Studies on Nepalese Crude Drugs. XXVI.[1] Chemical Constituents of Panch Aunle, the Roots of *Dactylorhiza hatagirea* D. Don.

Haruhisa Kizu, Ei-ichi Kaneko, and Tsuyoshi Tomimori*

Faculty of Pharmaceutical Sciences, Hokuriku University, Ho-3, Kanagawa-machi, Kanazawa 920–1181, Japan.
Received June 28, 1999; accepted August 16, 1999

From the dried roots of *Dactylorhiza hatagirea*, which are termed Panch Aunle in Nepal, five new compounds called dactylorhins A, B, C, D and E, and two new natural compounds named dactyloses A and B, have been isolated together with twelve known compounds. In addition to these compounds, two kinds of lipid mixture were also obtained.

The structures of the new compounds have been determined by spectroscopic and chemical methods as follows: dactylorhin A, (2*R*)-2-β-D-glucopyranosyloxy-2-(2-methylpropyl)butanedioic acid bis(4-β-D-glucopyranosyloxybenzyl) ester; dactylorhin B, (2*R*,3*S*)-2-β-D-glucopyranosyloxy-3-hydroxy-2-(2-methylpropyl)butanedioic acid bis(4-β-D-glucopyranosyloxybenzyl) ester; dactylorhin C, (2*R*)-2-β-D-glucopyranosyloxy-2-(2-methylpropyl)butanedioic acid; dactylorhin D, (2*R*,3*S*)-2-β-D-glucopyranosyloxy-3-hydroxy-2-(2-methylpropyl)butanedioic acid 1-(4-β-D-glucopyranosyloxybenzyl) ester; dactylorhin E, (2*R*)-2-β-D-glucopyranosyloxy-2-(2-methylpropyl)butanedioic acid 1-(4-β-D-glucopyranosyloxybenzyl) ester; dactylose A, 1-deoxy-1-(4-hydroxyphenyl)-L-sorbose; dactylose B, 1-deoxy-1-(4-hydroxyphenyl)-L-tagatose.

Although a mixture of dactyloses A and B has been already synthesized, this is the first example of their isolation from natural resources.

Dactyloses A and B were suggested to be biosynthesized from L-ascorbic acid and 4-hydroxybenzyl alcohol *via* 2-*C*-(4-hydroxybenzyl)-α-L-xylo-3-ketohexulofuranosono-1,4-lactone.

Key words *Dactylorhiza hatagirea*; 2-(2-methylpropyl)butanedioic acid derivative; 1-deoxy-1-(4-hydroxyphenyl)-L-sorbose; 1-deoxy-1-(4-hydroxyphenyl)-L-tagatose; Orchidaceae; Panch Aunle

Dactylorhiza hatagirea is a plant of the Orchidaceae family,[2] growing at 2800—4000 m in the area from Pakistan to southeast Tibet.[3] The dried tubers of this plant are called Panch Aunle in Nepal, being very often used as a tonic.[4] There have been few studies of the chemical constituents or pharmaceutical efficacy of this plant.[5] In the course of our studies on Nepalese crude drugs, we have investigated the chemical constituents of Panch Aunle.

An aqueous suspension of a hot MeOH extract of the material was extracted successively with ether, ethyl acetate and 1-butanol. Repeated chromatographic separation of the 1-butanol-soluble fraction led to the isolation of eleven compounds (**1**—**11**), including seven new ones named dactylose A (**1**), dactylose B (**2**), and dactylorhins A (**6**), B (**7**), C (**8**), D (**10**) and E (**11**), as described in the experimental section.

Compounds **3**, **4**, and **5** have been identified as 4-(β-D-glucopyranosyloxy) benzyl alcohol,[6] militarine[7] and loroglossin,[7,8] respectively, by comparison of the $[\alpha]_D$ values and NMR spectral data with those described in the literature. Compound **9** has been identified as (2*R*)-2-hydroxy-2-(2-methylpropyl)butanedioic acid, which has been obtained by enzymatic hydrolysis of militarine (**4**).

The ether- and ethyl acetate-soluble portions were combined and separated by various chromatographic methods (see experimental section) to give eight known compounds (**12**—**19**) and two lipid mixtures (substances A and B). Identification of known compounds are described in the experimental section.

Substance A was suggested to be a mixture of the 6′-*O*-acylates of **18** from the NMR spectra and alkaline-methanolysis followed by methylation afforded **18** and a mixture of methyl linoleate and methyl palmitate (molar ratios 1 : 1) as determined by GLC. Consequently, substance A proved to be a 1 : 1 molar mixture of 6′-*O*-linoleate and 6′-*O*-palmitate of **18**.

Substance B was suggested to be a mixture of the 1-*O*-α-galactopyranosyl-(1→6)-β-galactopyranoside of 2,3-diacylglycerols from comparison of its NMR spectral data with those of digalactosyl diacylglycerols isolated from an axenically cultured cyanobacterium, *Phormidium tenue*.[9] The structure of the glycosyl part was confirmed by two-dimensional MNR spectral data [^{1}H–^{1}H shift correlation spectroscopy (COSY), ^{1}H–^{13}C COSY and ^{1}H detected heteronuclear multiple bond connectivity (HMBC)]. The presence of a D-galactose moiety was confirmed by HPLC analysis of the acid-hydrolysate. Alkaline-methanolysis of substance B followed by methylation afforded a mixture of methyl palmitate, methyl oleate, methyl linoleate and methyl linolenate in a ratio of 6 : 2 : 18 : 7, respectively, as determined by GLC. Consequently, substance B is a mixture of several 1-*O*-α-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-2,3-diacylglycerols. No further separation has been performed.

Dactylose A (**1**) was suggested to be a monosaccharide derivative from its ^{13}C-NMR spectrum (in pyridine-d_5), which showed twelve carbon signals classified into one acetal carbon (δ 99.7, quaternary C), one methylene (δ 44.8), one oxygen-bonded methylene (δ 64.0), three oxygen-bonded methines (δ 71.8, 74.2, 76.7), and six sp^2 carbons [δ 115.6 (CH×2), 128.2 (quaternary C), 132.7 (CH×2), 157.4 (quaternary C)]. The presence of a 4-hydroxyphenyl group was deduced from the ^{13}C- and ^{1}H-NMR spectral data (Tables 1, 2). The molecular formula was determined to be $C_{12}H_{16}O_6$ from the FAB-MS and ^{13}C-NMR spectral data. The $^1J_{C-H}$ connectivity was clarified based on the ^{1}H–^{13}C COSY spectral data. The ^{1}H–^{1}H spin network from H-3 to H_2-6 was solved by analyses of the coupling pattern of each proton sig-

* To whom correspondence should be addressed.

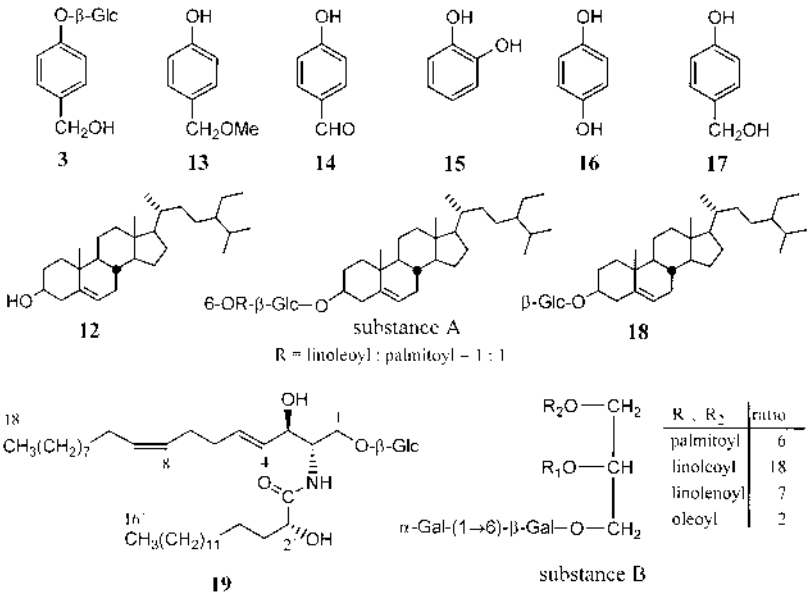

Chart 1

nal in pyridine-d_5 and D_2O (Tables 2, 3). Then, the remaining uncoupled-methylene was presumed to bond to both an acetal carbon and a 4-hydroxyphenyl group from its proton and carbon chemical shifts (Tables 1—3). From these data and by comparison with L-sorbose (α-pyranose form, predominant), **1** was deduced to be 1-deoxy-1-(4-hydroxyphenyl)-α-sorbopyranose. Furthermore, this was confirmed by difference nuclear Overhauser effect (DIFNOE) experiments, in which the following NOEs were observed: between H-4 and H_B-6 (axial); between H_A-1 and H-3; between H_2-1 and H-2′ (H-6′). The absolute configuration of **1** was inferred to be the L form by comparing the $[M]_D$ value of **1** ($-60.9°$ in H_2O) with that of L-sorbose ($-74.2°$ in H_2O) and this was confirmed by comparison with a synthetic sample as described later.

Dactylose B (**2**) was shown to exist as an equilibrium mixture of **2a** and **2b** (**2a** : **2b** = 10 : 1 in D_2O; 2 : 1 in pyridine-d_5) by its ^{13}C-NMR spectrum, which showed signals ascribable to a 4-hydroxyphenyl group and a deoxyhexose moiety, including a quaternary acetal carbon. From these findings and FAB-MS data, **2** was suggested to be a hexulose derivative and a stereoisomer of **1**.

Firstly, the structure of **2a** was examined based on the NMR spectral data in D_2O. The dominant component **2a** was suggested to be a pyranose from the ^{13}C-NMR spectrum in which an acetal carbon was observed at 102.2 ppm and no signal appeared in the relatively low-field area (around 80 ppm).[10] The ^{1}H–^{1}H spin network was solved from the coupling pattern of each proton (Table 3), and the configurations of H-3 (d, J = 3.5 Hz), H-4 (dd, J = 9.5, 3.5 Hz) and H-5 (ddd, J = 10.5, 9.5, 6 Hz) were deduced to be equatorial, axial and axial, respectively. Thus, **2a** was assumed to be 1-deoxy-1-(4-hydroxyphenyl)-tagatopyranose. This was supported by comparison of its ^{13}C-NMR spectral data with that of D-tagatopyranose. The configuration of the 4-hydroxybenzyl group at the anomeric position was determined to be equatorial by DIFNOE experiments, in which no NOE was observed between H_B-6 (δ 3.61, axial) and H_2-1 nor between H-4 and H_2-1.

Therefore, **2a** was assigned to be 1-deoxy-1-(4-hydroxyphenyl)-α-tagatopyranose.

The structure of the minor component (**2b**) in the equilibrium mixture was examined based on the NMR spectral data in pyridine-d_5. The ^{1}H–^{1}H spin network in **2b** as well as in **2a** was clarified from the ^{1}H–^{1}H COSY spectral data of **2** as shown in Table 2, and the ^{13}C resonances in **2a** and **2b** were assigned based on the ^{1}H–^{13}C COSY spectral data of **2**. The ^{13}C chemical shifts of C-4, C-5 and C-6 in **2b** showed that **2b** was a pyranose-type compound. The anomeric configuration and the ring conformation were determined as the β and C1 form,[11] respectively, by NOE experiments: irradiation of the H_2-1 signals enhanced the H-3 signal, but no enhancement of the H_2-6 signals was observed.

Consequently, **2b** is 1-deoxy-1-(4-hydroxyphenyl)-β-tagatopyranose.

Recently, Preobrazhenskaya *et al*. have reported the synthesis of a mixture of 1-deoxy-1-(4-hydroxyphenyl)-α-L-sorbose and 1-deoxy-1-(4-hydroxyphenyl)-α-L-tagatose.[12] In order to confirm the absolute configurations of **1** and **2**, these were synthesized by their method. The resulting mixture was separated by HPLC, giving a major (*syn*-**1**) and a minor (*syn*-**2**) product. The NMR spectral data and optical rotation of *syn*-**1** ($[\alpha]_D$ $-21.3°$) and *syn*-**2** ($[\alpha]_D$ $+14.0°$) were consistent with those of **1** ($[\alpha]_D$ $-23.8°$) and **2** ($[\alpha]_D$ $+15.0°$), respectively.

From these results, **1** and **2** were concluded to be 1-deoxy-1-(4-hydroxyphenyl)-L-sorbose and 1-deoxy-1-(4-hydroxyphenyl)-L-tagatose, respectively.

Dactylorhin C (**8**) was suggested to be a glycoside of 2-hydroxy-2-(2-methylpropyl)butanedioic acid from its NMR spectra. On enzymatic hydrolysis using cellulase, it afforded (2*R*)-2-hydroxy-2-(2-methylpropyl)butanedioic acid (**9**) and D-glucose. The FAB-MS spectrum of **8** exhibited quasi-molecular ion peaks at *m/z* 353 $[(M+H)^+]$ and 375 $[(M+Na)^+]$ and the ^{13}C-NMR spectrum showed signals due to one mole of a glucosyl moiety, confirming that **8** was a monoglucoside of **9**. The connecting position of the glucose moiety was deduced to be the C_2-oxygen by comparison of the ^{13}C-NMR spectrum with that of **9**, *i.e.* a glycosylation shift (+6.1

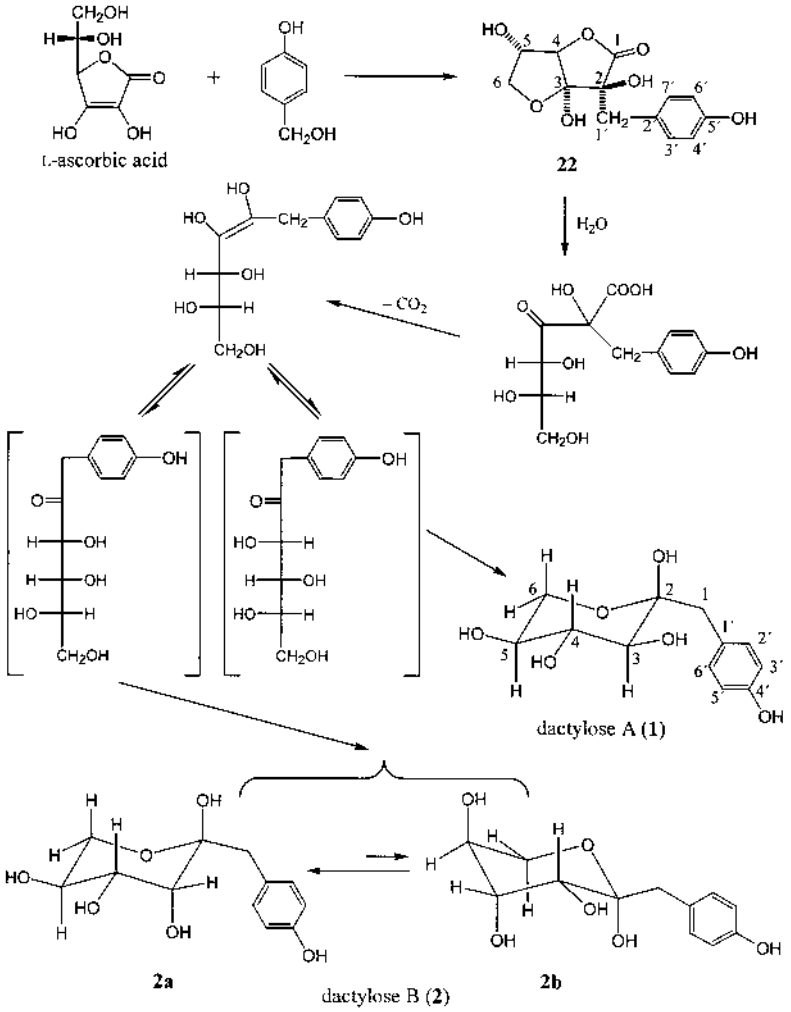

Chart 2

Table 1. ^{13}C-NMR Spectral Data for **1**, **2a**, **2b**, L-Sorbose and D-Tagatose[a)]

C-No.	**1**[b)]	α-Sor[b,c)]	**2a**[b)]	α-Tag[b,d)]	β-Tag[b,e)]	**1**[f)]	α-Sor[c,f)]	**2a**[f)]	α-Tag[d,f)]	**2b**[f)]	β-Tag[e,f)]
C-1	45.1	66.3	44.5	66.7	66.3	44.8	66.4	44.0	66.8	43.9	66.6
C-2	101.4	100.6	102.2	101.0	101.1	99.7	99.2	100.5	99.4	100.5	100.4
C-3	75.5	73.2	73.4	72.6	66.5	74.2	73.3	72.6	73.0	67.3	65.8
C-4	76.9	76.7	74.0	73.7	73.7	76.7	76.6	73.5	73.6	74.2	74.0
C-5	72.2	72.3	69.2	69.2	72.1	71.8	71.8	68.5	68.6	71.6	71.5
C-6	64.6	64.6	65.2	65.1	63.0	64.0	63.9	64.7	64.6	61.8	61.6
C-1′	129.9	—	130.0	—	—	128.2	—	128.4	—	128.0	—
C-2′, 6′	134.9	—	134.9	—	—	132.7	—	132.8	—	132.8	—
C-3′, 5′	117.9	—	118.0	—	—	115.6	—	115.7	—	115.6	—
C-4′	157.2	—	157.2	—	—	157.4	—	157.4	—	157.3	—

a) Data for L-sorbose and D-tagatose were obtained in our laboratory. *b*) Measured in D_2O. *c*) α-Sor, α-L-sorbopyranose. *d*) α-Tag, α-D-tagatopyranose. *e*) β-Tag, β-D-tagatopyranose. *f*) Measured in pyridine-d_5.

ppm)[13)] was observed at the C-2 position in **8**. The anomeric configuration was regarded as β from the *J* value (8 Hz) of the glucosyl H-1 and carbon signal pattern.

From this data, the structure of dactylorhin C was concluded to be (2*R*)-2-β-D-glucopyranosyloxy-2-(2-methylpropyl)butanedioic acid.

Dactylorhin A (**6**) was suggested to be related to **4** (militarine) based on the NMR data (Tables 4, 5). On enzymatic hydrolysis using almond emulsin, **6** afforded **8**, 4-hydroxybenzyl alcohol (**17**) and D-glucose. The FAB-MS of **6** exhibited a quasi-molecular ion peak at *m/z* 911 [$(M+Na)^+$] and the ^{13}C-NMR spectrum of **6** showed three anomeric carbon signals (δ 100.1, 102.0, 102.1), suggesting that **6** was a monoglucoside of **4**. The three anomeric configurations were deduced to be all β from their J_{H1-H2} values (Table 5). By comparison of the ^{13}C-NMR spectrum of **6** with that of **4**, **6** was suggested to be militarine 2-*O*-β-D-glucopyranoside because the C-2 in **6** was observed 4.4 ppm downfield from that of **4**. This was confirmed from the ^{1}H–^{13}C long-range COSY spectrum in which a long-range correlation was observed be-

Table 2. ^{1}H-NMR Spectral Data for **1**, **2a**, L-Sorbose and D-Tagatose in Pyridine-d_5[a)]

	1	α-Sor[b)]	**2a**	α-Tag[c)]	**2b**	β-Tag[d)]
H_A-1	3.72 d (13)	4.40 d (11.5)	3.44 d (13)	4.46 d (11.5)	3.58 d (13.5)	4.47 d (11.5)
H_B-1	3.49 d (13)	4.26 d (11.5)	3.88 d (13)	4.28 d (11.5)	3.60 d (13.5)	4.10 d (11.5)
H-3	4.01 d (9)	4.38 d (9)	4.44 d (3)	*ca.* 4.75	4.60 d (3)	4.99 d (3.5)
H-4	4.63 dd (9, 9)	4.65 dd (9, 9)	4.76 dd (9.5, 3)	*ca.* 4.75	4.70 m	*ca.* 4.78
H-5	4.08 ddd (10.5, 9, 6)	4.25 ddd (10.5, 9, 5.5)	4.81 ddd (10.5, 9.5, 5.5)	*ca.* 4.73	4.38 br d (6)	4.43 br s
H_A-6	4.14 dd (10.5, 6)	4.16 dd (10.5, 5.5)	4.28 dd (10.5, 5.5)	4.20 m	4.02 br d (12.5)	4.02 ddd (12.5, 1, 1)
H_B-6	4.47 dd (10.5, 10.5)	4.47 dd (10.5, 10.5)	4.47 dd (10.5, 10.5)	4.20 m	4.71 dd (12.5, 2)	*ca.* 4.83
H-2′, 6′	7.64 br d (8.5)[e)]	—	7.76 br d (8.5)[e)]	—	7.69 br d (8.5)[e)]	—
H-3′, 5′	7.10 br d (8.5)[e)]	—	7.13 br d (8.5)[e)]	—	7.11 br d (8.5)[e)]	—

a) Coupling constants (*J*) in Hz are given in parentheses. *b*) α-Sor, α-L-sorbopyranose. *c*) α-Tag, α-D-tagatopyranose. *d*) β-Tag, β-D-tagatopyranose. *e*) Deformed signals (AA′ BB′ type) because of virtual coupling.

Table 3. ^{1}H-NMR Spectral Data for **1**, **2a**, L-Sorbose and D-Tagatose in D_2O[a)]

	1	α-Sor[b)]	**2a**	α-Tag[c)]
H_A-1	3.03 d (14)	3.69 d (11.5)	3.07 d (14)	3.72 d (12)
H_B-1	2.98 d (14)	3.52 d (11.5)	2.84 d (14)	3.52 d (12)
H-3	3.27 d (9.5)	3.50 d (9.5)	3.52 d (3.5)	3.90 m
H-4	3.67 dd (9.5, 9.5)	3.66 m	3.77 dd (9.5, 3.5)	3.85 m
H-5	3.47 ddd (10, 9.5, 6)	3.61 m	3.88 ddd (10.5, 9.5, 6)	3.85 m
H_A-6	3.65 dd (11, 6)	3.72 m	3.74 dd (11, 6)	3.57—3.78 m
H_B-6	3.59 dd (11, 10)	3.66 m	3.61 dd (11, 10.5)	3.57—3.78 m
H-2′, 6′	7.22 m	—	7.23 m	—
H-3′, 5′	6.87 m	—	6.87 m	—

a) Coupling constants (*J*) in Hz are given in parentheses. *b*) α-Sor, α-L-sorbopyranose. *c*) α-Tag, α-D-tagatopyranose.

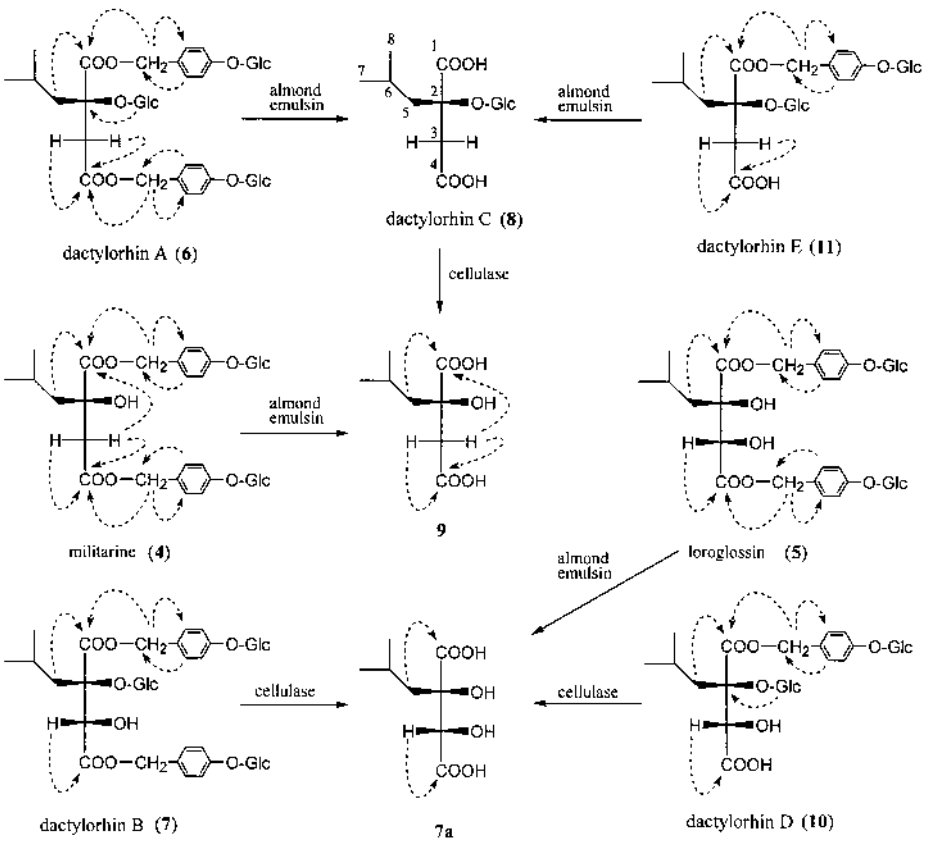

Fig. 1. Structures of Dactylorhins A—E,[a)] Enzymatic Hydrolysis[b)] and ^{1}H–^{13}C Long-range Correlations[c)]

a) Glc: β-D-glucopyranosyl. *b*) All compounds except for **8** afforded 4-hydroxybenzylalcohol (**17**) and D-glucose together with the respective 1,4-butanedioic acid derivatives. *c*) Long-range correlations were determined by ^{1}H–^{13}C long-range COSY (in the cases of **4**—**7**, **11**), HMBC (**10**) and the long-range selective proton decoupling method (**7a**, **9**), and are shown by dotted arrows.

tween C-2 and H-1 of the additional glucose moiety (Fig. 1).

Dactylorhin E (**11**), on enzymatic hydrolysis using emulsin, afforded the same products as for **6** and was suggested to be a mono(4-β-D-glucopyranosyloxybenzyl) ester of **8** from the FAB-MS and NMR spectral data. Then, **11** proved to be a 1-(4-β-D-glucopyranosyloxybenzyl) ester of **8** from the ^{1}H–^{13}C long-range COSY spectra which showed long-range correlations between C-1 and both H_2-5 and benzyl protons (Fig. 1).

Dactylorhin B (**7**) was suggested to be related to **5** (loroglossin) based on the NMR spectral data (Tables 4, 5). On enzymatic hydrolysis using cellulase, **7** gave **7a**, **17** and D-glucose. Compound **7a** was identified as (2*R*,3*S*)-2,3-dihydroxy-2-(2-methylpropyl)butanedioic acid which was obtained on enzymatic hydrolysis of **5**. The FAB-MS of **7** exhibited a quasi-molecular ion peak at *m/z* 927 [$(M+Na)^+$] and the ^{13}C-NMR spectrum showed three anomeric carbon signals. From these data, **7** was deduced to be loroglossin monoglucoside. On comparison of the ^{13}C-NMR spectral data of **7** with that of **5**, the signal due to C-2 was observed 4.6 ppm downfield. Consequently, the additional glucose proved to be linked to the C_2-oxygen, and then its anomeric configuration was determined to be β from the $J_{H1–H2}$ value (7.5 Hz).

Based on these results, **7** was concluded to be loroglossin 2-*O*-β-D-glucopyranoside.

Dactylorhin D (**10**), on enzymatic hydrolysis using cellulase, gave the same products as for **7** and was suggested to be a mono(4-β-D-glucopyranosyloxybenzyl) ester mono-β-D-glucopyranoside of **7a** from the FAB-MS and NMR spectral data. The connecting positions of a glucose residue and a 4-*O*-β-D-glucopyranosyloxybenzyl group were determined to be the C_2-oxygen and C_1-oxygen of **7a**, respectively, based on the HMBC spectral data as shown in Fig. 1.

Therefore, the structure of **10** is (2*R*,3*S*)-2-β-D-glucopyranosyloxy-3-hydroxy-2-(2-methylpropyl)butanedioic acid 1-(4-β-D-glucopyranosyloxybenzyl) ester.

As described above, two new hexulose derivatives and five new 2-(2-methylpropyl)butanedioic acid related compounds were isolated from the dried roots of *Dactylorhiza hatagirea*, and their structures were characterized.

1-Deoxy-1-substituted hexuloses like **1** and **2** are new types of natural derivatives of sugar, while their mixture has been synthesized. Some natural products, namely, 2-*C*-aryl-

Table 4. ^{13}C-NMR Spectral Data for **4**—**8**, **10** and **11**

C-No.	**4**[a]	**5**[a]	**6**[a]	**7**[a]	**8**[b]	**10**[b]	**11**[b]
C-1	175.6	174.8	173.5	172.2	180.4	178.1	178.2
C-2	76.1	80.9	80.5	85.5	84.6	82.9	84.8
C-3	45.8	77.9	42.8	76.0	45.3	80.2	48.5
C-4	170.6	172.4	171.0	171.6	177.1	179.3	180.0
C-5	48.5	44.9	47.8	45.0	50.0	46.8	49.5
C-6	24.3	24.5	24.02	23.8	26.3	26.4	25.9
C-7	23.7	23.8	23.95	23.4	25.8	25.6	25.6
C-8	24.6	24.7	24.5	24.9	26.6	26.1	26.5
1-*O*-(4-β-D-Glucopyranosyloxy)benzyl moiety[c]							
C-1	67.0	67.1	67.3	67.16	—	70.1	70.0
C-2	129.9	129.6	129.5	129.29	—	132.8	132.7
C-3,7	130.5	130.5	130.6	130.5	—	133.7	133.5
C-4,6	116.9	116.9	116.9	116.9	—	119.4	119.5
C-5	158.6	158.6	158.7[d]	158.6	—	159.6	159.6
Glc-1	101.96	102.1	102.0	102.0	—	102.9	103.0
Glc-2	74.8	74.9	74.9	74.8	—	75.8	75.8
Glc-3	78.4	78.4	78.4	78.39	—	78.4	78.5
Glc-4	71.1	71.2	71.2	71.1	—	72.3	72.3
Glc-5	78.8	78.8	78.8	78.8	—	79.0	79.0
Glc-6	62.2	62.3	62.3	62.2	—	63.4	63.4
4-*O*-(4-β-D-Glucopyranosyloxy)benzyl moiety[c]							
C-1	66.1	66.7	66.6	67.22	—	—	—
C-2	129.7	129.6	129.7	129.25	—	—	—
C-3,7	130.2	130.6	130.6	130.9	—	—	—
C-4,6	116.8	116.8	116.9	116.9	—	—	—
C-5	158.4	158.6	158.6[d]	158.7	—	—	—
Glc-1	102.02	102.1	102.1	102.1	—	—	—
Glc-2	74.8	74.9	74.9	74.8	—	—	—
Glc-3	78.4	78.4	78.4	78.36	—	—	—
Glc-4	71.1	71.2	71.1	71.1	—	—	—
Glc-5	78.8	78.8	78.8	78.7	—	—	—
Glc-6	62.2	62.3	62.3	62.2	—	—	—
3-*O*-β-D-Glucopyranosyl moiety							
Glc-1	—	—	100.1	99.2	101.1	—	100.7
Glc-2	—	—	75.7	75.6	76.5	—	76.5
Glc-3	—	—	78.7	78.5	79.1[d]	—	78.4
Glc-4	—	—	71.1	70.6	71.9	—	72.1
Glc-5	—	—	78.1	78.1	78.6[d]	—	78.8
Glc-6	—	—	62.5	61.9	63.1	—	63.4

a) Measured in pyridine-d_5. *b*) Measured in D_2O. *c*) Assignment of the glucopyranosyl moiety has not been confirmed. *d*) Assignments may be interchangeable.

Table 5. ^{1}H-NMR Spectral Data for **4**—**7** in Pyridine-d_5[a]

	4	**5**	**6**	**7**
H-3 (H_A-3)	3.01 d (15.5)	5.01 s	3.27 d (16)	5.01 s
(H_B-3)	3.24 d (15.5)	—	3.46 d (16)	—
H_A-5	1.86 m	2.12 m	1.88 m	2.06 dd (14.5, 7)
H_B-5	1.86 m	2.50 m	1.88 m	2.48 dd (14.5, 5)
H-6	2.05 m	2.09 m	1.99 m	2.08 m
H_3-7	0.90 d (6.5)	0.91 d (6.5)	0.83 d (6.5)	0.81 d (6.5)
H_3-8	0.97 d (6.5)	1.03 d (6.5)	0.90 d (6.5)	0.99 d (6.5)
1-*O*-(4-β-D-Glucopyranosyloxy)benzyl moiety[b]				
H_A-1	5.29 s	5.24 d (12)	5.33 d (12)	5.17 d (12)
H_B-1	5.29 s	5.18 d (12)	5.27 d (12)	5.29 d (12)
H-3,7	7.41 d (9)	7.39 d (9)	7.42 d (9)	7.42 d (9)
H-4,6	7.36 d (9)	7.37 d (9)	7.33 d (9)	7.37 d (9)
Glc-1	5.60 d (7.5)	5.63 d (7.5)	5.62 d (7.5)	5.61 d (7.5)
Glc-2	4.29 m	4.29 m	4.28 m	4.29 m
Glc-3	4.36 m	4.36 m	4.35 m	4.36 m
Glc-4	4.32 m	4.32 m	4.31 m	4.33 m
Glc-5	4.11 m	4.11 m	4.10 m	4.11 m
Glc-6	4.52 m	4.52 m	4.51 m	4.52 m
	4.38 m	4.38 m	4.37 m	4.38 m
4-*O*-(4-β-D-Glucopyranosyloxy)benzyl moiety[b]				
H_A-1	5.10 d (12.5)	5.15 d (12)	5.24 d (12)	5.14 d (12)
H_B-1	5.06 d (12.5)	5.03 d (12)	5.15 d (12)	5.27 d (12)
H-3,7	7.31 s	7.32 d (9)	7.39 d (9)	7.39 d (8.5)
H-4,6	7.31 s	7.30 d (9)	7.35 d (9)	7.31 d (8.5)
Glc-1	5.61 d (7.5)	5.59 d (7.5)	5.60 d (7.5)	5.62 d (7.5)
Glc-2	4.29 m	4.29 m	4.28 m	4.29 m
Glc-3	4.36 m	4.36 m	4.35 m	4.36 m
Glc-4	4.32 m	4.32 m	4.31 m	4.33 m
Glc-5	4.11 m	4.11 m	4.10 m	4.11 m
Glc-6	4.52 m	4.52 m	4.51 m	4.52 m
	4.38 m	4.38 m	4.37 m	4.38 m
3-*O*-β-D-Glucopyranosyl moiety				
Glc-1	—	—	5.58 d (7.5)	5.56 d (7.5)
Glc-2	—	—	4.01 dd (9, 7.5)	4.07 dd (9, 7.5)
Glc-3	—	—	4.22 m	4.19 dd (9, 9)
Glc-4	—	—	4.27 m	4.33 m
Glc-5	—	—	3.76 m	3.78 m
Glc-6	—	—	4.37 m	4.43 dd (12, 2.5)
			4.31 m	4.38 m

a) Coupling constants (*J*) in Hz are given in parentheses. *b*) Assignment of the glucopyranosyl moiety has not been confirmed.

Table 6. ^{1}H-NMR Spectral Data for **8**, **10** and **11** in D_2O[a)]

	8	10	11
H-3 (H_A-3)	3.12 d (17.5)	4.17 br s	2.82 d (17)
(H_B-3)	3.02 d (17.5)	—	2.94 d (17)
H_A-5	1.72 m	1.76 dd (15, 6)	1.67—1.81
H_B-5	1.82 m	1.77 dd (15, 7)	1.67—1.81
H-6	1.82 m	1.45 m	1.67—1.81
H_3-7	0.88 d (6.5)	0.68 d (6.5)	0.74 d (6.5)
H_3-8	0.92 d (6.5)	0.87 d (6.5)	0.86 d (6.5)
1-*O*-(4-β-D-Glucopyranosyloxy)benzyl moiety			
H_A-1	—	5.25 d (12)	5.23 d (12)
H_B-1	—	5.15 d (12)	5.13 d (12)
H-3,7	—	7.48 d (9)	7.46 d (8.5)
H-4,6	—	7.15 d (9)	7.16 d (8.5)
Glc-1	—	5.14 d (7.5)	5.15 d (7.5)
Glc-2	—	3.58 dd (9, 7.5)	3.58 dd (9, 7.5)
Glc-3	—	3.62 dd (9, 9)	3.62 dd (9, 9)
Glc-4	—	3.51 dd (10, 9)	3.51 dd (10, 9)
Glc-5	—	3.64 ddd (10, 6, 2.5)	3.64 m
Glc-6	—	3.94 dd (12.5, 2)	3.93 dd (12.5, 2)
	—	3.76 dd (12.5, 6)	3.76 dd (12.5, 6)
3-*O*-β-D-Glucopyranosyl moiety			
Glc-1	4.88 d (8)	—	4.94 d (8)
Glc-2	3.30 dd (9, 8)	—	3.30 dd (9, 8)
Glc-3	3.49 dd (9, 9)	—	3.49 dd (9, 9)
Glc-4	3.44 dd (9, 9)	—	3.40 dd (10, 9)
Glc-5	3.28 ddd (9, 4, 2.5)	—	3.30 m
Glc-6	3.81 dd (12.5, 2.5)	—	3.81 dd (12.5, 2)
	3.74 dd (12.5, 4)	—	3.68 dd (12.5, 5)

a) Coupling constants (*J*) in Hz are given in parentheses.

α-L-*xylo*-3-ketohexulofuranosono-1,4-lactones, are known to be related to such hexuloses.[14] Compounds **1** and **2** correspond to the products that 2-*C*-aryl-α-L-*xylo*-3-ketohexulofuranosono-1,4-lactones have been converted into during further biosynthesis. A mixture of **1** and **2** could be formed under very mild conditions and the molar ratios of the resulting *syn*-**1** to *syn*-**2** (4 : 1) were consistent with those of natural **1** to **2**.

From these facts, **1** and **2** are suggested to be biosynthesized from L-ascorbic acid and 4-hydroxybenzyl alcohol *via* 2-*C*-(4-hydroxybenzyl)-α-L-*xylo*-3-ketohexulofuranosono-1,4-lactone (**22**) (Chart 2).

The 2-alkylated butanedioic acid bis(4-β-D-glucopyranosyloxybenzyl) esters are characteristic constituents of Orchidaceae plants, however, this is the first report of the isolation of 2-*O*-glycoside-type compounds like **6**, **7**, **8**, **10** and **11**.

Experimental

General Procedures All melting points were determined on a Yanagimoto micromelting point apparatus and were uncorrected. NMR spectra were recorded on a JEOL GSX-400 spectrometer (^{1}H-NMR at 400 MHz and ^{13}C-NMR at 100 MHz) using 3-(trimethylsilyl)propionic acid sodium salt-d_4 (in D_2O) or a residual signal of the solvent [in pyridine-d_5, δ_C 123.5, δ_H 7.20 (β-CH)] as internal standard, and the chemical shifts are given in δ (ppm). MS were recorded on a JEOL JMS-D-300 or a JMS-SX-102A mass spectrometer. UV spectra were recorded in MeOH in a Shimadzu dual-wavelength/double beam recording spectrophotometer and peaks are given in λ_{max} nm (log ε). IR spectra were recorded in KBr disks on a Hitachi 270-30 infrared spectrophotometer and the data are given in cm^{-1}. Optical rotations were recorded in MeOH on a JASCO DIP-370 digital polarimeter. HPLC was performed on a Shimadzu LC-6AD pump system with a Shimadzu SPD-6AV UV detector. Preparative HPLC, unless otherwise stated, was performed on a YMC-Packed column, D-ODS-5 (20 i.d.×250 mm). HPLC conditions for sugar analysis: column, YMC-Pack Polyamine II (4.6 i.d.×250 mm); solvent, $CH_3CN : H_2O : H_3PO_4 = 86 : 14 : 0.05$; detector, Shimadzu RID-2A refractive index detector and JASCO OR-990 optical rotation detector; temperature, 50 °C. GLC was performed on a Shimadzu GC-6AM instrument with a flame-ionization detector, using a glass column (4 mm i.d.×2 m) packed with 15% 1,4-butanediol succinate on Chromosorb W (100—120 mesh); column temperature, 200 °C. For TLC, pre-coated plates of Silica gel 60F_{254}, RP-18 and HP Silica gel 60F_{254} (Merck) were used. For column chromatography, Wako gel C-200 (Wako Pure Chemical Ind.) and octadecyl silica gel (ODS) (Cosmosil 140 C_{18}-OPN, Nacalai Tesque) were used.

Isolation Panch Aunle (the dried roots of *Dactylorhiza hatagirea*, 4.5 kg) was purchased at the market in Kathmandu, Nepal, in 1991. The botanical identification was made by Dr. N. P. Manandhar, National Herbarium and Plant Laboratories, Godawari, Nepal. A voucher specimen has been deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Hokuriku University, Kanazawa, Japan. The materials were ground and extracted four times with MeOH under reflux. The MeOH extract was concentrated under reduced pressure and the resulting residue was suspended in water and then extracted successively with ether, AcOEt and 1-BuOH.

The 1-BuOH-soluble fraction (10 g) was chromatographed on silica gel (1 kg) eluting with a gradient of $CHCl_3$–MeOH (MeOH, 0→8%) and then with $CHCl_3$–MeOH–H_2O (25 : 3 : 0.3→25 : 6 : 0.7→25 : 8 : 1.2→25 : 11 : 2→25 : 14 : 3) to give seven fractions (frs. 1—7). Fraction 1 (500 mg) was chromatographed on an ODS (50 g) column and eluted with a gradient of MeOH–H_2O (MeOH, 0→10%) to give **1** (40 mg) and crude **2**. Crude **2** was submitted to HPLC [column, Purospher RP-18 (Merck); solvent, MeOH : H_2O=1 : 9] to give pure **2** (10 mg). Fraction 2 (500 mg) was chromatographed on an ODS (50 g) column and eluted with 5% MeOH to give crude **3**, which was recrystallized from isopropanol to give pure **3** (90 mg). Fraction 3 (1.2 g) was chromatographed on a silica gel column (120 g) and eluted with 1-BuOH–AcOEt–H_2O (4 : 1 : 1) to give fructose (26 mg) and a mixture. This mixture was submitted to ODS (50 g) column chromatography and eluted with a gradient of MeOH–H_2O (MeOH, 0→30%) to give fructose (13 mg) and **4** (280 mg). Fraction 4 (1.8 g) was chromatographed on an ODS (200 g) column and eluted with a gradient of MeOH–H_2O (MeOH, 0→30%) to give fructose (12 mg) and **5** (320 mg). Fraction 5 (2.2 g) was chromatographed on an ODS (200 g) column and eluted with a gradient of MeOH–H_2O (MeOH, 0→30%) to give crude **6**, which was further purified on a Toyopearl HW-40 (Tosoh Co.) column (solvent, H_2O) to give pure **6** (650 mg). Fraction 6 (250 mg) was chromatographed on an ODS (30 g) column and eluted with a gradient of MeOH–H_2O (MeOH, 0→30%) to give **7** (70 mg). Fraction 7 (350 mg) was recrystallized from MeOH to give sucrose (30 mg). The mother liquor of the crystallization was chromatographed on an ODS (30 g) column and eluted with a gradient of MeOH–H_2O (MeOH, 0→30%) to give two fractions (frs. 7-1, 7-2). Fraction 7-1 (130 mg) was partitioned between 0.1 N HCl aq. and a mixed solvent of hexane and ether (3 : 1). The aqueous layer was neutralized with 0.1 N NaOH and then concentrated. The residue was purified on an ODS (10 g) column (solvent, H_2O) and then on an ion-exchange resin (Organo Amberlite IR-120B) column (solvent, H_2O) to give **8** (25 mg). The organic layer was concentrated and the residue was purified in the same way as for **8** to give **9** (15 mg). Fraction 7-2 (110 mg) was submitted to preparative HPLC (solvent, 25% MeOH) to give two compounds which were purified on an Amberlite IR-120B column (solvent, H_2O) to give **10** (6 mg) and **11** (30 mg).

The ether-soluble and AcOEt-soluble fractions (12 g) were combined and submitted to silica gel (1.2 kg) column chromatography and eluted with a mixture of $CHCl_3$ saturated with H_2O and MeOH (MeOH, 2—8%) and then with $CHCl_3$–MeOH–H_2O (25 : 3 : 0.3→25 : 6 : 0.7→25 : 8 : 1.2) to give nine fractions (frs. 8—16). Fraction 8 (500 mg) was recrystallized from a mixture of $CHCl_3$ and MeOH to give **12** (95 mg). Fraction 9 (300 mg) was submitted to ODS (30 g) column chromatography and eluted with a gradient of MeOH–H_2O (MeOH, 5→100%) to give **13** (28 mg) and a mixture of fatty acid (106 mg). Fraction 10 (320 mg) was chromatographed on an ODS (30 g) column and eluted with a gradient of MeOH–H_2O (MeOH, 0→100%) to give **13** (20 mg) and **14** (13 mg). Fraction 11 (300 mg) was purified on an ODS (30 g) column and eluted with a gradient of MeOH–H_2O (MeOH, 10→50%) to give **15** (22 mg). Fraction 12 (230 mg) was purified on an ODS (30 g) column and eluted with a gradient of MeOH–H_2O (MeOH, 50→100%) and then on a silica gel column (solvent, $CHCl_3$: MeOH=9 : 1) to give substance A (31 mg). Fraction 13 (160 mg) was chromatographed on an ODS (30 g) column and eluted with a gradient of MeOH–H_2O (MeOH, 0→30%) to give **16** (7 mg) and a mixture of **16** and **17**. This mixture was separated on a silica gel column eluted with a gradient of benzene–AcOEt (AcOEt, 10→40%) to give **16** (7 mg) and **17** (7 mg). Fraction 14 (200 mg) was recrystallized from a mixture of $CHCl_3$ and MeOH to give **18** (50 mg). Frac-

tion 15 (400 mg) was submitted to repeated column chromatography [1. silica gel column (solvent, 1-BuOH : AcOEt : H_2O=2 : 7 : 0.5). 2. ODS column (solvent, 95% MeOH), 3. silica gel column (solvent, $CHCl_3$: MeOH=9 : 1)] to give **19** (11 mg). Fraction 16 (410 mg) was purified on an ODS (50 g) column and eluted with a gradient of MeOH–H_2O (MeOH 50→100%) and then on a silica gel column (solvent, $CHCl_3$: MeOH : H_2O=25 : 5 : 0.5) to give substance B (51 mg).

Identification of Known Compounds The NMR spectral data and optical rotation of compounds **3** [colorless needles from isopropanol, mp 157—159°, $[\alpha]_D^{30}$ −59.9° (c=1.00)], **4** [white amorphous powder, $[\alpha]_D^{27}$ −40.2° (c=1.00)] and **5** [colorless needles from water, mp 153—158°, $[\alpha]_D^{29}$ −30.0° (c=1.00)] are consistent with 4-β-D-glucopyranosyloxybenzyl alcohol,[6] militarine [(2*R*)-2-(2-methylpropyl)butanedioic acid bis(4-β-D-glucopyranosyloxybenzyl) ester][7] and loroglossin [(2*R*,3*S*)-2,3-dihydroxy-2-(2-methylpropyl)butanedioic acid bis(4-β-D-glucopyranosyloxybenzyl) ester].[7,8]

Compounds **12**—**18** were identified as β-sitosterol, 4-hydroxybenzyl methyl ether, 4-hydroxybenzaldehyde, pyrocatechol, hydroquinone, 4-hydroxybenzyl alcohol, and β-sitosterol 3-*O*-β-D-glucopyranoside, respectively, by direct comparison with the respective authentic samples.

Compound **19**, white amorphous powder, $[\alpha]_D^{26}$ +6.7° (c=0.30, 1-propanol). ^{1}H-NMR (pyridine-d_5): 0.86 (6H, t, J=6.5 Hz, H_3-16′ and H_3-18), 1.2—1.4 (34H, m), 1.72, 1.79 (each 1H, m, H_2-4′), 2.01, 2.20 (each 1H, m, H_2-3′), 2.06 (2H, m, H_2-10), 2.15 (2H, m, H_2-6), 2.17 (2H, m, H_2-7), 3.90 (1H, ddd, J=8.5, 5.5, 2.5 Hz, H-5″), 4.03 (1H, dd, J=8.5, 7.5 Hz, H-2″), 4.22 (1H, dd, J=8.5, 8.5 Hz, H-3″), 4.23 (1H, dd, J=8.5, 8.5 Hz, H-4″), 4.24 (1H, dd, J=10.5, 4 Hz, H_A-1), 4.35 (1H, dd, J=11.5, 5.5 Hz, H_A-6″), 4.51 (1H, dd, J=11.5, 2.5 Hz, H_B-6″), 4.58 (1H, dd, J=8, 3.5 Hz, H-2′), 4.71 (1H, dd, J=10.5, 6 Hz, H_B-1), 4.78 (1H, m, H-3), 4.80 (1H, m, H-2), 4.91 (1H, d, J=7.5 Hz, H-1″), 5.47 (2H, m, H-8, H-9), 5.92 (1H, dt, J=15.5, 5.5 Hz, H-5), 5.99 (1H, dd, J=15.5, 6 Hz, H-4), 8.36 (1H, d, J=9 Hz, NH). Determination of the coupling pattern was performed by referring to the data in pyridine-d_5+D_2O. ^{13}C-NMR (pyridine-d_5): 14.3 (C-18, 16′), 22.9 (C-17, 15′), 25.9 (C-4′), 27.3 (C-7), 27.6 (C-10), 29.6—30.0 (13C), 32.1 (C-16, 14′), 32.9 (C-6), 35.6 (C-3′), 54.6 (C-2), 62.6 (C-6″), 70.1 (C-1), 71.5 (C-4″), 72.3 (C-3), 72.5 (C-2′), 75.1 (C-2″), 78.4 (C-3″), 78.5 (C-5″), 105.6 (C-1″), 129.4 (C-8), 130.6 (C-9), 132.0 (C-5), 132.1 (C-4), 175.7 (C-1′). Signal assignment was confirmed based on 2D-NMR data (^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, HMBC). These data are consistent with 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*Z*)-2-[(2*R*)-2-hydroxyhexadecanoylamino]-4,8-octadecadiene-1,3-diol.[15]

Acid-Hydrolysis of Substance A A solution of substance A (3 mg) in 1 M H_2SO_4 (2 ml) was heated on a boiling water bath. After cooling, the reaction mixture was neutralized with saturated $Ba(OH)_2$. The precipitate deposited was removed by centrifuging and the supernatant was submitted to HPLC sugar analysis, which revealed the presence of D-galactose (t_R 18.6 min).

Alkaline-Methanolysis of Substance A A solution of substance A (20 mg) in 0.1 M NaOH–MeOH (10 ml) was left to stand for 12 h at room temperature. The reaction mixture was neutralized with 0.1 M HCl and evaporated. The residue was crystallized from MeOH to give a deacylated compound, which was identified as **18** by direct comparison. The mother liquor of the crystallization was evaporated and the residue was dissolved in ether, mixed with an ethereal solution of diazomethane and left to stand for 1 h at room temperature. The reaction mixture was evaporated and the residue was partitioned between H_2O and hexane. The hexane layer was examined by GLC, which revealed the presence of methyl palmitate (t_R 12.2 min) and methyl linoleate (t_R 30.0 min) in a ratio of 1 : 1.

Alkaline-Methanolysis of Substance B A solution of substance B (2 mg) in 0.1 M NaOH–MeOH (2 ml) was left to stand for 12 h at room temperature. A reaction mixture was neutralized with 0.1 M HCl and evaporated. The residue was partitioned between H_2O and ether and the ether phase was evaporated to dryness. The residue was dissolved in ether and processed in the same way as for substance A. GLC analysis of the fatty acid methyl esters revealed the presence of methyl palmitate (t_R 12.2 min), methyl oleate (t_R 24.9 min), methyl linoleate (t_R 30.0 min) and methyl linolenate (t_R 38.2 min), in a ratio of 6 : 2 : 18 : 7, respectively.

Dactylose A (1) White amorphous powder, $[\alpha]_D^{24}$ −23.8° (c=1.00, H_2O). IR: 3396, 1516, 1444, 1372, 1236, 1174, 1102, 1052, 816. UV: 220 (3.67), 274 (3.17). FAB-MS *m/z*: 279 [(M+Na)$^+$]. HR-FAB-MS *m/z*: 279.0851 [(M+Na)$^+$] (Calcd for $C_{12}H_{16}O_6$: 279.0845). ^{13}C-NMR: Table 1. ^{1}H-NMR: Tables 2, 3.

Dactylose B (2) White amorphous powder, $[\alpha]_D^{26}$ +15.0° (c=0.08, H_2O). IR: 3388, 2932, 1600, 1516, 1238, 1066, 830. UV: 220 (3.67), 274 (3.12). FAB-MS *m/z*: 279 [(M+Na)$^+$]. HR-FAB-MS *m/z*: 279.0850 [(M+Na)$^+$] (Calcd for $C_{12}H_{16}O_6$: 279.0845). ^{13}C-NMR: Table 1. ^{1}H-NMR: Tables 2, 3.

Synthesis of 1 and 2 A solution of L-ascorbic acid (4.4 g) and 4-hydroxybenzylalcohol (1 g) in H_2O was stirred in a nitrogen atmosphere at 37 °C for 4 d. The reaction mixture was partitioned between H_2O and AcOEt and the AcOEt layer was concentrated and chromatographed on a silica gel (80 g) column and eluted with a gradient of $CHCl_3$–MeOH (MeOH 0→5%) and then with $CHCl_3$–MeOH–H_2O (93 : 7 : 0.6→90 : 10 : 0.1) to give **22** (600 mg). Compound **22**, white amorphous powder, $[\alpha]_D^{23}$ +7.5° (c=3.41). ^{1}H-NMR (CD_3OD): 2.92, 3.11 (each 1H, d, J=13.5 Hz, H_2-1′), 3.73 (1H, br s, H-4), 3.99 (1H, dd, J=10, 3 Hz, H-6), 4.12 (1H, dd, J=10, 5.5 Hz, H′-6), 4.25 (1H, ddd, J=5.5, 3, 0.5 Hz, H-5), 6.69 (2H, m, H-4′, 6′), 7.12 (2H, m, H-3′, 7′). ^{13}C-NMR (CD_3OD): 41.1 (C-1′), 75.5 (C-4), 75.6 (C-6), 81.4 (C-2), 88.0 (C-5), 108.4 (C-3), 115.8 (C-4′, 6′), 125.7 (C-2′), 132.9 (C-3′, 7′), 157.6 (C-5′), 177.9 (C-1). Carbon signal assignment was confirmed based on ^{1}H–^{13}C COSY spectral data. The NMR data of **22** were consistent with 2-*C*-(4-hydroxybenzyl)-α-L-*xylo*-3-ketohexulofuranosono-1,4-lactone.

To a solution of **22** (500 mg) in MeOH (160 ml) was added triethylamine (0.16 ml) in H_2O (16 ml). After being allowed to stand at room temperature for 4 h, the reaction mixture was evaporated and the residue was submitted to HPLC [column, Purospher RP-18 (Merck); solvent, MeOH : H_2O=1 : 9] to give *syn*-**1** (223 mg) and *syn*-**2** (54 mg). The NMR data of *syn*-**1** [$[\alpha]_D^{26}$ −21.3° (c=1.36)] and *syn*-**2** [$[\alpha]_D^{19}$ +14.0° (c=1.17)] coincided with that of the natural products.

Enzymatic Hydrolysis of Militarine (4) To a solution of **4** (100 mg) in H_2O (20 ml), was added almond emulsin (P-L Biochemicals Inc., 50 mg) and the mixture was stirred at 37 °C for 7 d. The reaction mixture was extracted with AcOEt (40 ml×2) and the AcOEt phase was washed with H_2O (20 ml×2) and evaporated. The residue was chromatographed on an ODS (30 g) column and eluted with 30% MeOH to give **17** (35 mg) and (2*R*)-2-hydroxy-2-(2-methylpropyl)butanedioic acid (**9**, 23 mg).

Compound 9 Colorless needles, mp 161—162°, $[\alpha]_D^{25}$ −8.4° (c=0.51). ^{1}H-NMR (D_2O): 0.85 (3H, d, J=6.5 Hz, H_3-7), 0.91 (3H, d, J=6.5, H_3-8), 1.6—1.8 (3H, m, H_2-5, H-6), 2.70 (1H, d, J=16.5 Hz, H_A-3), 3.02 (1H, d, J=16.5 Hz, H_B-3). ^{13}C-NMR (D_2O): 25.6 (C-7), 26.3 (C-6), 26.7 (C-8), 47.5 (C-3), 50.5 (C-5), 78.5 (C-2), 177.3 (C-4), 181.6 (C-1). Spectral data of **9** agreed with that of (2*R*)-2-hydroxy-2-(2-methylpropyl)butanedioic acid, which was obtained by enzymatic hydrolysis of militarine (**4**).

Dactylorhin C (8) White amorphous powder, $[\alpha]_D^{24}$ −24.0° (c=1.15). IR: 3396, 1730, 1410, 1202, 1166, 1074, 1030, 910. FAB-MS *m/z*: 353 [(M+H)$^+$], 375 [(M+Na)$^+$]. HR-FAB-MS *m/z*: 375.1270 [(M+Na)$^+$] (Calcd for $C_{14}H_{24}O_{10}Na$: 375.1267). ^{1}H-NMR: (D_2O): 0.88 (3H, d, J=6.5 Hz, H_3-7), 0.92 (3H, d, J=6.5, H_3-8), 1.72 (1H, m, H_A-5), 1.82 (2H, m, H_B-5, H-6), 3.02 (1H, d, J=17.5 Hz, H_A-3), 3.12 (1H, d, J=17.5 Hz, H_B-3), 3.25 (1H, ddd, J=9, 4, 2.5 Hz, H-5′), 3.30 (1H, dd, J=9, 8 Hz, H-2′), 3.44 (1H, dd, J=9, 9 Hz, H-4′), 3.49 (1H, dd, J=9, 9 Hz, H-3′), 3.74 (1H, dd, J=12.5, 4 Hz, H-6′), 3.81 (1H, dd, J=12.5, 2.5 Hz, H′-6′), 4.88 (1H, d, J=8 Hz, H′-1). ^{13}C-NMR: Table 4.

Enzymatic Hydrolysis of 8 To a solution of **8** (10 mg) in H_2O (20 ml), was added cellulase (Sigma C-2415, 50 mg) and the mixture was stirred at 37 °C for 7 d. The reaction mixture was extracted with AcOEt (20 ml×2). HPLC analysis of the aqueous layer revealed the presence of D-glucose (t_R 16.9 min). The AcOEt layer was washed with H_2O (10 ml×2) and evaporated. The residue was chromatographed on an ODS (10 g) column and eluted with 30% MeOH to give **9** (2 mg).

Dactylorhin A (6) White amorphous powder, $[\alpha]_D^{24}$ −53.9° (c=1.00). IR: 3444, 1734, 1616, 1516, 1234, 1076, 828. UV: 223 (4.29), 271 (3.17), 277 (3.09). FAB-MS *m/z*: 911 [(M+Na)$^+$]. HR-FAB-MS *m/z*: 911.3162 [(M+Na)$^+$] (Calcd for $C_{40}H_{56}O_{22}Na$: 911.3161). ^{13}C-NMR: Table 4. ^{1}H-NMR: Table 5.

Enzymatic Hydrolysis of 6 To a solution of **6** (200 mg) in H_2O (50 ml) was added almond emulsin (150 mg) and the mixture was processed in a similar way as for **8** to give D-glucose, **17** and **8** (68 mg).

Dactylorhin B (7) White amorphous powder, $[\alpha]_D^{24}$ −41.9° (c=1.00). IR: 3412, 1736, 1616, 1516, 1230, 1076, 832. UV: 224 (4.32), 271 (3.24), 277 (3.71). FAB-MS *m/z*: 927 [(M+Na)$^+$]. HR-FAB-MS *m/z*: 927.3111 [(M+Na)$^+$] (Calcd for $C_{40}H_{56}O_{22}$: 927.3110). ^{13}C-NMR: Table 4. ^{1}H-NMR: Table 5.

Enzymatic Hydrolysis of 7 To a solution of **7** (30 mg) in H_2O (30 ml), was added cellulase (50 mg) and the mixture was stirred at 37 °C for 7 d. The reaction mixture was processed in a similar way as for **8** to give D-glucose, **7a** (4 mg) and **17**. Compound **7a**, white amorphous powder, $[\alpha]_D^{23}$ +4.3° (c=1.30). ^{1}H-NMR (D_2O): 0.88 (3H, d, J=6.5 Hz, H_3-7), 0.95 (3H, d, J=6.5 Hz, H_3-8), 1.72 (1H, m, H-6), 1.78—1.85 (2H, m, H_2-5). ^{13}C-NMR

(D_2O): 25.6 (C-7), 26.48 (C-8), 26.52 (C-6), 46.6 (C-5), 78.8 (C-3), 82.8 (C-2), 177.3 (C-4), 180.0 (C-1). Compound **7a** was identified as (2*R*,3*S*)-2,3-dihydroxy-2-(2-methylpropyl)butanedioic acid, which was obtained from loroglossin (**5**) in the same way as for **7**.

Dactylorhin D (10) White amorphous powder, $[\alpha]_D^{25}$ −25.7° (*c*=0.30). IR: 3400, 1730, 1716, 1636, 1234, 1072. UV: 221 (4.06), 271 (3.29), 277 (3.20). FAB-MS *m/z*: 497 [(M+Na)$^+$]. HR-FAB-MS *m/z*: 497.1638 [(M+Na)$^+$] (Calcd for $C_{40}H_{56}O_{22}$: 497.1635). ^{13}C-NMR: Table 4. ^{1}H-NMR: Table 6.

Enzymatic Hydrolysis of 10 To a solution of **10** (2 mg) in H_2O (5 ml), was added cellulase (10 mg) and the mixture was stirred at 37 °C for 7 d. The reaction mixture was processed in a similar way as for **5** to give **7a** and **17**.

Dactylorhin E (11) White amorphous powder, $[\alpha]_D^{25}$ −39.7° (*c*=0.73). IR: 3420, 1736, 1590, 1404, 1234, 1076. UV: 223 (3.94), 271 (2.89), 277 (2.82). FAB-MS *m/z*: 643 [(M+Na)$^+$]. HR-FAB-MS *m/z*: 643.2217 [(M+Na)$^+$] (Calcd for $C_{27}H_{40}O_{16}$: 643.2215). ^{13}C-NMR: Table 4. ^{1}H-NMR: Table 6.

Enzymatic Hydrolysis of 11 To a solution of **11** (15 mg) in H_2O (50 ml) was added almond emulsin (30 mg) and the mixture was processed in a similar way as for **4** to give **17** and **8** (7 mg).

Acknowledgements The authors are grateful to Dr. N. P. Manandhar, National Herbarium and Plant Laboratories, Ministry of Forests, His Majesty's Government of Nepal, for his botanical identification of the crude drug and to members of the analytical center of this university for MS measurements.

References and Notes

1) Part XXV: Segawa A., Miyaichi Y., Tomimori T., Kiuchi F., Ohta T., *Chem. Pharm. Bull*., **47**, 1404—1411 (1999).
2) *Dactylorhiza hatagirea* was termed *Orchis latifolia* in the past and the earlier name is occasionally still used.
3) Polunin O., Stainton A., "Flowers of the Himalaya," Oxford University Press, New Delhi, 1984, p. 395.
4) Department of Medicinal Plants, Ministry of Forests and Soil Conservation, His Majesty's Govt. of Nepal (ed.), "Medicinal Plants of Nepal," 4th ed., Kathmandu, 1993, p. 88.
5) Isolation of loroglossin, and analyses of carbohydrates and general components are reported: Delauney P., *Compt. Rend*., **176**, 598—600 (1923); Stanimirovic S., Stanimirovic D., Miric M., Lalic Z., *Arh. Farm*., **38**, 13—18 (1988) [*Chem. Abstr*., **109**, 208334p (1988)].
6) Inoue S., Wakai A., Konishi T., Kiyosawa S., Sawada T., *Yakugaku Zasshi*, **104**, 42—49 (1984).
7) Aasen A., Behr D., Leander K., *Acta Chem. Scand*., *Ser. B*, **29**, 1002—1004 (1975).
8) Gray von R. W., Guggisberg A., Segebarth K. P., Hesse M., Schmid H., *Helv. Chim. Acta*, **60**, 1304—1311 (1977).
9) Murakami N., Morimoto T., Imamura H., Ueda T., Nagai S., Sakakibara J., *Chem. Pharm. Bull*., **39**, 2277—2281 (1991).
10) Angyal S. J., Bethell G. S., *Aust. J. Chem*., **29**, 1249—1265 (1976).
11) Here, the assumption is adopted that **2** is a derivative of L-tagatose.
12) Preobrazhenskaya M. N., Rozhkov I. I., Lazhko E. I., Yudina L. N., Korolev A. M., *Tetrahedron*, **53**, 6971—6976 (1997).
13) Tanaka O., *Yakugaku Zasshi*, **105**, 323—351 (1985).
14) Poss A. J., Belter R., *J. Org. Chem*., **53**, 1535—1540 (1988).
15) Inagaki M., Harada Y., Yamada K., Isobe R., Higuchi R., Matsuura H., Itakura Y., *Chem. Pharm. Bull*., **46**, 1153—1156 (1998).