ("Ebine" in Japanese) grows in Japan, China, and Korea and is widely cultivated as an ornamental. The dried whole plant of *Calanthe discolor* has been used for antiinflammatory, antibacterial, and antitoxic purposes in Chinese traditional medicine (Chinese herbal name 九子連環草),[1] but its pharmacological property and chemical constituent are left uncharacterized. In the course of our studies in search of bioactive constituents of natural medicines,[2] we found that the methanolic extract of this plant showed potent hair restoring activity in C3H mice and also promoted the blood flow in the dorsal skin of rats.[3] From the methanolic extract from the underground part of *C. discolor*, two indole glycosides termed calanthoside (**1**) and glucoindican (**2**) and a dihydrophenanthrene called calaphenanthreol (**4**) were isolated, while two bioactive known alkaloids, tryptanthrin (**6**)[4,5] and indirubin (**7**),[6] were isolated from the aerial part of this plant together with vomifoliol.[7] Calanthoside (**1**) was also isolated together with calaliukiuenoside (**3**), **6**, **7**, isatin (**8**),[4e] indican (**9**), benzyl glucopyranoside,[8] 2,6-dimethoxy-4-hydroxyphenol 1-*O*-*β*-D-glucopyranoside,[9] and 3-hydroxyoctyl *β*-D-glucoside[10] from the underground part of *C. liukiuensis* (Figure 1). The two indole glycosides (**1**, **2**) were found to exhibit an activating effect on skin blood flow.[3] In addition, we found that enzymatic hydrolysis of **1** with *β*-glucosidase furnished tryptanthrin (**6**) together with a small amount of indirubin (**7**) and isatin (**8**), while acid hydrolysis of **1** provided **7** and **8**. In this paper, we present the structure elucidation of **1**, **2**, **3**, and **4** and the transformation from **1** to **6**, **7**, and **8**.

Calanthoside (**1**) was isolated as a hygroscopic white powder. In the positive-ion FAB-MS and SIMS of **1**, quasimolecular ion peaks and molecular ion peak were observed at *m/z* 512 $(M+Na)^+$, *m/z* 490 $(M+H)^+$, and *m/z* 489 (M^+) in addition to fragment ion peaks at *m/z* 327 $(M-C_6H_{11}O_5+H)^+$ and *m/z* 165 $(M-2xC_6H_{11}O_5+2H)^+$. The negative-ion FAB-MS of **1** showed a quasimolecular ion peak at *m/z* 488 $(M-H)^-$ and a fragment ion peak at *m/z* 326 $(M-C_6H_{11}O_5)^-$. High-resolution MS analysis of the quasimolecular ion peak $(M+H)^+$ and molecular ion peak (M^+) revealed the molecular formula of **1** to be $C_{20}H_{27}NO_{11}S$. The IR spectrum of **1** showed absorption bands due to aromatic function at 1624, 1560, and 748 cm^{-1} and strong bands at 3389 and 1043 cm^{-1} suggestive of glycoside structure. The UV spectrum of **1** showed absorption maxima at 222 nm (log ε 3.5) and 290 nm (log ε 2.7), which suggested the presence of an indole function. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra of **1**, which were completely assigned with the aid of various NMR analytical methods,[11] indicated the presence of a 2,3-disubstituted indole moiety [δ 7.00, 7.12 (dd-like, 5,6-H), 7.25, 7.79 (both d, *J*=8.3 Hz, 7, 4-H)]

† Dedicated to Professor Sho Ito, Bunri University of Tokushima, in cerebration of his 77th birthday

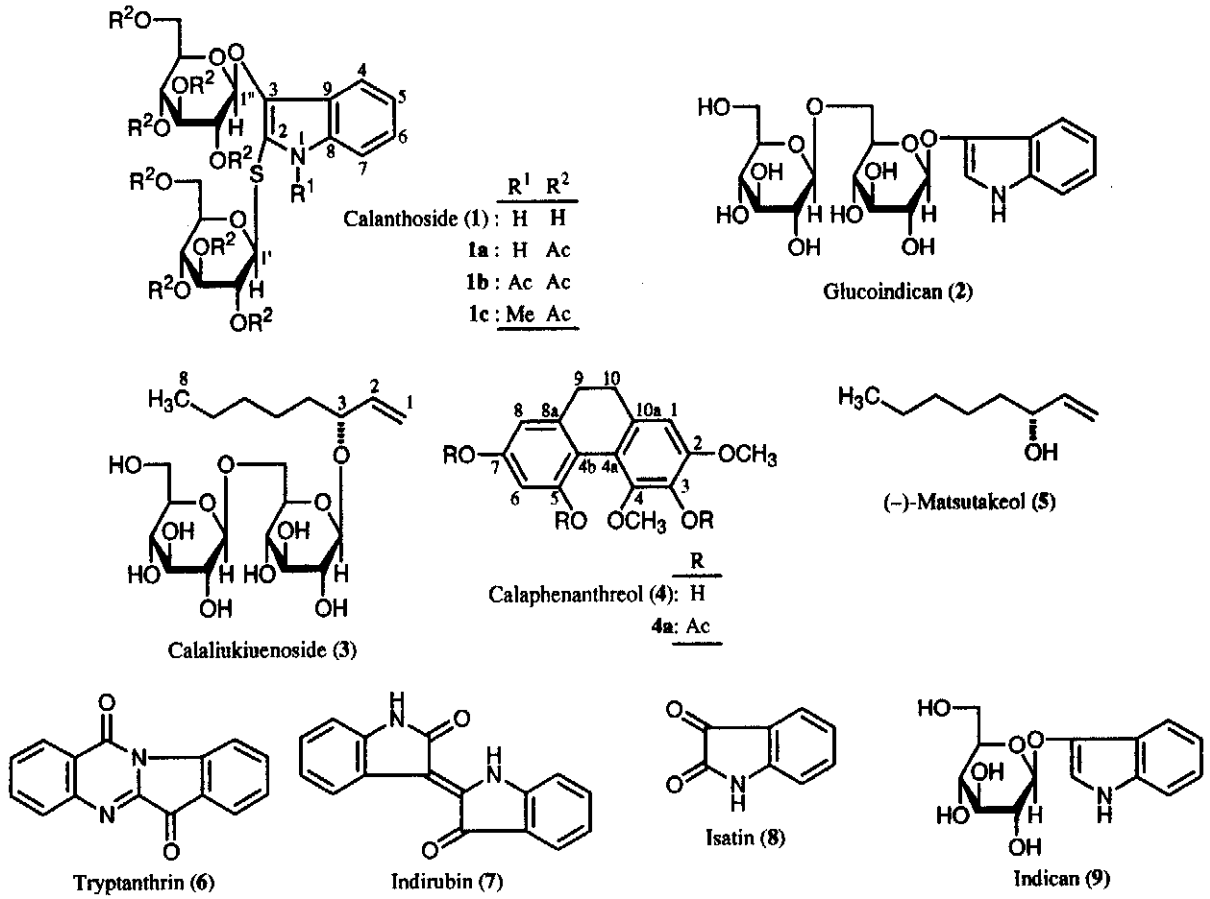

Figure 1

together with an *O*-β-D-glucopyranosyl part [δ 4.92 (d, J=6.9 Hz, 1"-H)] and an *S*-β-D-glucopyranosyl part [δ 4.43 (d, J=9.6 Hz, 1'-H)], for which the ^{13}C-NMR signals corresponded to those of methyl 1-thio-β-D-glucopyranoside.[12] Acetylation of **1** with acetic anhydride and dimethylaminopyridine (DMAP) in pyridine furnished the octaacetate (**1a**) and nonaacetate (**1b**). The octaacetate (**1a**) was subjected to methylation with methyl iodide and potassium carbonate in dimethylformamide (DMF) to provide the *N*-methyl octaacetate (**1c**). The ^{1}H-NMR ($CDCl_3$) spectrum of **1b** showed signals assignable to an *N*-acetyl group [δ 2.87 (s)] together with eight *O*-acetyl groups, while the ^{1}H-NMR ($CDCl_3$) spectrum of **1c** showed due to an *N*-methyl group [δ 3.77 (s)] and eight *O*-acetyl groups. The positions of the *S*- and *O*-glucoside linkages in **1** were clarified by heteronuclear multiple bond correlation (HMBC) experiments on **1**, **1a**, **1b**, and **1c**, which showed long-range correlations between the following protons and carbons: 1'-H and 2-C; 1"-H and 3-C; *N*-CH_3 and 2, 8-C; 4-H and 3-C (Figure 2).

Exhaustive acid hydrolysis of **1** with 5% aqueous sulfuric acid liberated D-glucose, which was identified by GLC analysis of the trimethylsilyl (TMS) thiazolidine derivative.[13] Acid hydrolysis of **1** with 5% aqueous hydrochloric acid in dioxane (1 : 1) yielded indirubin (**7**) and isatin (**8**) together with 1-thio-β-D-glucose and D-glucose in a *ca.* 1 : 1 ratio. On the other hand, enzymatic hydrolysis of **1** with β-glucosidase (sweet almond) in 0.2 M acetate buffer (pH 4.4) furnished **6** together with a small amount of **7** and **8** (Figure 3). Although **6**, **7**, and **8** were isolated from various plants[4,6] and also from the culture solution of a yeast,[5] **1** is considered to be a common precursor glycoside of **6**, **7**, and **8** in the plant.

Glucoindican (**2**) was isolated as a white powder. The IR spectrum of **2** showed absorption bands at 3490, 1618, 1554, and 747 cm^{-1}, while the absorption maxima were observed at 224 nm (log ε 5.4) and 282 nm (log ε 4.7) in the UV spectrum. The molecular formula $C_{20}H_{27}NO_{11}$ of **2** was determined by the positive-ion and negative-ion FAB-MS and by high-resolution MS measurement. Namely, in the positive-ion FAB-MS of **2**, quasimolecular ion peaks were observed at m/z 458 $(M+H)^+$ and m/z 480 $(M+Na)^+$, whereas the negative-ion FAB-MS of **2** showed a quasimolecular ion peak at m/z 456 $(M-H)^-$. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra[11] of **2** showed signals assignable to an indole moiety [δ 7.00 (ddd, J=1.2, 7.9,

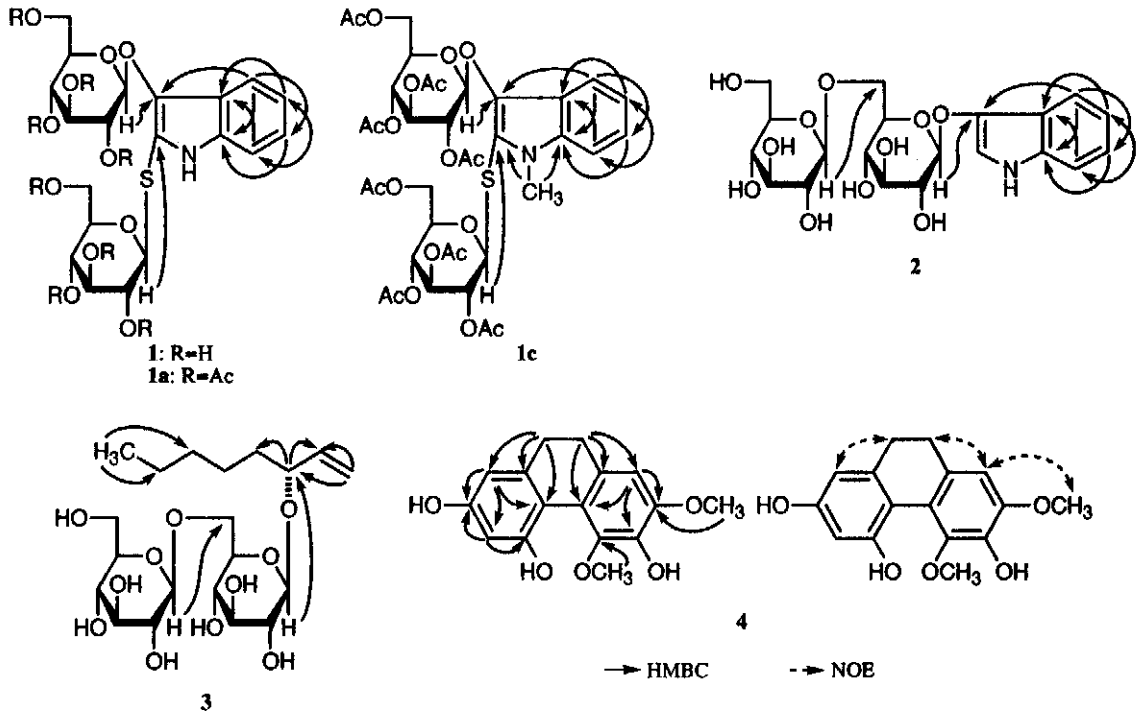

Figure 2. HMBC Correlations and NOE Correlations of **1**, **1a**, **1c**, **2**, **3**, and **4**

7.9 Hz, 5-H), 7.13 (ddd, J=1.2, 7.9, 7.9 Hz, 6-H), 7.15 (s, 2-H), 7.26 (dd, J=1.2, 7.9 Hz, 7-H), 7.79 (dd, J=1.2, 7.9 Hz, 4-H)] and two β-D-glucopyranosyl groups [δ 4.43 (d, J=9.2 Hz, 1"-H), 4.92 (d, J=7.3 Hz, 1'-H)]. The gentiobiosyl structure and the linkaged position of **2** were elucidated by HMBC experiment, which showed long-range correlations between the 1"-proton and the 6'-carbon and between the 1'-proton and the 3-carbon. Acid hydrolysis of **2** liberated D-glucose.[13] Finally, on the basis of comparison of the ^{13}C-NMR data of **2** with those of indican (**9**), the structure of glucoindican (**2**) was elucidated.

Calaliukiuenoside (**3**), also isolated as a white powder, showed absorption bands ascribable to hydroxyl and olefin functions at 3410, 1655, 1075, and 1040 cm^{-1} in the IR spectrum. Here again, the molecular formula $C_{20}H_{36}O_{11}$ of **3** was clarified from its positive- and negative-ion FAB-MS [quasimolecular ions: m/z 475 $(M+Na)^+$, m/z 451 $(M-H)^-$] and by high-resolution MS measurements. Enzymatic hydrolysis of **3** with β-glucosidase liberated (–)-matsutakeol (**5**).[14] The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra[11] of **3** showed the presence of a (–)-matsutakeol moiety and a β-gentiobiosyl part [δ 4.32, 4.40 (both d, J=7.9 Hz, 1', 1"-H)]. Finally, **3** was synthesized by glycosidation of **5** with *O*-(hepta-*O*-acetyl)-gentiobiosyltrichloroacetamide in the presence of boron trifluoride (BF_3·Et_2O) and molecular sieves 4A in dichloromethane (CH_2Cl_2) followed by deacetylation with sodium methoxide (NaOMe). Consequently, the structure of calaliukiuenoside (**3**) was determined as shown.

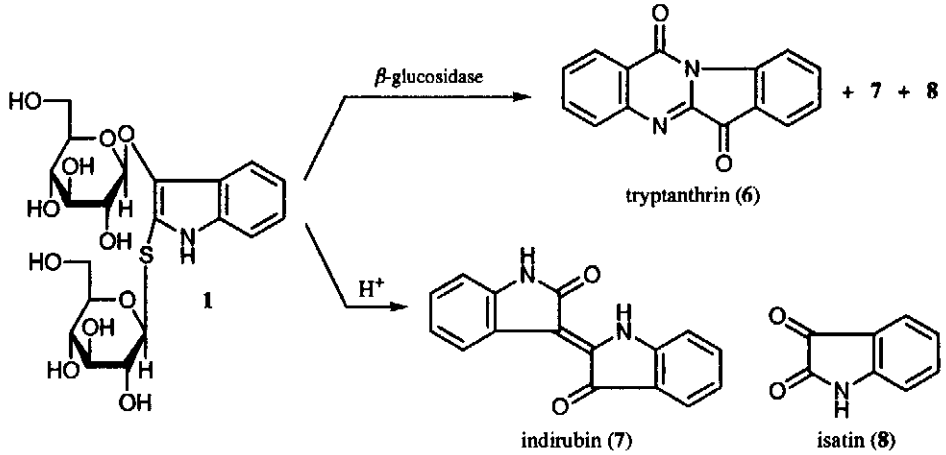

Figure 3

Table 1. ^{13}C-NMR Data for Calanthoside (**1**) and Its Derivatives (**1a**, **1b**, **1c**), Glucoindican (**2**), Calaliukiuenoside (**3**), Calaphenanthreol (**4**), and Indican (**9**)

	1[a]	**1a**[b]	**1b**[b]	**1c**[b]	**2**[a]	**9**[a]		**3**[a]		**4**[b]
C-2	113.3	110.8	114.4	115.8	112.2	112.2	C-1	116.3	C-1	108.0
3	142.0	141.0	146.7	140.5	138.8	138.8	2	140.9	2	145.7
4	119.5	119.0	118.2	118.3	118.6	118.6	3	82.7	3	154.7
5	120.3	119.9	123.5	119.8	119.5	119.5	4	25.7	4	142.1
6	124.2	123.9	127.3	123.9	122.8	122.8	5	35.7	4a	119.2
7	112.2	111.1	116.6	110.0	112.4	112.4	6	33.7*	4b	113.0
8	136.3	135.1	136.5	136.0	135.4	135.4	7	23.7*	5	142.0
9	121.5	120.9	121.6	118.9	121.3	121.3	8	14.4	6	104.3
C-1'	89.4	83.7	86.8	86.9	105.4	105.6	C-1'	104.7	7	156.0
2'	73.7	70.0	70.4	70.6	75.0	75.0	2'	75.1	8	108.0
3'	78.0	74.1	72.8	72.5	77.9	77.9	3'	78.0	8a	131.4
4'	71.2	67.6	68.1	68.3	71.5	71.4	4'	71.6	9	31.6
5'	81.9	74.1	73.6	73.8	77.3	77.9	5'	77.1	10	30.5
6'	62.7	62.2	62.2	62.3	69.9	62.5	6'	69.5	10a	137.2
C-1"	106.5	102.7	101.4	102.4	104.7		C-1"	103.3	2-OMe	56.4
2"	75.4	71.9	71.6	71.6	75.1		2"	75.3	4-OMe	61.8
3"	77.8	72.8	72.7	73.0	77.9		3"	78.1		
4"	71.2	68.6	68.4	68.5	71.5		4"	71.4		
5"	79.1	76.5	75.9	75.8	77.9		5"	78.0		
6"	62.4	61.6	61.8	62.0	62.7		6"	62.7		
NMe				30.3						

125 MHz, CD_3OD,[a] $CDCl_3$[b]

* maybe interchangeable

Calaphenanthreol (**4**) was isolated as an amorphous powder and its IR spectrum showed absorption bands due to hydroxyl and anomeric functions at 3446, 2854, 1508, and 1082 cm^{-1}. In the UV spectrum of **4**, absorption maxima were observed at 217 (log ε 4.4), 275 (4.0), and 305 (3.9) nm, suggestive of a biphenyl structure. The molecular formula $C_{16}H_{16}O_5$ of **4** was clarified by the positive- and negative-ion FAB-MS [*m/z* 289 $(M+H)^+$, 311 $(M+Na)^+$; *m/z* 287 $(M-H)^-$] and by high-resolution MS measurements. Acetylation of **4** with acetic anhydride in pyridine gave triacetate (**4a**). The ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra[11] of **4** and **4a** showed signals due to the pentasubstituted 9,10-dihydrophenanthrene moiety [**4**: δ 2.63 (br s, 9, 10-H_2), 6.40, 6.47 (both d, *J*=2.6 Hz, 8, 6-H), 6.68 (s, 1-H)] and two methoxyl groups [**4**: δ 3.75, 3.91 (both s, 4, 2-OMe)]. The positions of two methoxyl and three hydroxyl groups were characterized by detailed analysis of the HMBC data and difference NOE experiments as shown in Figure 2. Finally, the structure of calaphenanthreol (**4**) was characterized by comparison of the ^{1}H-NMR and ^{13}C-NMR data for **4** with those of related known dihydrophenanthrenes.[15]

EXPERIMENTAL

The instruments used for obtaining physical data and experimental conditions for chromatography were the same as described in our previous paper.[2]

Isolation of Calanthoside (1), Glucoindican (2), and Calaphenanthreol (4) from the Underground Part of *C. discolor*

The fresh underground part of *C. discolor* (7.5 kg, cultivated in Miyazaki Prefecture) was cut and extracted three times with MeOH (10 L) under reflux for 3 h. Evaporation of the solvent under reduced pressure provided the MeOH extract (330 g, 4.4%) and the MeOH extract (300 g) was partitioned into an AcOEt-H_2O (1 : 1, v/v) solution. Removal of the solvent *in vacuo* from the AcOEt-soluble and aqueous-soluble portions yielded 75 g (1.0%) and 220 g (2.9%) of the residue, respectively. The H_2O extract (220 g) was subjected to reversed-phase silica gel column chromatography [Chromatorex ODS DM1020T (Fuji Silysia Ltd., 1.0 kg), $H_2O \rightarrow$ MeOH] to give the H_2O eluate and MeOH eluate (13 g, 0.17%). Normal-phase silica gel column chromatography [BW-200 (Fuji Silysia Ltd., 1.0 kg), $CHCl_3$–MeOH (10 : 1 → 3 : 1 → 1 : 1, v/v) → $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower layer → 6 : 4 : 1, v/v) → MeOH] of the MeOH eluate (13 g) gave five fractions

[fr. 1 (493 mg, 0.0066%), fr. 2 (4.1 g, 0.055%), fr. 3 (2.7 g, 0.036%), fr. 4 (2.0 g, 0.027%), fr. 5 (4.0 g, 0.053%)]. Fraction 2 (4.1 g) was separated by repeated normal-phase silica gel column chromatography [1] 15 g, $CHCl_3$–MeOH (20 : 1 → 10 : 1 → 5 : 1, v/v) → MeOH; 2] 4 g, *n*-hexane–AcOEt (3 : 1 → 1 : 1, v/v) → AcOEt] to give calaphenanthreol (**4**, 43 mg, 0.00057%). Fraction 4 (4.3 g) was separated by normal-phase silica gel column chromatography [40 g, $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower layer → 65 : 35 : 10, lower layer → 6 : 4 : 1, v/v)] and purified by HPLC [YMC-Pack ODS-A (YMC Co., Ltd., 250 x 20 mm i.d.), MeOH-H_2O (25 : 75, v/v)] to give calanthoside (**1**, 33 mg, 0.00044%) and glucoindican (**2**, 82 mg, 0.0011%).

Calanthoside (**1**): a hygroscopic white powder, $[\alpha]_D^{25}$ –12.0° (*c*=0.2, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{20}H_{28}NO_{11}S$ $(M+H)^+$: 490.1301. Found: 490.1342. High-resolution SIMS: Calcd for $C_{20}H_{27}NO_{11}S$ (M^+): 489.1302. Found: 489.4289. UV λ_{max}^{MeOH} nm (log ε): 290 (2.7), 222 (3.5). IR (KBr): 3389, 1624, 1560, 1043, 748 cm^{-1}. ^{1}H-NMR (CD_3OD, 500 MHz) δ: 4.43 (1H, d, *J*=9.2 Hz, 1'-H), 4.92 (1H, d, *J*=6.9 Hz, 1''-H), 7.00 (1H, dd-like, 5-H), 7.12 (1H, dd-like, 6-H), 7.25 (1H, d, *J*=8.3 Hz, 7-H), 7.79 (1H, d, *J*=8.3 Hz, 4-H). ^{13}C-NMR (CD_3OD, 125 MHz) δc: given in Table 1. Negative-ion FAB-MS: *m/z* 488 $(M–H)^–$, 326 $(M–C_6H_{11}O_5)^–$. Positive-ion FAB-MS: *m/z* 512 $(M+Na)^+$, 490 $(M+H)^+$. SIMS: *m/z* 489 (M^+), 327 $(M–C_6H_{11}O_5+H)^+$, 165 $(M–2xC_6H_{11}O_5+2H)^+$.

Glucoindican (**2**): an amorphous powder, $[\alpha]_D^{25}$ +164.0° (*c*=0.01, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{20}H_{28}NO_{11}$ $(M+H)^+$: 458.1638. Found: 458.1650. UV λ_{max}^{MeOH} nm (log ε): 282 (4.7) , 224 (5.4). IR (KBr): 3490, 1618, 1554, 1458, 1028, 747 cm^{-1}. ^{1}H-NMR (CD_3OD, 500 MHz) δ: 4.43 (1H, d, *J*=9.2 Hz, 1''-H), 4.92 (1H, d, *J*=7.3 Hz, 1'-H), 7.00 (1H, ddd, *J*=1.2, 7.9, 7.9 Hz, 5-H), 7.13 (1H, ddd, *J*=1.2, 7.9, 7.9 Hz, 6-H), 7.15 (1H, s, 2-H), 7.26 (1H, dd, *J*=1.2, 7.9 Hz, 7-H), 7.79 (1H, dd, *J*=1.2, 7.9 Hz, 4-H). ^{13}C-NMR (CD_3OD, 125 MHz) δc: given in Table 1. Negative-ion FAB-MS: *m/z* 456 $(M–H)^–$. Positive-ion FAB-MS: *m/z* 480 $(M+Na)^+$, 458 $(M+H)^+$.

Calaphenanthreol (**4**): an amorphous powder. High-resolution positive-ion FAB-MS: Calcd for $C_{16}H_{17}O_5$ $(M+H)^+$: 289.1114. Found: 289.1095. UV λ_{max}^{MeOH} nm (log ε): 305 (3.9), 275 (4.0), 217 (4.4). IR (KBr): 3446, 2854, 1508, 1082 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 2.63 (4H, s, 9, 10-H_2), 3.75, 3.91 (3H each, both s, 4, 2-OMe), 6.40, 6.47 (1H each, both d, *J*=2.6 Hz, 8, 6-H), 6.68 (1H, s, 1-H). ^{13}C-NMR ($CDCl_3$, 125 MHz) δc: given in Table 1. Negative-ion FAB-MS: *m/z* 287 $(M–H)^–$. Positive-ion FAB-MS: *m/z* 289 $(M+H)^+$, 311 $(M+Na)^+$.

Isolation of Tryptanthrin (6), Indirubin (7), and Vomifoliol from the Aerial Part of *C. discolor*

The fresh aerial part of *C. discolor* L. (2.9 kg, cultivated in Miyazaki Prefecture) was cut and extracted three times with MeOH (10 L) under reflux for 3 h. Evaporation of the solvent under reduced pressure provided the MeOH extract (150 g, 5.2%) and the MeOH extract (150 g) was partitioned into an AcOEt-H_2O (1 : 1, v/v) solution. Removal of the solvent *in vacuo* from the AcOEt-soluble and aqueous-soluble portions yielded 62 g (2.1%) and 87 g (3.0%) of the residue, respectively. The AcOEt extract (62 g) was subjected to normal-phase silica gel column chromatography [1.0 kg, $CHCl_3$ → $CHCl_3$–MeOH (50 : 1 → 10 : 1 → 5 : 1, v/v) → MeOH] to give five fractions [fr. 1 (15.9 g, 0.55%), fr. 2 (14.2 g, 0.49%), fr. 3 (8.0 g, 0.26%), fr. 4 (10.7 g, 0.37%), fr. 5 (10.3 g, 0.36%)]. Fraction 2 (14.2 g) was purified by normal-phase silica gel column chromatography [15 g, $CHCl_3$ → $CHCl_3$–MeOH (20 : 1 → 10 : 1 → 5 : 1, v/v) → MeOH] to give tryptanthrin (**6**, 7 mg, 0.00024%) and indirubin (**7**, 128 mg, 0.0044%). Fraction 4 (10.7 g) was separated by reversed-phase silica gel column chromatography [Chromatorex DM1020T (Fuji Silysia Ltd., 130 g), MeOH–H_2O (40 : 60 → 50 : 50 → 80 : 20, v/v)] and HPLC [MeOH-H_2O (45 : 55, v/v)] to give vomifoliol (39 mg, 0.0013%). The known compounds (**6**) was identified by TLC, IR, ^{1}H-NMR, and ^{13}C-NMR spectral comparisons with an authentic sample,[4] while **7** and vomifoliol were identified by comparisons of their physical date ($[\alpha]_D$, ^{1}H-, ^{13}C-NMR) with reported values.[6,7]

Isolation of Calanthoside (1), Calaliukiuenoside (3), and Seven Known Compounds from the Underground Part of *C. liukiuensis*

The fresh underground part of *C. liukiuensis* (12.5 kg, collected in Okinawa Prefecture) was cut and extracted with MeOH (10 L) at rt (28 °C) for 10 h. Evaporation of the solvent under reduced pressure provided the MeOH extract (194 g, 1.6%) and the MeOH extract (182 g) was partitioned into AcOEt-H_2O (1 : 1, v/v) solution. Removal of the solvent *in vacuo* from the AcOEt-soluble and aqueous-soluble portions yielded 38 g (0.3%) and 140 g (1.1%) of the residue, respectively. The AcOEt extract (35 g) was subjected to normal-phase silica gel column chromatography [1 kg, *n*-hexane–AcOEt (10 : 1 → 5 : 1 → 3 : 1, v/v) → $CHCl_3$–MeOH (20 : 1 → 10 : 1, v/v) → MeOH → $CHCl_3$] gave seven fractions [fr. 1 (3.6 g, 0.029%), fr. 2 (3.3 g, 0.026%), fr. 3 (2.2 g, 0.018%), fr. 4 (24.5 g, 0.20%)]. Fraction 3 (2.2 g) was further separated by reversed-phase [66 g, MeOH–H_2O (30 : 70 → 50 : 50 → 80 : 20, v/v) → MeOH] and normal-phase silica gel column chromatography [10 g, *n*-hexane–AcOEt (4 : 1 → 1 : 1, v/v) → MeOH] to give tryptanthrin (**6**, 13 mg, 0.00010%), indirubin (**7**, 14 mg, 0.00011%), and isatin (**8**, 445 mg, 0.0036%). The H_2O extract (133 g) was subjected to Diaion HP-20 column chromatography [Diaion HP-20 (Nippon Rensui Co., 1.0 kg), H_2O → MeOH] gave the H_2O eluate and MeOH eluate (11 g, 0.088%). Reversed-phase silica gel column chromatography [300 g, MeOH–H_2O (30 : 70 →50 : 50, v/v) →$CHCl_3$ → MeOH] of the MeOH eluate (13 g) gave eleven fractions [fr. 1 (2.1 g, 0.017%), fr. 2 (1.6 g, 0.013%), fr. 3 (2.9 g, 0.023%), fr. 4 (1.1 g, 0.0088%), fr. 5 (3.0 g, 0.024%)]. Fraction 1 (900 mg) was separated by normal-phase silica gel column chromatography [100 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer) → MeOH] and HPLC [MeCN–H_2O (5 : 95, v/v)] to give 2,6-dimethoxyphenol 1-*O*-β-D-glucopyranoside (14 mg, 0.00011%). Fraction 2 (1.5 g) was separated by normal-phase silica gel column chromatography [100 g, $CHCl_3$–MeOH (20 : 1 → 10 : 1 → 5 : 1, v/v) → MeOH] and HPLC [1) MeCN-H_2O (15 : 85, v/v); 2) MeCN–H_2O (30 : 70, v/v)] to give calanthoside (**1**, 200 mg, 0.0016%), indican (**9**, 62 mg, 0.00050%), and benzyl glucopyranoside (37 mg, 0.00030%). Fraction 4 (150 mg) was purified by HPLC [MeCN–H_2O (25 : 75, v/v)] to give calaliukiuenoside (**3**, 21 mg, 0.00017%). Fraction 5 (1.2 g) was separated by normal-phase silica gel column chromatography [100 g, $CHCl_3$–MeOH (20 : 1 → 10 : 1 → 5 : 1, v/v) → $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower layer) → MeOH] and HPLC [MeCN–H_2O (20 : 80, v/v)] to give 3-hydroxyoctyl β-D-glucoside (15 mg, 0.00012%). Seven known compounds were identified by TLC, ^{1}H-NMR, and ^{13}C-NMR spectral comparisons with authentic samples obtained from *C. discolor* or by comparison of their physical date ($[\alpha]_D$, ^{1}H-, ^{13}C-NMR) with reported values.[8,9,10]

Calaliukiuenoside (**3**): a white powder, $[\alpha]_D^{28}$ –45.4° (*c*=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{20}H_{36}O_{11}Na$ $(M+Na)^+$: 475.2155. Found: 475.2166. IR (KBr): 3410, 1655, 1075, 1040 cm^{-1}. ^{1}H-NMR (CD_3OD, 270 MHz) δ: 0.90 (3H, t, *J*=5.6 Hz, 8-H_3), 4.32 (1H, d, *J*=7.9 Hz, 1''-H), 4.40 (1H, d, *J*=7.9 Hz, 1'-H), 4.36 (1H, ddd-like, 3-H), 5.11 (1H, d, *J*=10.5 Hz), 5.22 (1H, d, *J*=17.2 Hz) (1-H_2), 5.87 (1H, ddd, *J*=6.9, 10.5, 17.2 Hz, 2-H). ^{13}C-NMR (CD_3OD, 125 MHz) δc: given in Table 1. Negative-ion FAB-MS: *m/z* 451 $(M-H)^-$, 289 $(M-C_6H_{11}O_5)^-$. Positive-ion FAB-MS: *m/z* 475 $(M+Na)^+$.

Acetylation of Calanthoside (1)

A solution of **1** (10 mg) in pyridine (1.0 mL) was treated with Ac_2O (0.5 mL) in the presence of DMAP (5 mg) and the mixture was stirred at rt (24 °C) for 2 days. The reaction mixture was poured into ice water and the whole was extracted with AcOEt. The AcOEt extract was washed with 5% aq. HCl, sat. aq. $NaHCO_3$, and brine, then dried over $MgSO_4$. Removal of solvent under reduced pressure gave a product, which was purified by normal-phase silica gel column chromatography [500 mg, *n*-hexane–AcOEt (6 : 1, v/v) → MeOH] and HPLC [MeCN-H_2O (50 : 50, v/v)] to give the octaacetate (**1a**, 3.6 mg, 21.0%) and the nonaacetate (**1b**, 3.8 mg, 21.4%).

1a: an amorphous powder. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.98, 2.04, 2.08, 2.20 (3H each, all s, Acx4), 2.03, 2.15 (6H each, both s, Acx4), 4.57 (1H, d, *J*=9.7 Hz, 1'-H), 5.29 (1H, d, *J*=6.7 Hz, 1''-H), 7.08 (1H, ddd-like, 5-H), 7.23 (1H, ddd-like, 6-H), 7.32 (1H, dd-like, 7-H), 7.73 (1H, dd-like, 4-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δc: given in Table 1.

1b: an amorphous powder, $[\alpha]_D^{25}$ +9.3° (*c*=0.1, $CHCl_3$). High-resolution positive-ion FAB-MS: Calcd for $C_{38}H_{45}NO_{20}NaS$ $(M+Na)^+$: 890.2173. Found: 890.2153. UV λ_{max}^{MeOH} nm (log ε): 286 (3.0). IR (KBr): 1753, 1451, 1219, 1040, 772 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.96, 1.98, 1.99, 2.04, 2.14, 2.18 (3H each, all s, Acx6), 2.05 (6H, s, Acx2), 2.87 (3H, s, N-Ac),

4.70 (1H, d, *J*=10.1 Hz, 1'-H), 5.29 (1H, d, *J*=8.6 Hz, 1''-H), 7.29 (1H, dd-like, 5-H), 7.41 (1H, dd-like, 6-H), 7.71 (1H, d, *J*=7.9 Hz, 4-H), 8.34 (1H, d, *J*=8.6 Hz, 7-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δc: given in Table 1. Positive-ion FAB-MS: *m/z* 890 $(M+Na)^+$.

Methylation of 1a

A solution of **1a** (2.6 mg) in dry DMF (0.5 mL) was treated with CH_3I (0.036 mL) in the presence of K_2CO_3 (16.6 mg) and the mixture was stirred at N_2 atmosphere under reflux for 3 h. The reaction mixture was poured into brine and the whole was extracted with AcOEt. The AcOEt extract was washed with sat. aq. $Na_2S_2O_3$ and brine, then dried over $MgSO_4$. Removal of solvent under reduced pressure gave a product, which was purified by HPLC [MeCN-H_2O (50 : 50, v/v)] to give **1c** (2.5 mg, 94.5%).

1c: an amorphous powder, $[\alpha]_D^{25}$ –125.0° (*c*=0.2, $CHCl_3$). High-resolution positive-ion FAB-MS: Calcd for $C_{37}H_{46}NO_{19}S$ $(M+H)^+$: 839.2307. Found: 839.2308. UV λ_{max}^{MeOH} nm (log ε): 293 (3.0), 228 (3.3). IR (KBr): 2853, 1750, 1466, 771 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.87, 2.03, 2.04, 2.08, 2.13, 2.19 (3H each, all s, Acx6), 1.99 (6H, s, Acx2), 3.77 (3H, s, N-Me), 4.62 (1H, d, *J*=10.1 Hz, 1'-H), 5.13 (1H, d-like, 1"-H), 7.10 (1H, dd-like, 5-H), 7.29 (1H, dd-like, 6-H), 7.30 (1H, d, *J*=6.4 Hz, 7-H), 7.73 (1H, d, *J*=7.9 Hz, 4-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δc: given in Table 1. Positive-ion FAB-MS: *m/z* 839 $(M+H)^+$, 862 $(M+Na)^+$.

Acid Hydrolysis of Calanthoside (1) and Glucoindican (2)

A solution of **1** and **2** (2 mg each) in 5% aq. H_2SO_4–1,4-dioxane (1 : 1, v/v, 1 mL) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the resin was filtered. After removal of the solvent under reduced pressure from the filtrate, the residue was passed through a Sep-Pak C18 cartridge with H_2O and MeOH. The H_2O eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (0.01 mL) in pyridine (0.02 mL) at 60 °C for 1 h. After reaction, the solution was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.01 mL) at 60 °C for 1 h. The supernatant was then subjected to GLC analysis to identify the derivatives of D-glucose from **1** and **2**. GLC conditions: column, Supeluco^{TM}-1, 0.25 mm (i.d.) x 30 m; column temperature, 230 °C; t_R, D-glucose: 24.4 min.

Acid Hydrolysis of Calanthoside (1)

A solution of **1** (10 mg) in 5% aqueous HCl–1,4-dioxane (1 : 1, v/v, 2 mL) was stirred under reflex for 1 h. The reaction mixture was poured into ice water and the whole was extracted with AcOEt. The AcOEt extract was washed with sat. aq. $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a crude product. The crude product was separated by normal-phase silica gel column chromatography [300 mg, *n*-hexane–AcOEt (6 : 1 → 4 : 1, v/v)] to give indirubin (**7**, 1.3 mg, 24.3%) and isatin (**8**, 1.5 mg, 49.9%), which were identified by comparison of their physical date ($[\alpha]_D$, IR, ^{1}H-NMR, MS) with authentic samples.

The H_2O layer was subjected by HPLC analysis to identify the 1-thio-D-glucose and D-glucose. HPLC conditions: column, YMC-Pack Polyamine II, 250 x 4.6 mm i.d.; flow rate, 1.0 mL/min; mobile phase, MeCN–H_2O (75 : 25, v/v); t_R: D-glucose, 13.7 min, 1-thio-D-glucose, 17.9 min.

Enzymatic Hydrolysis of Calanthoside (1)

A solution of **1** (40 mg) in 0.2 M acetate buffer (pH 4.4, 8 mL) was treated with β-glucosidase (sweet almonds, Oriental Yeast Co., 80 mg) and the whole mixture was stirred at 38 °C for 7 days. The reaction mixture was poured into EtOH and removal of solvent under reduced pressure gave a product. Crude product was purified by reversed-phase (1 g, H_2O → MeOH) and normal-phase silica gel column chromatography [1 g, *n*-hexane–AcOEt (6 : 1, v/v)] to give tryptanthrin (**6**, 3.2 mg, 15.5%), indirubin (**7**, trace), and isatin (**8**, trace), which were identified by comparison of TLC with authentic samples.

Enzymatic Hydrolysis of Calaliukiuenoside (3)

A solution of **3** (5 mg) in 0.2 M acetate buffer (pH 4.4, 1 mL) was treated with β-glucosidase (sweet almonds, Oriental Yeast Co., 80 mg) and the whole mixture was stirred at 38 °C for 5 days. The reaction mixture was poured into EtOH and removal of solvent under reduced pressure gave a product. Crude product was purified by normal-phase silica gel column chromatography [*n*-hexane–AcOEt (5 : 1, v/v)] to give (–)-matsutakeol (**5**, 0.4 mg, 26.6%), which was identified by comparison of the physical date ($[\alpha]_D$, ^{1}H-NMR) with reported values.[12]

Glycosilation of Matsukakeol (5)

1) A solution of D-glucose (1 g) in dry pyridine (40 mL) was treated with 4-methoxytrityl chloride (MMTr-Cl, 3.4 g) and the mixture was stirred at rt (24 °C) under N_2 atmosphere for 1 day. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with brine, then dried over $MgSO_4$. Removal of solvent under reduced pressure gave a product, which was purified by normal-phase silica gel column chromatography [*n*-hexane–AcOEt (1 : 2, v/v)] to furnish 6-*O*-MMTr-D-glucose (1.37 g). A solution of 6-*O*-MMTr-D-glucose (600 mg) in pyridine (15 mL) was treated with Ac_2O (5 mL) and the mixture was stirred at rt (24 °C) for 5 h. It was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave 1,2,3,4-tetra-*O*-acetyl-6-*O*-MMTr-D-glucose (692 mg). A solution of 1,2,3,4-tetra-*O*-acetyl-6-*O*-MMTr-D-glucose (250 mg) in dry THF–Et_2O (1 : 2, v/v, 15 mL) was treated with $BF_3{\cdot}Et_2O$ (7 mL) and the mixture was stirred at rt (24 °C) under an N_2 atmosphere for 20 min. It was poured into AcOEt and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a product, which was purified by normal-phase silica gel column chromatography [*n*-hexane-AcOEt (1 : 1)] to give 1,2,3,4-tetra-*O*-acetyl-D-glucose (83 mg). A solution of 1,2,3,4-tetra-*O*-acetyl-D-glucose (83 mg) in dry CH_2Cl_2 (5 mL) in the presence of molecular sieves-4A (1 g) was treated with *O*-(2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl)-trichloroacetimidate[15] (260 mg) and the mixture was stirred at 0 °C under an N_2 atmosphere for 10 min. It was treated with $BF_3{\cdot}Et_2O$ (21 μl) and stirring was continued for 3 h. It was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a product, which was separated by reversed-phase silica gel column chromatography [MeOH–H_2O (1 : 1, v/v)] to give 1,2,3,4,2',3',4',6'-octa-*O*-acetylgentiobiose (207 mg). A solution of 1,2,3,4,2',3',4',6'-octa-*O*-acetylgentiobiose (207 mg) in DMF (5 mL) was treated with $NH_2NH_2{\cdot}CH_3COOH$ (8.1 mg) and the mixture was stirred at rt (24 °C) under an N_2 atmosphere for 2 h, then poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave 2,3,4,2',3',4',6'-hepta-*O*-acetylgentiobiose (147 mg). A solution of 2,3,4,2',3',4',6'-hepta-*O*-acetylgentiobiose (147 mg) in CH_2Cl_2 (10 mL) was treated with K_2CO_3 (96 mg) and the mixture was stirred at rt (24 °C) under an N_2 atmosphere for 10 min. It was treated with $BF_3{\cdot}Et_2O$ (125 μL) and stirring was continued for 1 day. It was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave *O*-(hepta-*O*-acetyl-gentiobiosyl)trichloroacetamide (54 mg).

2) A solution of *O*-(hepta-*O*-acetyl-gentiobiosyl)trichloroacetamide (54 mg) in dry CH_2Cl_2 (5 mL) in the presence of molecular sieves-4A (1 g) was treated 1-octen-3-ol [(±)-matsutakeol, 20 mg] and the mixture was stirred at 0 °C under an N_2 atmosphere for 10 min. It was treated with $BF_3{\cdot}Et_2O$ (30 μL), and stirring was continued for 3 h. The reaction mixture was poured into ice-water and the whole was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with brine, then dried over $MgSO_4$. Removal of solvent under reduced pressure gave a product, which was purified by normal-phase silica gel column chromatography [*n*-hexane–AcOEt (1 : 1, v/v)] to give (±)-mixture. (19 mg). A solution of (±)-mixture (19 mg) in 0.1% NaOMe–MeOH (3 mL) was stirred at rt (24 °C) for 1 h. After cooling, the reaction mixture was neutralized with Dowex HCR W2 (H^+ form) and the insoluble portion was removed by filtration. After removal of the solvent from filtrate under reduced pressure, the residue was purified by HPLC [MeCN–H_2O (20 : 80, v/v)] to give calaliukiuenoside (**3**, 5.3 mg), which was found to be identical with an authentic sample by TLC, HPLC, $[\alpha]_D$, and ^{1}H-NMR comparisons, and its 3-epimer (**3'**, 4.3 mg).

3': a white powder. ^{1}H-NMR (CD_3OD, 270 MHz) δ: 0.91 (3H, t, J=6.6 Hz, 8-H_3), 4.33 (1H, d, J=7.6 Hz, 1''-H), 4.40 (1H, d, J=7.6 Hz, 1'-H), 4.14 (1H, ddd-like, 3-H), 5.11 (1H, d, J=10.3 Hz), 5.22 (1H, d, J=15.3 Hz) (1-H_2), 5.87 (1H, ddd, J=7.9, 10.3, 15.3 Hz, 2-H).

Acetylation of Calaphenanthreol (4)

A solution of **4** (2 mg) in pyridine (1.0 mL) was treated with Ac_2O (0.5 mL) and the mixture was stirred at rt (24 °C) for 1 day. The reaction mixture was poured into ice water and the whole was extracted with AcOEt. The AcOEt extract was washed with 5% aq. HCl, sat. aq. $NaHCO_3$, and brine, then dried over $MgSO_4$. Removal of solvent under reduced pressure gave a product, which was purified by normal-phase silica gel column chromatography [100 mg, *n*-hexane–AcOEt (6 : 1, v/v) → MeOH] to give the triacetate (**4a**, quant.).

4a:. an amorphous powder. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 2.21, 2.29, 2.36 (3H each, all s, Ac), 2.71 (4H, s, 9, 10-H_2), 3.35, 3.86 (3H each, both s, 4, 2-OMe), 6.90, 6.96 (1H each, both d, J=2.6 Hz, 8, 6-H), 6.70 (1H, s, 1-H).

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