NOVEL INDOLE *S,O*-BISDESMOSIDE, CALANTHOSIDE, THE PRECURSOR GLYCOSIDE OF TRYPTANTHRIN, INDIRUBIN, AND ISATIN, WITH INCREASING SKIN BLOOD FLOW PROMOTING EFFECTS, FROM TWO *CALANTHE* SPECIES (ORCHIDACEAE)

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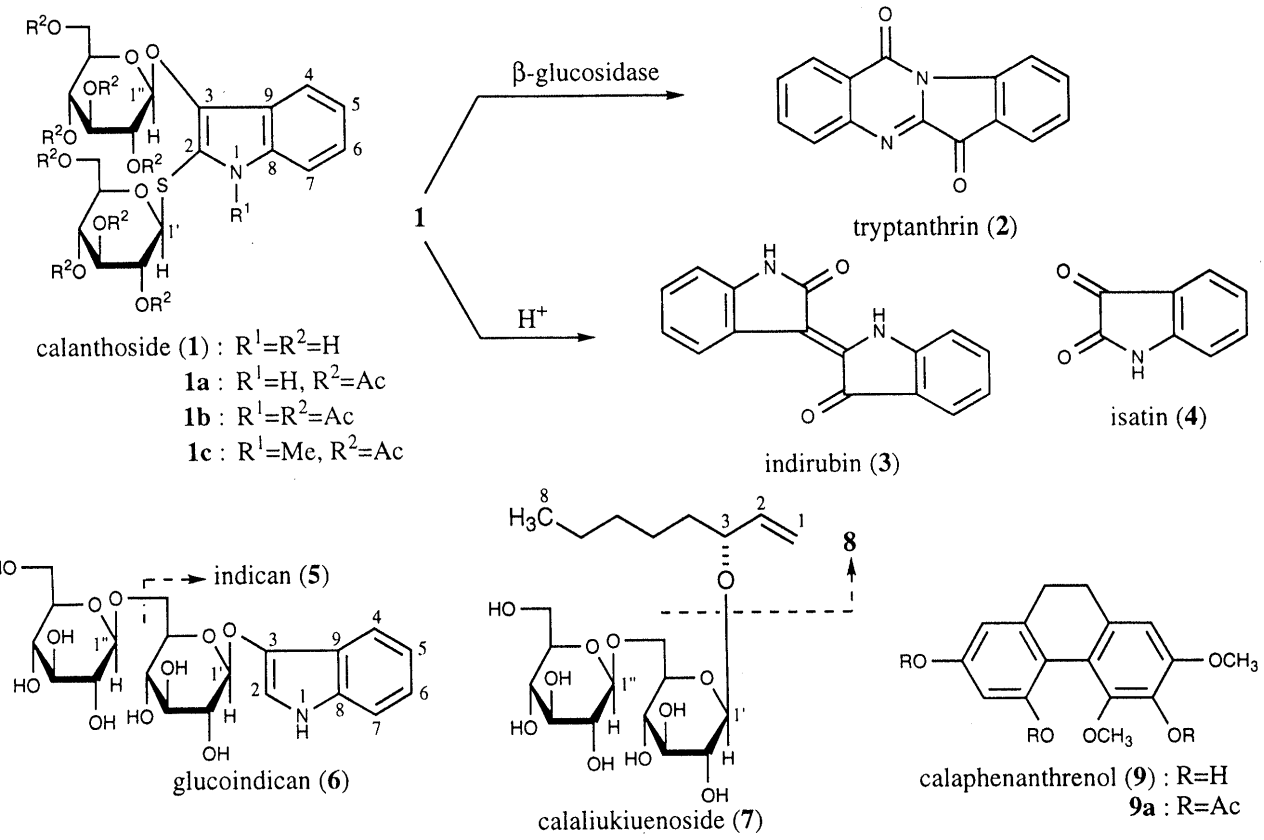
The methanolic extracts from *Calanthe discolor* LINDL. and *C. liukiuensis* SCHLTR. were found to exhibit hair restoring and skin blood flow promoting activities. Through bioassay-guided separation using the skin blood flow increasing effect, a novel indole *S,O*-bisdesmoside, calanthoside, was isolated together with three new components, glucoindican, calaliukiuenoside, and calaphenanthrenol, and known compounds such as tryptanthrin, indirubin, isatin, and indican. The structures of the new compounds were determined on the basis of physicochemical and chemical evidence and they showed an activating effect on skin blood flow. In addition, it was found that enzymatic hydrolysis of calanthoside with β-glucosidase furnished tryptanthrin together with a small amount of indirubin and isatin, whereas indirubin and isatin were obtained from calanthoside by acid hydrolysis. Based on their contents in the fresh and dried plant, calanthoside may be a common genuine glycoside of tryptanthrin, indirubin, and isatin in the plant.

KEY WORDS calanthoside; indole *S,O*-bisdesmoside; tryptanthrin; indirubin; *Calanthe discolor*; *Calanthe liukiuensis*

The dried whole plant of *Calanthe discolor* LINDL. (Orchidaceae) has been used for antiinflammatory, antibacterial, and antitoxic purposes in Chinese traditional medicine, but its pharmacological properties and chemical constituents remained uncharacterized. As a part of our characterization studies on the bioactive constituents of natural medicines,[1] we have found that the methanolic extracts of this plant and *C. liukiuensis* SCHLTR. exhibited potent hair restoring activity in C3H mice and promoted the blood flow in the dorsal skin of rats. Through bioassay-guided separation from the methanolic extract using the skin blood flow increasing effect, we have isolated[2] calanthoside (**1**), glucoindican (**6**), calaliukiuenoside (**7**), and calaphenanthrenol (**9**) together with the antibiotic alkaloid tryptanthrin (**2**),[3,4] antileukemia alkaloids indirubin (**3**),[5] isatin (**4**),[3e] indican (**5**), vomifoliol, 2,6-methoxyphenol 1-*O*-β-D-glucopyranoside, 3-hydroxyoctyl β-D-glucoside, and benzyl β-D-glucopyranoside.

Calanthoside (**1**), a white powder, $[\alpha]_D^{25}$ -21.0° (MeOH), $C_{20}H_{27}NO_{11}S$,[6] UV (MeOH, log ε): 222 (3.5), 290 (2.7) nm, IR (KBr): 3389, 1624, 1560, 1448, 1342, 1240, 1043, 748 cm^{-1}, showed quasimolecular ion peaks at *m/z* 512 $(M+Na)^+$ and 490 $(M+H)^+$ in addition to fragment ion peaks at *m/z* 327 $(M-C_6H_{11}O_5+H)^+$ and 165 $(M-2xC_6H_{11}O_5+2H)^+$ in positive-ion FAB-MS and SIMS, while negative-ion FAB-MS showed a quasimolecular ion peak at *m/z* 488 $(M-H)^-$ and a fragment ion peak at *m/z* 326 $(M-C_6H_{11}O_5)^-$. 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,[6] showed the presence of a 2,3-disubstituted indole moiety [δ 7.00, 7.12 (both dd-like, 5, 6-H), 7.25, 7.79 (both d, *J*=8.3 Hz, 7, 4-H)] together with an *O*-β-D-glucopyranosyl part [δ 4.92 (d, *J*=6.9 Hz, 1"-H)] and *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.[7] Acetylation of **1** with Ac_2O and DMAP in pyridine furnished the octaacetate (**1a**)[8] and nonaacetate (**1b**).[9] Methylation of **1a** with CH_3I and K_2CO_3 in DMF provided the *N*-methyl octaacetate (**1c**).[10] The positions of the *S*- and *O*-glucoside linkages in **1** were clarified by 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. On the basis of this evidence, the structure of calanthoside (**1**) was determined.



Acid hydrolysis of **1** with 5% HCl in dioxane yielded **3** (39%) and **4** (38%) together with 1-thio-β-D-glucopyranose and D-glucose in a *ca.* 1 : 1 ratio, which were identified by HPLC and GLC analysis of the trimethylsilyl thiazolidine derivative.[11] On the other hand, enzymatic hydrolysis of **1** with β-glucosidase (sweet almond, Oriental Yeast Co.) in 0.2 M acetate buffer (pH 4.4) furnished **2** (63%) and a small amount of **3** and **4** together with 1-thio-β-D-glucopyranose. **1** could not be isolated from the dried plant, whereas the contents of **2**, **3**, and **4** increased during the drying process. Although **2**, **3**, and **4** were isolated from various plants[3] and also from the culture solution of a yeast,[4] **1** is considered to be a common genuine glycoside of **2**, **3**, and **4** in the plant. Furthermore, the antibiotic activity of **2** produced from **1** in the drying process may be important evidence substantiating the traditional effect of this natural medicine, the dried whole plant of *Calanthe discolor*.

Table 1. ^{13}C-NMR Data of **1**, **1a**, **1b**, **1c**, **6**, and **7**

	1[a]	1a[b]	1b[b]	1c[b]	6[a]	7[a]
C-1						116.3
C-2	113.3	110.8	114.4	115.8	112.2	140.9
C-3	142.0	141.0	146.7	140.5	138.8	82.7
C-4	119.5	119.0	118.2	118.3	118.6	25.7
C-5	120.3	119.9	123.5	119.8	119.5	35.7
C-6	124.2	123.9	127.3	123.9	122.8	33.7
C-7	112.2	111.1	116.6	110.0	112.4	23.7
C-8	136.3	135.1	136.5	136.0	135.4	14.1
C-9	121.5	120.8	121.6	118.9	121.3	
C-1'	89.3	83.7	86.8	86.9	105.4	104.7
C-2'	73.7	70.0	70.4	70.6	75.0	75.1
C-3'	78.0	74.1	72.8	72.5	77.9	78.0
C-4'	71.2	67.6	68.1	68.3	71.5	71.6
C-5'	81.9	74.1	73.6	73.8	77.3	77.1
C-6'	62.7	62.2	62.2	62.3	69.9	69.5
C-1"	106.5	102.7	101.4	102.4	104.7	103.3
C-2"	75.4	71.9	71.6	71.6	75.1	75.3
C-3"	77.8	72.8	72.7	73.0	77.9	78.1
C-4"	71.2	68.6	68.4	68.5	71.5	71.4
C-5"	79.1	76.5	75.9	75.8	77.9	78.0
C-6"	62.4	61.6	61.8	62.0	62.7	62.7
NMe				30.3		

a) Measured in CD_3OD. *b*) $CDCl_3$.

Glucoindican (**6**), a white powder, $[\alpha]_D^{25}$ +164.0° (MeOH), $C_{20}H_{27}NO_{11}$,[6] UV (MeOH, log ε): 224 (5.4), 282 (4.7) nm, IR (KBr): 3490, 1618, 1554, 1458, 1348, 1235, 1028 cm^{-1}, liberated D-glucose by acid hydrolysis. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra[6] on **6** showed signals due to the 3-substituted indole moiety [δ 6.97 (ddd, *J*=1.3, 6.9, 7.9 Hz, 5-H), 7.07 (ddd, *J*=1.3, 6.9, 8.3 Hz, 6-H), 7.15 (s, 2-H), 7.27 (d, *J*=8.3 Hz, 7-H), 7.67 (d, *J*=7.9 Hz, 4-H)] and gentiobiosyl moiety [δ 4.40 (d, *J*=7.6 Hz, 1"-H), 4.74 (d, *J*=7.3 Hz, 1'-H)]. In the HMBC experiment on **6**, long-range correlations were observed between the following protons and carbons: 1"-H and 6'-C, 1'-H and 3-C, 2-H and 3, 8, 9-C, 4-H and 3-C. This evidence and comparison of the ^{13}C-NMR data for **6** with those for indican (**5**) led us to determine the structure of glucoindican (**6**).

Calaliukiuenoside (**7**), a white powder, $[\alpha]_D^{28}$ -45.4 (MeOH), $C_{20}H_{36}O_{11}$,[6] IR (KBr): 3410, 1655, 1075, 1040 cm^{-1}, negative-ion and positive-ion FAB-MS *m/z*: 289 (M-

$C_6H_{11}O_5)^-$, 451 $(M-H)^-$, 475 $(M+Na)^+$, liberated (-)-matsutakeol (**8**)[12] by enzymatic hydrolysis. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra[6] of **7** showed the presence of a 1-octen-3-ol moiety and gentiobiosyl moiety [δ 4.32, 4.40 (both d, *J*=7.9 Hz, 1', 1''-H)]. Glycosidation of **8** with *O*-(hepta-*O*-acetyl-gentiobiosyl)trichloroacetimidate in the presence of $BF_3{\cdot}Et_2O$ and molecular sieves 4A in CH_2Cl_2 followed by deacetylation furnished **7**, which was identified with calaliukiuenoside. Consequently, the structure of calaliukiuenoside (**7**) was elucidated.

Calaphenanthrenol (**9**), an amorphous powder, $C_{16}H_{16}O_5$,[6] UV ($CHCl_3$, log ε): 217 (4.4), 275 (4.0), 305 (3.9) nm, IR (KBr): 3446, 2854, 1508, 1082 cm^{-1}, gave the triacetate (**9a**)[13] by acetylation with Ac_2O in pyridine. The ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR spectra[6,14] of **9** showed signals due to the pentasubstituted 9,10-dihydrophenanthrene moiety [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 [δ 3.75, 3.91 (both s, 4, 2-OCH_3)]. The positions of two methoxyl and three hydroxyl groups was characterized by detailed analysis of the HMBC data and difference NOE experiments. Consequently, the structure of calaphenanthrenol (**9**) was characterized.

Table 2. Effects of **1**, **6**, **7**, and **9** on Blood Flow in Dorsal Skin of Intact Rats

Treatment	Conc. (%)	Increase (%)
Control	-	8.8±5.0
Calanthoside (**1**)	0.2	6.2±3.2
	0.5	25.7±3.8*
	1.0	27.5±3.3*
Glucoindican (**6**)	0.2	22.5±3.0*
Calaliukiuenoside (**7**)	0.5	7.4±5.2
Calaphenanthrenol (**9**)	0.2	10.3±5.0
	0.5	16.9±6.7
Carpronium chloride	0.5	26.4±6.7*

Significantly different from control group, * $p<0.05$ (n=8-9).

Table 2 summarizes the promoting effects of the new constituents (**1**, **6**, **7**, **9**) on skin blood flow in rats. Two indole glycosides (**1**, **6**) were found to increase the skin blood flow and this activity may be beneficial in hair restoration.

ACKNOWLEDGMENT

The authors are grateful to Professor Gisho Honda, Faculty of Pharmaceutical Sciences, University of Kyoto, for the kind gift of authentic tryptanthrin.

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2) Isolation yields of new compounds from *C. discolor*: **1** (0.0004% from the fresh whole plant), **6** (0.0011%), **9** (0.0006%); from *C. liukiuensis*: **1** (0.0017% from the fresh underground part), **7** (0.0012%), **9** (trace).

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6) The molecular compositions of new compounds were confirmed by high-resolution mass spectrometry. The ^{1}H and ^{13}C-NMR spectra were assigned with the aid of homo- and hetero-correlation spectroscopy (^{1}H-^{1}H, ^{1}H-^{13}C COSY), distortionless enhancement by polarization transfer (DEPT), and HMBC experiments.

7) The ^{13}C-NMR data on methyl 1-thio-β-D-glucopyranoside (68 MHz, CD_3OD) δc: 87.4 (1-C), 73.8 (2-C), 79.6 (3-C), 71.6 (4-C), 82.1 (5-C), 63.0 (6-C), 12.0 (1-SCH_3).

8) **1a**: a white powder. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.98, 2.08, 2.15, 2.19 (all s, OAc), 2.03, 2.04 (both s, OAc), 4.57 (d, *J*=9.8, 1'-H), 4.98 (d, *J*=7.6, 1''-H), 7.08, 7.23 (both dd-like, 5, 6-H), 7.32, 7.73 (both d-like, 4, 7-H). ^{13}C-NMR (68 MHz, $CDCl_3$) δc: given in Table 1.

9) **1b**: a white powder, $[\alpha]_D^{25}$ +9.3° ($CHCl_3$), $C_{38}H_{45}NO_{20}S$, IR (film): 1753, 1451. 1219, 1040, 772 cm^{-1}. UV (MeOH, log ε): 286 (3.0) nm. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.96, 1.98, 1.99, 2.04, 2.14, 2.18 (all s, OAc), 2.05 (s, OAc), 2.87 (s, NAc), 4.70 (d, *J*=10.1, 1'-H), 5.29 (1H, d, *J*=8.6, 1''-H), 7.29, 7.41 (both dd-like, 5, 6-H), 7.71, 8.34 (both d-like, 4, 7-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δc: given in Table 1. Positive-ion FAB-MS *m/z*: 890 $(M+Na)^+$.

10) **1c**: a white powder, $[\alpha]_D^{25}$ -134.0° ($CHCl_3$), $C_{37}H_{45}NO_{19}S$, IR (film): 2853, 1750, 1466, 771 cm^{-1}. UV (MeOH, log ε): 228 (3.3), 293 (3.0) nm. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.87, 2.03, 2.04, 2.08, 2.13, 2.19 (all s, OAc), 1.99 (s, OAc), 4.17 (s, NMe), 4.62 (d, *J*=10.1, 1'-H), 5.15 (d, *J*=7.6, 1''-H), 7.10, 7.29 (both dd-like, 5, 6-H), 7.29, 7.73 (both s, 7, 4-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δc: given in Table 1. Positive-ion FAB-MS (*m/z*) : 839 $(M+H)^+$, 862 $(M+Na)^+$.

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13) **9a**: ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 2.21, 2.29, 2.36 (all s, OAc), 2.71 (br s, 9, 10-H_2), 3.35, 3.87 (all s, 2, 4-OMe), 6.70 (s, 1-H), 6.91, 6.95 (both d, *J*=2.6, 6, 8-H). ^{13}C-NMR (68 MHz, $CDCl_3$) δc: 169.7, 169.2, 168.9, 151.4, 151.0, 149.0, 147.5, 141.3, 139.1, 122.4, 117.7, 117.4x2, 115.4, 106.9, 61.7, 56.1, 30.5, 30.4, 21.5, 21.2, 20.5.

14) **9**: ^{13}C-NMR (68 MHz, CD_3OD) δ: 156.0 (7-C), 154.7 (5-C), 145.7 (2-C), 142.0 (3-C), 142.1 (4-C), 137.2 (10a-C), 131.4 (8a-C), 119.2 (4a-C), 113.0 (4b-C), 108.0 (1-C), 108.0 (8-C), 104.0 (6-C), 61.8 (2-OMe), 56.4 (4-OMe), 31.6 (9-C), 30.5 (10-C).

(Received March 23, 1998; accepted April 14, 1998)