Indonesian Medicinal Plants. XX.[1] Chemical Structures of Eleuthosides A, B, and C, Three New Aromatic Glucosides from the Bulbs of *Eleutherine palmifolia* (Iridaceae)

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Three new aromatic glucosides named eleuthosides A (5), B (6), and C (7), have been isolated from the bulbs of *Eleutherine palmifolia* (Iridaceae), an Indonesian medicinal plant. Their chemical structures have been elucidated on the bases of physicochemical properties and chemical evidence.

Key words *Eleutherine palmifolia*; Iridaceae, Indonesian medicinal plant; eleuthoside; aromatic glucoside

The bulbs of *Eleutherine palmifolia* (L.) MERR. (Iridaceae) have traditionally been used to treat cancer in the Sambas district of West Kalimantan, Indonesia. In the course of our chemical study of Indonesian medicinal plants,[2] we have investigated the chemical constituents of the bulbs and have isolated three new aromatic glucosides named eleuthosides A (**5**), B (**6**) and C (**7**). This paper deals with the elucidation of their structures.

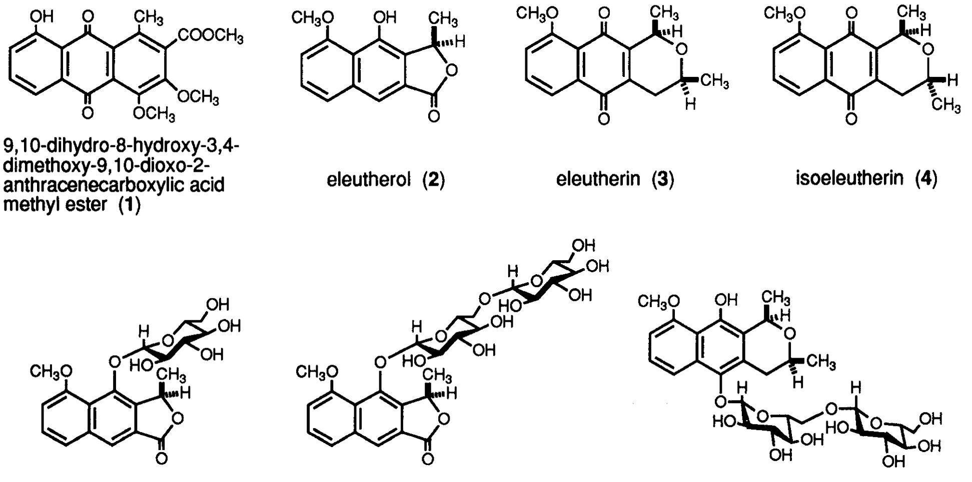
The methanolic extract of the bulbs of *Eleutherine palmifolia* was partitioned into ethyl acetate and water. The ethyl acetate-soluble portion was repeatedly separated by silica gel column chromatography to afford 9,10-dihydro-8-hydroxy-3,4-dimethoxy-9,10-dioxo-2-anthracenecarboxylic acid methyl ester (**1**, 0.002% from the bulb),[3] eleutherol (**2**, 0.04%),[4] eleutherin (**3**, 0.04%)[4,5] and isoeleutherin (**4**, 0.005%).[4,5] The water-soluble portion was extracted with 1-butanol to give the 1-butanol-soluble extract, which was separated by MCI-gel chromatography to afford eleuthosides A (**5**, 0.024% from the bulb), B (**6**, 0.009%) and C (**7**, 0.027%).

Eleuthoside A (5) Eleuthoside A (**5**) showed a quasimolecular ion peak at *m*/*z* 429 [$C_{20}H_{22}NaO_9$ $(M+Na)^+$] in the FAB-MS. The IR spectrum showed absorption bands due to a hydroxyl (3370 cm^{-1}) group and a conjugated γ-lactone (1750 cm^{-1}) group.

The peracetate (**5a**) of eleuthoside A (**5**) showed signals of four acetoxymethyl groups in the ^{1}H-NMR spectrum, which indicated that **5** possessed four hydroxyl groups.

The ^{13}C-NMR, distortionless enhancement by polarization transfer (DEPT) and ^{13}C–^{1}H correlation spectroscopy (COSY) spectra of **5** revealed the presence of ten aromatic carbons, one secondary methyl carbon, one methine carbon attached to the methyl group, one methoxyl carbon, one carbonyl carbon, and one glucosyl residue (see Table 1). Furthermore, the ^{1}H-NMR showed signals of four aromatic protons [δ 7.13 (1H, d, J=7.9 Hz, 6-H), δ 7.48 (1H, dd, J=7.9, 7.9 Hz, 7-H), δ 7.61 (1H, d, J=7.9 Hz, 8-H), δ 8.17 (1H, s, 9-H)]. The above-mentioned findings, together with the heteronuclear multiple-bond correlation spectroscopy (HMBC) and ^{1}H–^{1}H COSY spectra (Fig. 2), indicate that eleuthoside A (**5**) is a naphthalene glucoside.

Methanolysis of eleuthoside A (**5**) with 10% hydrogen chloride in methanol gave eleutherol (**2**), which was identical with an authentic sample (including the optical rotation), and methyl D-glucopyranoside, for which the



9,10-dihydro-8-hydroxy-3,4-dimethoxy-9,10-dioxo-2-anthracenecarboxylic acid methyl ester (**1**)

eleutherol (**2**)

eleutherin (**3**)

isoeleutherin (**4**)

eleuthoside A (**5**)

eleuthoside B (**6**)

eleuthoside C (**7**)

Fig. 1

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Table 1. ^{13}C-NMR Data for Eleutherol (**2**), Eleuthoside A (**5**) and Eleuthoside B (**6**)

	2	5	6
C-1	172.2 (s)	172.3 (s)	172.3 (s)
C-3	78.5 (d)	80.9 (d)	81.1 (d)
C-4	149.7 (s)	147.8 (s)	147.9 (s)
C-5	157.1 (s)	157.4 (s)	157.5 (s)
C-6	107.1 (d)	109.8 (d)	109.8 (d)
C-7	127.4 (d)	128.4 (d)	128.4 (d)
C-8	123.8 (d)	123.7 (d)	123.7 (d)
C-9	116.8 (d)	124.1 (d)	124.2 (d)
C-10	125.7 (s)	125.9 (s)	126.0 (s)
C-11	128.1 (s)	140.7 (s)	140.8 (s)
C-12	118.0 (s)	124.0 (s)	124.1 (s)
C-13	137.8 (s)	139.3 (s)	139.3 (s)
3-CH_3	19.2 (q)	19.4 (q)	19.3 (q)
5-OCH_3	56.7 (q)	56.1 (q)	56.2 (q)
glc-1			
C-1′		106.2 (d)	106.0 (d)
C-2′		76.1 (d)	76.1 (d)
C-3′		77.8 (d)	77.4 (d)
C-4′		71.6 (d)	71.1 (d)
C-5′		78.3 (d)	78.0 (d)
C-6′		62.5 (t)	69.1 (s)
glc-2			
C-1″			104.3 (d)
C-2″			75.2 (d)
C-3″			76.6 (d)
C-4″			71.7 (d)
C-5″			78.0 (d)
C-6″			62.9 (t)

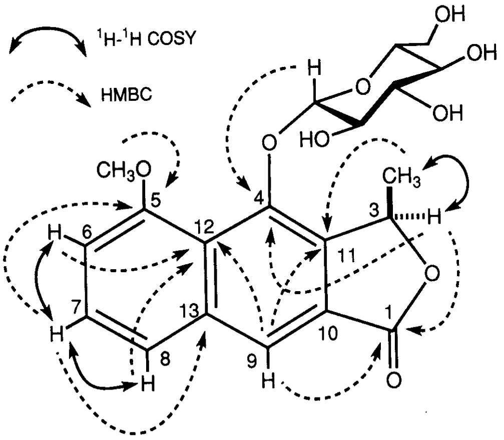

Fig. 2. Correlations Observed in the ^{1}H–^{1}H COSY and HMBC Spectra of Eleuthoside A (**5**)

absolute configuration was determined by the gas chromatographic method.[6] In addition, the HMBC spectrum showed a correlation peak between the anomeric proton (1′-H) and 4-C, and the configuration at the anomeric position was clarified as β by the coupling constant (J = 7.6 Hz) of anomeric proton in the ^{1}H-NMR spectrum.

Consequently, the chemical structure of eleuthoside A was elucidated as **5**.

Eleuthoside B (6) Eleuthoside B (**6**) showed a quasimolecular ion peak at m/z 591 [$C_{26}H_{32}NaO_{14}$ $(M+Na)^+$] in the FAB-MS and the IR spectrum showed a similar absorption pattern to that of eleuthoside A (**5**). The peracetate (**6a**) showed the signals of seven acetoxymethyl groups in the ^{1}H-NMR spectrum.

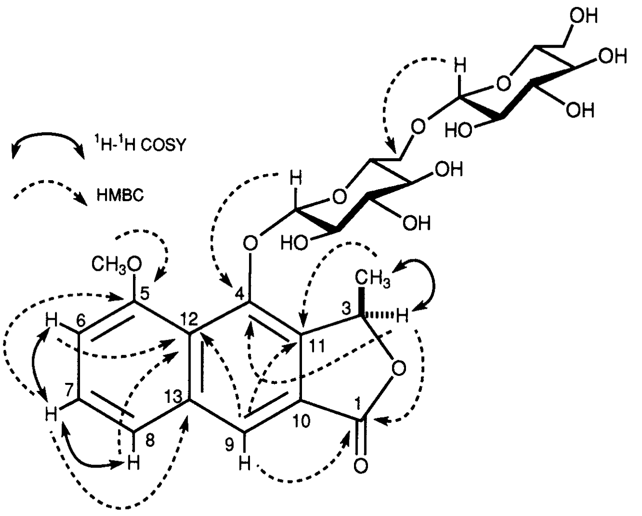

Fig. 3. Correlations Observed in the ^{1}H–^{1}H COSY and HMBC Spectra of Eleuthoside B (**6**)

Table 2. ^{13}C-NMR Data for Eleutherin (**3**) and Eleuthoside C (**7**)

	3	7
C-1	70.2 (d)	72.6 (d)
C-3	68.7 (d)	71.2 (d)
C-4	29.9 (t)	34.8 (t)
C-5	184.0 (s)	141.8 (s)
C-6	117.7 (d)	116.9 (d)
C-7	134.6 (d)	126.6 (d)
C-8	119.0 (d)	105.0 (d)
C-9	159.3 (s)	157.7 (s)
C-10	183.7 (s)	148.4 (s)
C-11	148.6 (s)	121.6 (s)
C-12	139.9 (s)	131.2 (s)
C-13	133.9 (s)	130.1 (s)
C-14	120.2 (s)	114.9 (s)
1-CH_3	20.8 (q)	56.2 (q)
3-CH_3	21.3 (q)	19.3 (q)
9-OCH_3	56.4 (q)	56.2 (q)
glc-1		
C-1′		106.6 (d)
C-2′		75.9 (d)
C-3′		78.0 (d)
C-4′		71.3 (d)
C-5′		77.9 (d)
C-6′		69.5 (t)
glc-2		
C-1″		104.6 (d)
C-2″		75.2 (d)
C-3″		78.2 (d)
C-4″		71.3 (d)
C-5″		76.5 (d)
C-6″		62.5 (t)

The ^{13}C-NMR spectrum of **6** showed a quite similar signal pattern to that of eleutherol (**2**), except for signals due to two glucosyl residues including two anomeric carbons [δ_C 106.0 (1′-C), δ_C 104.3 (1″-C)]. Furthermore, methanolysis of **6** with 10% hydrogen chloride in methanol yielded eleutherol (**2**) and methyl D-glucopyranoside, for which the absolute configuration was determined by the GC method.[6]

Finally, in the HMBC spectrum (Fig. 3), correlation peaks were observed between 1″-H and 6′-C, and 1′-H and 4-C. The coupling constants of two anomeric protons

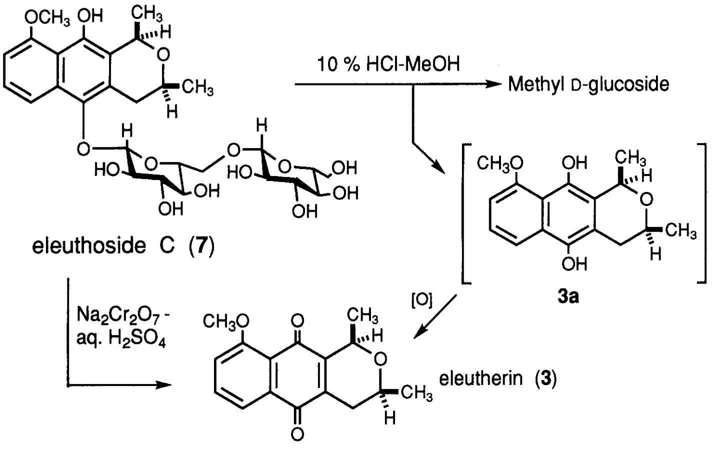

Chart 1

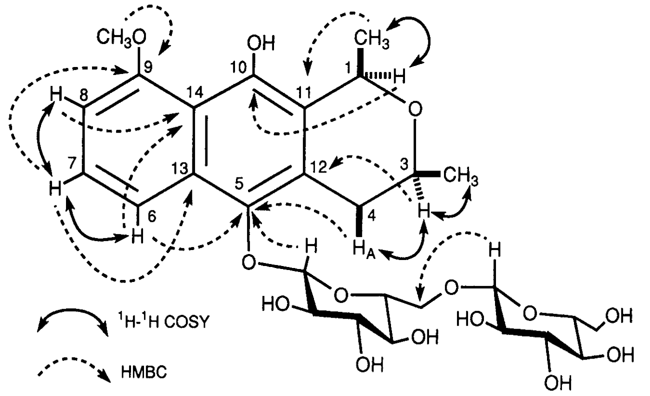

Fig. 4. Correlations Observed in the ^{1}H–^{1}H COSY and HMBC Spectra of Eleuthoside C (7)

[J=7.6 Hz for 1′-H and J=7.6 Hz for 1″-H] revealed that the configurations at the anomeric positions were both β.

Consequently, the chemical structure of eleuthoside B was elucidated as **6**.

Eleuthoside C (7) Eleuthoside C (**7**) showed a quasi-molecular ion peak at m/z 621 [$C_{28}H_{38}NaO_{14}$ $(M+Na)^+$] in the FAB-MS. The IR spectrum suggested the presence of a hydroxyl group (3390 cm^{-1}).

The ^{13}C-NMR, DEPT, and ^{13}C–^{1}H COSY spectra of **7** showed signals of ten aromatic carbons, two secondary methyl carbons, one methoxyl carbon, one methylene carbon, and two glucosyl residues, including the anomeric carbons (δ_C 106.6, d, 1′-C; δ_C 104.6, d, 1″-C) (see Table 2). The ^{1}H-NMR spectrum showed signals of two secondary methyl (δ 1.34, d, J=6.1 Hz; δ 1.62, d, J=6.1 Hz), one methoxyl (δ 4.06, s), two methine protons (δ 3.60, m; δ 5.16, q, J=6.1 Hz) attached to an oxygen function, one pair of methylene protons (δ 2.86, A in ABX, J_{AB}=16.9 Hz, J_{AX}=11.4 Hz; δ 3.06, B in ABX, J_{BA}=16.9 Hz, J_{BX}=1.1 Hz), three aromatic protons (δ 6.87, d, J=7.6 Hz, 8-H; δ 7.30, dd, J=8.6, 7.6 Hz, 7-H; δ 8.02, d, J=8.6 Hz, 6-H), and two anomeric protons (δ 4.67, d, J=7.6 Hz, 1′-H; δ 4.25, d, J=7.6 Hz, 1″-H). Furthermore, the ^{1}H–^{1}H COSY and the HMBC spectra suggested that the structure of eleuthoside C (**7**) is as shown in Fig. 4.

Methanolysis of eleuthoside C (**7**) with 10% hydrogen chloride in methanol afforded methyl D-glucopyranoside and eleutherin (**3**), which was also obtained by oxidation of **7** with sodium dichromate in aqueous sulfuric acid. Thus, it was presumed that eleutherin (**3**) was produced from the unstable hydronaphthoquinone intermediate (**3a**) in the methanolysis.

Finally, correlation peaks were observed between 4-H$_A$ and 5-C, 1′-H and 5-C, and 1″-H and 6′-C in the HMBC spectrum. From the above-mentioned findings, the chemical structure of eleuthoside C was determined as **7**, a hydronaphthoquinone-type glucoside.

Experimental

Melting points were determined with a Yanagimoto micro melting point apparatus without correction. Optical rotations were measured with a JASCO DIP-360 digital polarimeter. FAB-MS and electron impact (EI)-MS were recorded on JEOL SX-102A spectrometer. IR spectra were recorded on a Shimadzu FT-IR 8500 spectrometer. UV spectra were recorded on a Hitachi U-3500 spectrometer. ^{1}H- and ^{13}C-NMR spectra were obtained with a JEOL JNM-Lambda 500 spectrometer operating at 500 MHz and 125 MHz for ^{1}H and ^{13}C nuclei, respectively; chemical shifts are reported in ppm relative to that of tetramethylsilane (δ=0) as an internal standard, and coupling constants are given in hertz. GC-MS was carried out with a Shimadzu QP-5000 apparatus. Column chromatography was carried out on Silica gel 60 (70—230 mesh, Merck) and MCI-gel (CHP20P, 37—75 μ). Thin-layer chromatography on Silica gel 60F$_{254}$ (Merck) was used to ascertain the purity of the compounds. The spots were visualized by spraying the plates with 1% $Ce(SO_4)_2$ in 10% aqueous sulfuric acid and then heating.

Plant Material The bulbs of *Eleutherine palmifolia* (L.) MERR. (Iridaceae) were collected in the Sambas district, West Kalimantan Province, Indonesia in August 1994. The plant was identified at Herbarium Bogoriense, Research and Development Centre for Biology-LIPI, Indonesia. Voucher specimens have been deposited at the Herbarium Bogoriense and the Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University.

Isolation of Eleuthosides A (5), B (6), C (7) and Other Constituents The fresh bulbs (2.2 kg) of the plant were extracted three times with hot methanol under reflux. The combined solution was evaporated under reduced pressure to give the MeOH extract (130 g, 5.9% from the bulb). The MeOH extract (100 g) was partitioned into ethyl acetate and water (1 : 1), and the upper layer (EtOAc phase) was taken and concentrated under reduced pressure to give the EtOAc extract (28.7 g, 1.7%). The lower layer (aqueous phase) was further partitioned with 1-butanol to give the 1-butanol phase and the water phase, which were each concentrated under reduced pressure to give the 1-butanol extract (43.5 g, 2.5%) and the aqueous extract (32.9 g, 1.9%).

The EtOAc extract (10 g) was subjected to silica gel column chromatography (SiO_2 500 g, hexane : EtOAc = 3 : 1→2 : 1, EtOAc, and then MeOH) to give fr. EA-1 (856 mg), fr. EA-2 (156 mg), fr. EA-3 (1.49 g), fr. EA-4 (254 mg), fr. EA-5 (151 mg) and fr. EA-6 (1.77 g). Fr. EA-2 (150 mg) was again chromatographed on silica gel (SiO_2 15 g, benzene : EtOAc = 25 : 1→9 : 1, EtOAc, then MeOH) to afford 9,10-

dihydro-8-hydroxy-3,4-dimethoxy-9,10-dioxo-2-anthracenecarboxylic acid methyl ester (**1**, 9 mg, 0.002%). Separation of fr. EA-3 (1.49 g) by silica gel column chromatography (SiO_2 150 g, benzene : EtOAc = 10 : 1→5 : 1→3 : 1→1 : 1, EtOAc, then MeOH) afforded fr. EA-3-1 (45 mg), fr. EA-3-2 (286 mg), fr. EA-3-3 (56 mg), fr. EA-3-4 (352 mg), fr. EA-3-5 (385 mg), fr. EA-3-6 (155 mg), and fr. EA-3-7 (350 mg). Crystallization of fr. EA-3-2 (280 mg) from EtOAc gave eleutherol (**2**, 241 mg, 0.04%). Crystallization of fr. EA-3-4 (350 mg) and fr. EA-3-6 (150 mg) with acetone gave eleutherin (**3**, 231 mg, 0.04%) and isoeleutherin (**4**, 30 mg, 0.005%), respectively. 9,10-Dihydro-8-hydroxy-3,4-dimethoxy-9,10-dioxo-2-anthracenecarboxylic acid methyl ester (**1**), eleutherol (**2**), eleutherin (**3**) and isoeleutherin (**4**) were identified by comparing their physical data including the optical rotations, with those reported.[3-5]

The 1-butanol extract (8.7 g) was subjected to MCI-gel (CHP20P 60 g, MeOH : H_2O = 1 : 1→2 : 1, then MeOH) to give fr. BA-1 (130 mg), fr. BA-2 (129 mg), fr. BA-3 (150 mg), fr. BA-4 (178 mg), fr. BA-5 (123 mg), and fr. BA-6 (175 mg). Fr. BA-4 (100 mg) was again chromatographed on silica gel (SiO_2 10 g, $CHCl_3$: MeOH = 6 : 1) to afford eleuthoside A (**5**, 45 mg, 0.024%). Separation of fr. BA-2 (120 mg) and fr. BA-5 (100 mg) with silica gel (both, SiO_2 10 g, $CHCl_3$: MeOH = 5 : 2) afforded eleuthoside B (**6**, 30 mg, 0.009%) and eleuthoside C (**7**, 74 mg, 0.027%), respectively.

Eleuthoside A (**5**): an amorphous solid, $[\alpha]_D$ $-8.4°$ ($c=0.89$, in MeOH at 23 °C). IR (KBr) cm^{-1}: 3370, 1750, 1276, 1058. UV (MeOH) nm (ε): 215 (15000), 248 (14700), 273 (4,100), 342 (2,500). ^1H-NMR (CD_3OD) δ: 1.73 (3H, d, $J=6.7$ Hz, 3-CH_3), 3.13 (1H, ddd, $J=2.1$, 5.8, 9.4 Hz, 5′-H), 3.43 (1H, dd, $J=9.4$, 9.5 Hz, 4′-H), 3.50 (1H, dd, $J=9.2$, 9.5 Hz, 3′-H), 3.62 (1H, dd, $J=5.8$, 11.6 Hz, 6′-H_A), 3.63 (1H, dd, $J=7.6$, 9.2 Hz, 2′-H), 3.71 (1H, dd, $J=2.1$, 11.6 Hz, 6′-H_B), 4.01 (3H, s, OCH_3), 5.01 (1H, d, $J=7.6$ Hz, 1′-H), 6.07 (1H, q, $J=6.7$ Hz, 3-H), 7.13 (1H, d, $J=7.9$ Hz, 6-H), 7.48 (1H, dd, $J=7.9$, 7.9 Hz, 7-H), 7.61 (1H, d, $J=7.9$ Hz, 8-H), 8.17 (1H, s, 9-H). ^{13}C-NMR: in Table 1. FAB-MS m/z: 429 $(M+Na)^+$. High-resolution FAB-MS m/z: Calcd for $C_{20}H_{22}NaO_9$: 429.1162. Found: 429.1175 $(M+Na)^+$.

Eleuthoside B (**6**): an amorphous solid, $[\alpha]_D$ $-35.7°$ ($c=1.05$, in MeOH at 23 °C). IR (KBr) cm^{-1}: 3568, 1749, 1276, 1055. UV (MeOH) nm (ε): 210 (21000), 248 (18500), 271 (5100), 344 (2800). ^1H-NMR (CD_3OD) δ: 1.71 (3H, d, $J=6.7$Hz, 3-CH_3), 3.15 (1H, dd, $J=7.6$, 7.9 Hz, 2″-H), 3.19 (1H, m, 5″-H), 3.24 (1H, dd, $J=8.6$, 9.5 Hz, 4″-H), 3.30 (1H, m, 3″-H), 3.31 (1H, m, 5′-H), 3.50 (1H, dd, $J=9.2$, 9.2 Hz, 3′-H), 3.58 (1H, m, 4′-H), 3.59 (1H, m, 6″-H_A), 3.63 (1H, dd, $J=7.6$, 9.2 Hz, 2′-H), 3.70 (1H, dd, $J=4.6$, 11.0 Hz, 6′-HA), 3.83 (1H, dd, $J=2.1$, 11.9 Hz, 6″-HB), 4.00 (1H, dd, $J=2.1$, 11.0 Hz, 6′-H_B), 4.00 (3H, s, OCH_3), 4.19 (1H, d, $J=7.6$ Hz, 1″-H), 5.01 (1H, d, $J=7.6$ Hz, 1′-H), 6.15 (1H, q, $J=6.7$ Hz, 3-H), 7.14 (1H, d, $J=7.6$ Hz, 6-H), 7.49 (1H, dd, $J=7.6$, 7.9 Hz, 7-H), 7.63 (1H, d, $J=7.9$ Hz, 8-H), 8.19 (1H, s, 9-H). ^{13}C-NMR: in Table 1. FAB-MS m/z: 591 $(M+Na)^+$. High-resolution FAB-MS m/z: Calcd for $C_{26}H_{32}NaO_{14}$: 591.1699. Found: 591.1706 $(M+Na)^+$.

Eleuthoside C (**7**): an amorphous solid, $[\alpha]_D$ $+40.6°$ ($c=1.05$, in MeOH at 23 °C). IR (KBr) cm^{-1}: 3390, 1166, 1367, 1066. UV (MeOH) nm (ε): 235 (15700), 310 (2900), 323 (2800), 338 (2800). ^1H-NMR (CD_3OD) δ: 1.34 (3H, d, $J=6.1$ Hz, 1-CH_3), 1.62 (3H, d, $J=6.1$ Hz, 3-CH_3), 2.86 (1H, A in ABX, $J_{AB}=16.9$ Hz, $J_{AX}=11.4$ Hz, 4-H_A), 3.06 (1H, B in ABX, $J_{BA}=16.9$ Hz, $J_{BX}=1.1$ Hz, 4-H_B), 3.22 (3H, m, 5′-H, 2″-H, 5″-H), 3.35 (2H, m, 3″-H, 4″-H), 3.43 (1H, dd, $J=9.5$, 9.2 Hz, 3′-H), 3.50 (1H, m, 4′-H), 3.60 (1H, m, 3-H), 3.63 (1H, dd, $J=7.6$, 9.5 Hz, 2′-H), 3.68 (1H, dd, $J=5.2$, 12.2 Hz, 6″-H_A), 3.74 (1H, dd, $J=4.1$, 10.7 Hz, 6′-H_A), 3.83 (1H, dd, $J=2.1$, 12.2 Hz, 6″-H_B), 4.06 (3H, s, OCH_3), 4.06 (1H, dd, $J=2.1$, 10.7 Hz, 6′-H_B), 4.25 (1H, d, $J=7.6$ Hz, 1″-H), 4.67 (1H, d, $J=7.6$ Hz, 1′-H), 5.16 (1H, q, $J=6.1$ Hz, 1-H), 6.87 (1H, d, $J=7.6$ Hz, 8-H), 7.30 (1H, dd, $J=7.6$, 8.6 Hz, 7-H), 8.02 (1H, d, $J=8.6$ Hz, 6-H). ^{13}C-NMR: in Table 2. EI-MS m/z (%): 274 (M^+ −2glc, 59), 259 (M^+ −2glc − CH_3, 100). FAB-MS m/z: 621 $(M+Na)^+$. High-resolution FAB-MS m/z: Calcd for $C_{28}H_{38}NaO_{14}$: 621.2159. Found: 621.2155 $(M+Na).^+$

Acetylation of Eleuthoside A (5) A solution of eleuthoside A (**5**, 15 mg) in pyridine (1.0 ml) was treated with acetic anhydride (0.5 ml) at room temperature for 2 h without stirring, then the reaction mixture was concentrated under reduced pressure to give **5a** (9 mg, 43%).

5a: an amorphous solid, $[\alpha]_D$ $-17.6°$ ($c=0.63$, in $CHCl_3$ at 23 °C). IR (KBr) cm^{-1}: 1759, 1276, 1250, 1225, 1058, 1043. UV (MeOH) nm (ε): 215 (23300), 250 (28500), 313 (2900), 356 (3400). ^1H-NMR ($CDCl_3$) δ: 1.74 (3H, d, $J=6.7$ Hz, 3-CH_3), 2.02, 2.05, 2.07, 2.18 (3H each, all s, $OCOCH_3 \times 4$), 3.56 (1H, ddd, $J=2.4$, 4.9, 10.1 Hz, 5′-H), 3.85 (1H, dd, $J=2.4$, 12.5 Hz, 6′-H_A), 4.04 (3H, s, OCH_3), 4.25 (1H, dd, $J=4.9$, 12.5 Hz, 6′-H_B), 5.18 (1H, dd, $J=9.2$, 10.1 Hz, 4′-H), 5.30 (1H, d, $J=7.6$ Hz, 1′-H), 5.33 (1H, dd, $J=9.2$, 9.8 Hz, 3′-H), 5.43 (1H, dd, $J=7.6$, 9.8 Hz, 2′-H), 5.84 (1H, q, $J=6.7$ Hz, 3-H), 7.04 (1H, d, $J=7.3$ Hz, 6-H), 7.50 (1H, dd, $J=7.3$, 7.9 Hz, 7-H), 7.63 (1H, d, $J=7.9$ Hz, 8-H), 8.24 (1H, s, 9-H). ^{13}C-NMR ($CDCl_3$) δ_C: 19.1 (q, 3-CH_3), 20.4, 20.6, 20.7, 20.9 (all q, $OCO\underline{C}H_3 \times 4$), 55.5 (q, OCH_3), 61.2 (t, 6′-C), 68.6 (d, 4′-C), 71.6 (d, 2′-C), 72.0 (d, 5′-C), 72.6 (d, 3′-C), 78.8 (d, 3-C), 101.5 (d, 1′-C), 108.2 (d, 6-C), 122.4 (s, 12-C), 123.1 (d, 8-C), 123.8 (d, 9-C), 125.0 (d, 7-C), 127.1 (s, 10-C), 137.9 (s, 13-C), 139.7 (s, 11-C), 144.8 (s, 4-C), 155.6 (s, 5-C), 169.3, 169.4 (both s, $O\underline{C}OCH_3 \times 2$), 169.9 (s, 1-C), 170.3 (2C, s, $O\underline{C}OCH_3 \times 2$). FAB-MS m/z: 597 $(M+Na)^+$, 575 $(M+H)^+$. High-resolution FAB-MS m/z: Calcd for $C_{28}H_{30}NaO_{13}$: 597.1585. Found: 597.1555 $(M+Na)^+$.

Methanolysis of Eleuthoside A (5) A solution of eleuthoside A (**5**, 10 mg) in 10% hydrogen chloride in methanol (5 ml) was heated with stirring at 70 °C for 12 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 powder and the precipitate was removed by filtration. The filtrate was evaporated off under reduced pressure to give a product, which was purified by silica gel column chromatography (SiO_2 1 g, benzene : EtOAc = 10 : 1) to afford a saccharic product (3 mg) and an aglycone {4 mg, 66%, $[\alpha]_D$ $+80°$ ($c=0.4$, in $CHCl_3$)} which was identified as eleutherol (**2**) by comparing the physical data, including optical rotation with the reported values.[4]

The saccharic product (2 mg) was treated with the hexamethyldisilazane (HMDS)–trimethylchlorosilane(TMCS)–pyridine(Py) reagent (0.1 ml, Tokyo Chemical Industry Co.). After standing at room temperature for 10 min, the reaction mixture was directly subjected to GC-MS to determine methyl glucopyranoside (t_R 19.28 min, 19.68 min). GC conditions: column, TC-5 (GL Science Inc., 0.25 mm × 30 m); column temperature, 80—300 °C (7 °C/min); carrier gas, He; flow rate, 1.3 ml/min; injection temperature, 300 °C.

A solution of the saccharic product (1 mg) in pyridine (0.1 ml) was treated with l-cysteine methyl ester hydrochloride (1 mg) and the whole was heated at 60 °C for 1 h. After cooling, the reaction mixture was treated with the HMDS–TMCS–Py reagent (0.1 ml) at room temperature for 10 min, and then subjected to GC-MS; the absolute configuration of glucose was determined to be D-form. GC conditions: column, TC-17 (GL Science Inc., 0.25 mm × 30 m); column temperature, 170—270 °C (8 °C/min); carrier gas, He; flow rate, 0.92 ml/min; injection temperature, 270 °C.

Acetylation of Eleuthoside B (6) A solution of eleuthoside B (**6**, 10 mg) in pyridine (1.0 ml) was treated with acetic anhydride (0.5 ml) at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure to give **6a** (8 mg, 53%).

6a: an amorphous solid, $[\alpha]_D$ $-28.1°$ ($c=0.57$, in $CHCl_3$ at 23 °C). IR (KBr) cm^{-1}: 1759, 1275, 1248, 1221, 1055, 1040. UV (MeOH) nm (ε): 215 (21000), 250 (28700), 313 (2600), 356 (2900). ^1H-NMR ($CDCl_3$) δ: 1.74 (3H, s, $OCOCH_3$), 1.77 (3H, d, $J=6.7$ Hz, 3-CH_3), 1.97, 2.02, 2.05, 2.06, 2.07, 2.18 (3H each, all s, $OCOCH_3 \times 6$), 3.48 (1H, m, 5′-H), 3.50 (1H, m, 5″-H), 3.53 (1H, m, 6′-H_A), 3.71 (1H, m, 6′-H_B), 4.04 (3H, s, OCH_3), 4.09 (1H, m, 6″-H_A), 4.18 (1H, m, 6″-H_B), 4.20 (1H, d, $J=7.9$ Hz, 1″-H), 4.77 (1H, dd, $J=7.9$, 9.2 Hz, 2″-H), 4.92 (1H, dd, $J=9.2$, 9.5 Hz, 3″-H), 4.97 (1H, dd, $J=9.5$, 9.5 Hz, 4″-H), 5.10 (1H, dd, $J=9.2$, 9.8 Hz, 4′-H), 5.31 (1H, d, $J=7.6$ Hz, 1′-H), 5.34 (1H, m, 3′-H), 5.38 (1H, dd, $J=7.6$, 9.8 Hz, 2′-H), 5.86 (1H, q, $J=6.7$ Hz, 3-H), 7.02 (1H, d, $J=7.9$ Hz, 6-H), 7.49 (1H, dd, $J=7.9$, 8.4 Hz, 7-H), 7.66 (1H, d, $J=8.4$ Hz, 8-H), 8.33 (1H, s, 9-H). ^{13}C-NMR ($CDCl_3$) δ_C: 19.1 (q, 3-CH_3), 20.1 (q, $OCO\underline{C}H_3$), 20.6 (2C, q, $OCO\underline{C}H_3 \times 2$), 20.7 (3C, q, $OCO\underline{C}H_3 \times 3$), 20.9 (q, $OCO\underline{C}H_3$), 55.5 (q, OCH_3), 61.8 (t, 6″-C), 66.1 (t, 6′-C), 68.3 (d, 4′-C), 68.8 (d, 4″-C), 70.8 (d, 2″-C), 71.6 (d, 2′-C), 72.0 (d, 3′-C), 72.6 (d, 5′-C), 72.7 (d, 3″-C), 73.8 (d, 5″-C), 78.9 (d, 3-C), 100.0 (d, 1″-C), 101.3 (d, 1′-C), 108.2 (d, 6-C), 122.4 (s, 12-C), 125.1 (d, 7-C), 123.4 (d, 8-C), 124.2 (d, 9-C), 127.0 (s, 10-C), 137.9 (s, 13-C), 139.6 (s, 11-C), 144.8 (s, 4-C), 155.6 (s, 5-C), 169.1, 169.2 (both s, $O\underline{C}OCH_3 \times 2$), 169.3 (s, 1-C), 169.5, 169.7, 170.0, 170.4, 170.6 (all s, $O\underline{C}OCH_3 \times 5$). FAB-MS m/z: 885 $(M+Na)^+$, 863 $(M+H)^+$. High-resolution FAB-MS m/z: Calcd for $C_{40}H_{46}NaO_{21}$: 885.2429. Found: 885.2404 $(M+Na)^+$.

Methanolysis of Eleuthoside B (6) A solution of eleuthoside B (**6**, 11 mg) in 10% hydrogen chloride in methanol (5 ml) was heated with stirring at 70 °C for 12 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 powder and the precipitate was removed by filtration. The filtrate was evaporated under reduced pressure to give a product, which was purified by silica gel column chromatography (SiO_2 1 g, benzene : EtOAc = 10 : 1) to afford a saccharic product (3 mg) and an aglycone {4 mg, 65%, $[\alpha]_D$ +81° (c=0.4, in $CHCl_3$)} which was identified as eleutherol (**2**) by comparing the physical data, including optical rotation, with the reported values.[4)]

The saccharic product (2 mg) was treated with the HMDS–TMCS–Py reagent (0.1 ml) at room temperature for 10 min. The reaction mixture was directly subjected to GC-MS to determine methyl glucopyranoside (t_R 19.28 min, 19.68 min). GC conditions: same as those for eleuthoside A (**5**). A solution of the saccharic product (1 mg) in pyridine (0.1 ml) was treated with L-cysteine methyl ester hydrochloride (1 mg) and the whole was heated at 60 °C for 1 h. After cooling, the reaction mixture was treated with the HMDS–TMCS–Py reagent (0.1 ml) at room temperature for 10 min, and then subjected to GC-MS; the absolute configuration of glucose was determined to be D-form. GC conditions: same as those for eleuthoside A (**5**).

Methanolysis of Eleuthoside C (7) A solution of eleuthoside C (**7**, 10 mg) in 10% hydrochloride in methanol (5 ml) was heated at 70 °C for 12 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 powder and the precipitate was removed by filtration. The filtrate was evaporated under reduced pressure to give a product, which was purified by silica gel column chromatography (SiO_2 1 g, benzene : EtOAc = 10 : 1) to afford a saccharic product (3 mg) and an aglycone {3 mg, 69%, $[\alpha]_D$ +345° (c=0.3, in $CHCl_3$)}, which was identified as eleutherin (**3**) by comparing the physical data, including optical rotation, with the reported values.[4)]

The saccharic product (2 mg) was treated with the HMDS–TMCS–Py reagent (0.1 ml) at room temperature for 10 min, then subjected to GC-MS to determine methyl glucopyranoside (t_R 19.28 min, 19.68 min). GC conditions: same as those for **5**. A solution of the saccharic product (1 mg) in pyridine (0.1 ml) was treated with l-cysteine methyl ester hydrochloride (1 mg) and the whole was heated at 60 °C for 1 h, then allowed to cool. The HMDS–TMCS–Py reagent (0.1 ml) was added to the reaction mixture at room temperature. The whole was subjected to GC-MS, and the absolute configuration of glucose was determined as D-form. GC conditions: same as those in the case of **5**.

Oxidation of Eleuthoside C (7) Eleuthoside C (**7**, 9.6 mg) was treated with sodium dichromate dihydrate (20 mg) and 5% aqueous sulfuric acid (2.0 ml) at room temperature for 3 h, and then the reaction was quenched with 2-propanol (5 ml). The reaction mixture was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a product, which was purified by silica gel column chromatography (SiO_2 1 g, hexane : EtOAc = 10 : 1) to afford an oxidized product (2 mg, 45%). The oxidized product was identified as eleutherin (**3**) by comparing the physical data, including optical rotation, with those of an authentic sample.

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