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THREE NEW SECOIRIDOID GLUCOSIDES FROM *EUSTOMA RUSSELLIANUM**

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Key Word Index—*Eustoma russellianum*; Gentianaceae; secoiridoid glucosides; eustomoside; eustoside; eustomorusside; ^{13}C NMR.

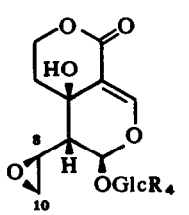
Abstract—Three new secoiridoid glucosides, eustomoside, eustoside and eustomorusside, have been isolated along with three known glucosides, sweroside, swertiamarin and gentiopicroside, as well as one unknown glycoside from *Eustoma russellianum*.

INTRODUCTION

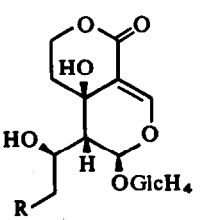
Previously, Inouye *et al.* [1–5] investigated the structure of the secoiridoid glucosides from *Swertia japonica* (family Gentianaceae) and further examined a variety of plants of the genera *Gentiana* and *Swertia* for secoiridoid glucosides [6]. As a part of this series, we have now investigated the constituents of *Eustoma russellianum* (Japanese name: Torukogikyo) of the same family which is native to North America and cultivated as a garden flower plant in Japan. This paper describes the structure elucidation of three new secoiridoid glucosides isolated from this plant.

RESULTS AND DISCUSSION

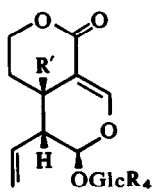
From the MeOH extract of the aerial parts of *E. russellianum* three new secoiridoid glucosides: eustomoside (1), eustoside (2) and eustomorusside (3), were isolated along with sweroside (4), swertiamarin (5) [1–3] and one unknown glycoside. From the MeOH extract of the root stocks and roots of the plants, swertiamarin (5) and gentiopicroside (6) [1–3] were isolated.



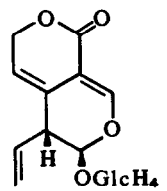
1 R = H
7 R = Ac



2 R = Cl
3 R = OH



4 R = R′ = H
5 R = H; R′ = OH
8 R = Ac; R′ = OH



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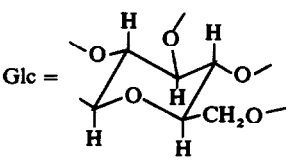
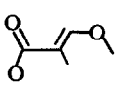


The first glucoside, eustomoside (1), was a colourless amorphous bitter principle, $C_{16}H_{22}O_{11}\cdot H_2O$, $[\alpha]_D$ −123.2° (MeOH). It showed a UV absorption at 235.5 nm and IR bands at 3400 (OH) as well as 1695 and 1620 cm^{-1} for the structure  which is characteristic of iridoid glucosides. The ^1H NMR spectrum of 1 shown in Table 1 along with those of the other compounds was similar to that of swertiamarin (5) suggesting that 1 was closely related to 5. Acetylation of eustomoside (1) gave the tetraacetate (7), $C_{24}H_{30}O_{15}$, which had an additional oxygen as compared with swertiamarin tetraacetate (8). The ^1H NMR spectrum of 7 was similar to that of 8 except for the absence of signals (δ 5.30–5.45) of the three olefinic protons on C-8 and C-10 of 8 and the presence of three-proton signals besides a signal of the C-9 proton (δ 2.45–3.00). Furthermore, as shown in Table 2, the chemical shifts of the ^{13}C NMR signals of 7 were in good agreement with those of 8 except for the signals arising from C-8 and C-10. These facts strongly suggested that 1 had a structure similar to that of swertiamarin (5) but possessed an additional oxygen function which was

* Part 38 in the series "Studies on Monoterpene Glucosides and Related Natural Products". For Part 37 see Inouye, H., Ueda, S. and Uesato, S. (1978) *Chem. Pharm. Bull. (Tokyo)* **26**, 3384.

† To whom correspondence should be addressed.

Table 1. ^{1}H NMR data* for the secoiridoid glucosides of *E. russellianum* and their derivatives

Compound (solvent)	1-H	3-H	5-OH	6-H_2	8-H	9-H	10-H_2	OAc groups
1	5.96	7.73		1.83–2.42	2.70–3.08	2.70–3.08	2.70–3.08	
(D_2O)	*d* (1.0)	*s*		*m*	*m*	*m*	*m*	
2	6.03	7.69		1.93–2.33		2.68	*ca* 3.48	
(D_2O)	*d* (1.0)	*s*		*m*		*dd* (6.0, 1.0)		
3	6.00	7.70		2.00–2.28		2.60	*ca* 3.50	
(D_2O)	*d* (0.5)	*s*		*m*		*dd* (6.0, 0.5)		
5	5.75	7.68			5.35–5.50		5.35–5.50	
(D_2O)	*d* (1.5)	*s*			*m*		*m*	
7	5.63	7.49	3.72		2.45–3.00	2.45–3.00	2.45–3.00	2.01–2.10
($CDCl_3$)	*d* (1.5)	*s*	*br s*		*m*	*m*	*m*	
8	5.50	7.50	3.77		5.30–5.45	2.67–3.30	5.30–5.45	2.02–2.10
($CDCl_3$)	*d* (1.5)	*s*	*br s*		*m*	*m*	*m*	
10	5.81	7.45	3.88			2.80	3.57–3.75	2.00–2.11
($CDCl_3$)	*d* (0.5)	*s*	*br s*			*dd* (6.0, 0.5)	*m*	
12	5.45	5.85			*ca* 4.07	*ca* 3.55	3.05 *dd* (11.0, 9.0)	1.95–2.09
($CDCl_3$)	*d* (2.5)	*s*					3.51 *dd* (11.0, 5.0)	
14	5.50	5.87		*ca* 2.67			3.46	1.96–2.08
($CDCl_3$)	*d* (2.5)	*s*					*dd* (9.0, 5.0)	
15	5.80	7.47	3.83			2.74		2.02–2.11
($CDCl_3$)	*d* (0.5)	*s*	*br s*			*dd* (6.0, 0.5)		

* Numbers in parentheses denote coupling constants in Hz.

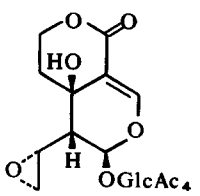
Table 2. ^{13}C NMR data* for the glucoside acetates **7**, **8**, **10**, **12**, **14** and **15** in $CDCl_3$

Compound	C-1	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
7	95.8	150.0	110.0	63.5	32.0	64.6	49.1†	49.0†	45.0	164.4	96.7	70.6	71.6	68.0	72.3	61.4
	(*d*)	(*d*)	(*s*)	(*s*)	(*t*)	(*t*)	(*d*)	(*d*)	(*t*)	(*s*)	(*d*)	(*d*)	(*d*)	(*d*)	(*d*)	(*t*)
8	97.6†	150.5	109.7	63.9	32.5	64.6	131.4	50.7	121.2	164.8	96.9†	70.6	71.7	68.1	72.3	61.5
	(*d*)	(*d*)	(*s*)	(*s*)	(*t*)	(*t*)	(*d*)	(*d*)	(*t*)	(*s*)	(*d*)	(*d*)	(*d*)	(*d*)	(*d*)	(*t*)
10	94.7	150.5	110.5	62.9	31.5	64.2	69.9	46.8	43.6	164.0	96.9	70.6	71.6	68.2	72.3	61.6
	(*d*)	(*d*)	(*s*)	(*s*)	(*t*)	(*t*)	(*d*)	(*d*)	(*t*)	(*s*)	(*d*)	(*d*)	(*d*)	(*d*)	(*d*)	(*t*)
12	92.5	86.8	129.9	151.5	42.5	66.5	69.5	41.2	27.3	159.0	95.6	70.5	72.0	68.2	72.3	61.7
	(*d*)	(*d*)	(*s*)	(*s*)	(*t*)	(*t*)	(*d*)	(*d*)	(*t*)	(*s*)	(*d*)	(*d*)	(*d*)	(*d*)	(*d*)	(*t*)
14	89.6	87.2	129.4	154.4	42.3	66.6	70.0	39.6	26.5	159.3	95.5	70.5	72.0	68.2	72.3	61.7
	(*d*)	(*d*)	(*s*)	(*s*)	(*t*)	(*t*)	(*d*)	(*d*)	(*t*)	(*s*)	(*d*)	(*d*)	(*d*)	(*d*)	(*d*)	(*t*)
15	94.5	150.3	110.6	62.6	31.1	64.3	68.5	47.0	62.4	164.0	96.8	70.6	71.6	68.1	72.4	61.4
	(*d*)	(*d*)	(*s*)	(*s*)	(*t*)	(*s*)	(*d*)	(*d*)	(*t*)	(*s*)	(*d*)	(*d*)	(*d*)	(*d*)	(*d*)	(*t*)

* Off resonance patterns are given in parentheses.
† Assignment may be interchanged.
All compounds have additional signals arising from acetoxy groups at *ca* 170 and 20.5 ppm.

presumably an 8,10-oxiran ring. This was verified by the finding that, of the two isomeric 8,10-epoxides, **7** and **9**, obtained by oxidation of swertiamarin tetraacetate (**8**) with *m*-chloroperbenzoic acid, the product with mp 201–203° was identical with eustomoside tetraacetate (**7**). The absolute structure of eustomoside (**1**) was thus established except for the stereochemistry at C-8, which will be discussed later.

The second glucoside, eustoside (**2**), was a colourless amorphous bitter principle, $C_{16}H_{23}O_{11}Cl{\cdot}H_2O$, $[\alpha]_D$ −100.0° (MeOH). It showed a UV absorption at 237.0 nm. The IR and ^{1}H NMR spectra of **2** were very similar to those of eustomoside (**1**). The ^{1}H NMR spectrum of its pentaacetate (**10**) showed the absence of signals attributable to the C-8 and C-10 oxiran protons in **7** and the signals present at δ 3.57–3.75 originated from $-CH_2Cl$. The ^{1}H NMR spectrum of **10** further showed signals for five acetoxy groups but no signals for the methylene attached to an acetoxy group except for the C-6′ methylene in the glucose moiety. These facts indicated the presence of one secondary hydroxy group in the aglycone moiety of **2**. In accord with the above ^{1}H NMR findings, the ^{13}C NMR spectrum of **10** showed a signal (43.6 ppm) of a methylene carbon attached to Cl and that (69.9 ppm) of a methine carbon linked to an acetoxy group. Therefore, **2** was assumed to be a chlorohydrin corresponding to eustomoside (**1**). This assumption was proved by the fact that eustoside pentaacetate (**10**) was obtained by treatment of eustomoside tetraacetate (**7**) with conc HCl in EtOAc yielding the chlorohydrin (**11**)



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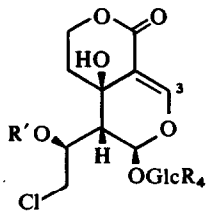
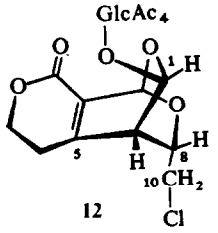


11 R = Ac; R′ = H
10 R = R′ = Ac



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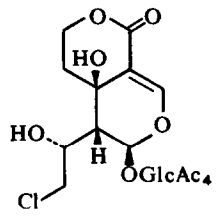

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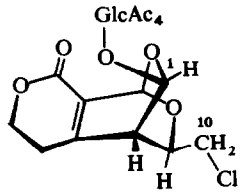
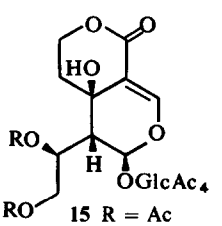


14

followed by acetylation. The absolute configuration of all chiral centres in **2** were thus established to be identical with those of **1**.

The absolute configuration of C-8, the only unsolved problem of the structures of eustomoside (**1**) and eustoside (**2**), was clarified in the following way. On treatment with BF_3 in THF, the chlorohydrin (**11**) afforded colourless needles (**12**), which showed a UV absorption at 226.5 nm and IR bands at 1760 and 1740 cm^{-1}. The ^1H NMR spectrum of **12** lacked the signals attributable to the C-5 and C-8 hydroxy groups appearing in **11**. On the other hand, the signal arising from the C-3 proton was shifted from δ 7.45 (*br s*) in **11** to δ 5.85 (*sh s*) in **12**. Consequently, **12** was thought to be the tricyclic compound which was formed from **11** by the allylic rearrangement caused by the elimination of the C-5 hydroxy group followed by an attack of the C-8 hydroxy group upon C-3. In addition, the epoxide (**9**) (the C-8 epimer of **7**) with mp 228–229°, after conversion into the chlorohydrin (**13**), was treated with BF_3 giving rise to a product (**14**), which should be the C-8 epimer of **12**. It was therefore attempted to elucidate the stereochemistry at C-8 of **12** and **14** by means of ^{13}C NMR spectroscopy and NOE experiments. In the ^{13}C NMR spectra (Table 2) of both compounds, the signal for C-5 in **12** or that for C-1 in **14** appeared upfield by 2.9 ppm relative to the frequency for the corresponding carbon in the counterpart. These phenomena are explained by the reciprocal γ-effect [7] of C-5 and C-10 in **12** as well as C-1 and C-10 in **14**. On the other hand, in the NOE experiments carried out in C_5D_5N, NOEs were observed between the following protons: C-1-H and C-8-H in **12**; C-1-H and C-10-H_2 in **14**, but no enhancement was observed between the following protons: C-1-H and C-10-H_2 in **12**; C-1-H and C-8-H in **14**. These results definitely indicate that the configuration at C-8 of **12** and that of **14** should be *S* and *R*, respectively, and further, that the stereochemistry at C-8 of eustomoside (**1**) as well as eustoside (**2**) should be *S*. Thus, the absolute structures **1** and **2** were assigned to eustomoside and eustoside, respectively.

The third glucoside, eustomorusside (**3**), was a colourless amorphous bitter principle, $C_{16}H_{24}O_{12}\cdot H_2O$, $[\alpha]_D$ −72.9° (MeOH). Its UV, IR and ^1H NMR spectra were very similar to those of the glucosides **1** and **2** suggesting that **3** also belongs to the secoiridoid glucosides of the swertiamarin type. The ^1H NMR spectrum of its hexaacetate (**15**) showed signals for six acetoxy groups, indicating the presence of two hydroxy groups in the aglycone moiety. Furthermore, compatible with this fact, the ^{13}C NMR spectrum of **15** showed signals (68.5 and 62.4 ppm) of the methylene and methine carbons bearing an acetoxy group in the aglycone moiety, respectively. Therefore, **3** was assumed to be an 8,10-dihydroxy compound corresponding to swertiamarin (**5**). This was proved by the chemical correlation described below. The two isomeric 8,10-dihydroxy compounds **16** and **17** obtained by oxidation of swertiamarin tetraacetate (**8**) with OsO_4 were acetylated to afford their acetates **15** and **18**, respectively. The acetate (**15**) with mp 142–144° was found to be identical with eustomorusside hexaacetate. The configuration at C-8 of eustomorusside (**3**) is assumed to be *S*-oriented as in the case of eustoside (**2**) since it is likely that **3**, as well as **2**, would be biosynthetically formed by the opening of the oxiran ring of **1**



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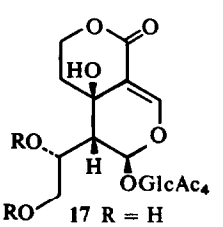
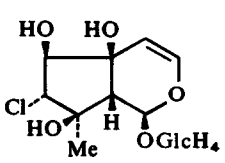
16 R = H



17 R = H
18 R = Ac



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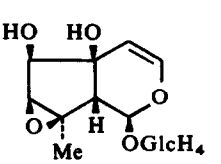

20

caused by an attack of an anion (OH^- or Cl^-) upon the C-atom 10. This was verified by the fact that eustoside tetraacetate (**11**) was formed by treatment of the tetraacetate (**16**) of eustomorusside (**3**) with CCl_4–triphenylphosphine [8]. Thus, the absolute structure **3** was assigned to eustomorusside.

The only glucoside of the iridoid series possessing a chlorohydrin moiety previously reported is linarioside (**19**) from three members of the Scrophulariaceae, *Linaria japonica* [9], *Cymbalaria muralis* [10] and *Kickxia elatine* [11]. Therefore, **2** is the second example of this type. Co-occurrence of **19** with the corresponding epoxide, antirrhinoside (**20**), in the three plants suggests that **19** would also be biosynthesized by the attack of a chloride anion on the oxiran ring of **20**.

EXPERIMENTAL

General procedures. Mps were uncorr. Unless otherwise stated ^1H NMR spectra were recorded at 60 MHz. ^{13}C NMR spectra were determined at 25.0 MHz. TMS was used in $CDCl_3$ or in C_5D_5N, whereas TSP was employed in D_2O as the internal standard. Chemical shifts were given in ppm (δ) relative to the internal standard. Si gel G was employed for TLC and spots were visualized by exposure to I_2 vapour or spraying with a mixture of anisaldehyde (0.5 ml), conc H_2SO_4 (0.5 ml), HOAc (few drops) and 95% EtOH (9 ml) followed by heating. Si gel PF_{254} was used for PLC (20 × 20 cm, 0.75 mm in thickness) and spots were visualized under a UV light (254 nm).

Plant material. Eustoma russellianum Griseb. which was cultivated in Ueda (Nagano Pref.) was collected in November 1977. Plant material was identified by Mr. G. Murata of Faculty of Science, Kyoto University. Voucher specimens of *Eustoma russellianum* (S. Uesato and T. Hashimoto, No. 1) have been deposited in the Herbarium of the Institute of Botany, Faculty of Science, Kyoto University (KYO), Kitashirakawa-oiwake-cho, Sakyo-ku, Kyoto, Japan.

Isolation of glucosides from Eustoma russellianum. (i) *From the aerial parts.* Fresh aerial parts (1.30 kg) of *E. russellianum* were extracted with boiling MeOH (10 l. × 4). After concn of the combined extracts *in vacuo*, H_2O (2 l.) was added and the insoluble material was filtered off through a celite layer, which was washed with H_2O (5 l.). The combined filtrate and washings were extracted successively with C_6H_6 (0.5 l. × 3) and *n*-BuOH (0.5 l. × 3). The H_2O and *n*-BuOH layers were concd *in vacuo* to give residues, 77.70 and 36.10 g, respectively. The residue of the H_2O layer was chromatographed on a charcoal (140 g) column with MeOH–H_2O as eluent with an increasing MeOH content to give the following eluates: 20% MeOH (Chrom-1), 30% MeOH (Chrom-2), 30–40% MeOH (Chrom-3) and 50–70% MeOH (Chrom-4). An aliquot (0.59 g) of the residue (2.45 g) of Chrom-1 was acetylated (Ac_2O–Py) and the product was recrystallized from Me_2CO yielding the acetate (0.48 g) of an unknown glycoside, mp 222.5–224°. In addition, the residue of the mother liquor was purified by PLC (3 plates) with $CHCl_3$–Et_2O (1:1) as eluent to afford eustomorusside hexaacetate (**15**) (0.22 g) as colourless plates, mp 142–144°. An aliquot (1.63 g) of the residue (4.74 g) of Chrom-2 was subjected to PLC (16 plates, 3 developments) with MeOH–$CHCl_3$ (1:3) as eluent. Of the three major bands, the most mobile one was scraped off and extracted with MeOH–$CHCl_3$ (2:8). The extract, after concn *in vacuo*, was submitted to chromatography on a charcoal (5 g) column, eluted successively with H_2O and MeOH. The MeOH eluate, on concn *in vacuo*, gave eustomoside (**1**) (0.93 g) as a white powder. The middle and the least mobile bands were worked up in the same way as above yielding eustoside (**2**) (0.23 g) and eustomorusside (**3**) (0.21 g) as white powders, respectively. The residue (15.42 g) of Chrom-3 was composed of pure eustomoside (**1**). The residue (3.37 g) of Chrom-4, which was mainly composed of sweroside (**4**) and swertiamarin (**5**) was acetylated in the usual way and the product was chromatographed on a Si gel (200 g) column with Et_2O–$CHCl_3$ as eluent with an increasing Et_2O content. The eluate with Et_2O–$CHCl_3$ (25:75) was concd *in vacuo* and the residue was recrystallized from EtOH yielding the tetraacetate (0.79 g) of sweroside (**4**) as colourless needles, mp 167–168°. Likewise, the eluate with Et_2O–$CHCl_3$ (3:7) afforded the tetraacetate (**8**) (0.82 g) of swertiamarin (**5**) as colourless needles, mp 176–178°. The residue of the *n*-BuOH layer was also chromatographed on a charcoal (80 g) column with MeOH–H_2O as eluent with an increasing MeOH content. The combined 80–100% MeOH eluates were concd *in vacuo* and the residue (1.50 g) consisting mainly of sweroside (**4**) and swertiamarin (**5**) was acetylated. The product was chromatographed on a Si gel (50 g) column in the same way as stated above giving two fractions which were recrystallized from EtOH to afford the tetraacetate (0.38 g, mp 167–168°) of sweroside (**4**) and the tetraacetate (**8**) (0.39 g, mp 176–178°) of swertiamarin (**5**), respectively.

Eustomoside (**1**), a hygroscopic bitter-tasting white powder; $[\alpha]_D^{23}$ −123.2° (MeOH, c = 1.01); UV λ_{max}^{MeOH} nm (log ε): 235.5 (3.94); IR ν_{max}^{KBr} cm^{-1}: 3400, 1695, 1620; ^1H NMR: see Table 1. (Found: C, 47.19; H, 6.05. $C_{16}H_{22}O_{11}\cdot H_2O$ requires: C, 47.06; H, 5.92%).

Eustoside (**2**), a hygroscopic bitter-tasting white powder; $[\alpha]_D^{23}$ −100.0° (MeOH, c = 1.00); UV λ_{max}^{MeOH} nm (log ε): 237.0 (3.86); IR ν_{max}^{KBr} cm^{-1}: 3350, 1685, 1610; ^1H NMR: see Table 1. (Found: C, 43.50; H, 5.78; Cl, 7.54. $C_{16}H_{23}O_{11}Cl\cdot H_2O$ requires: C, 43.20; H, 5.67; Cl, 7.97%).

Eustomorusside (**3**), a hygroscopic bitter-tasting white powder; $[\alpha]_D^{20}$ −72.9° (MeOH, c = 1.10); UV λ_{max}^{MeOH} nm (log ε): 236.5 (3.76); IR ν_{max}^{KBr} cm^{-1}: 3350, 1690, 1615; ^1H NMR: see Table 1. (Found: C, 44.76; H, 6.53. $C_{16}H_{24}O_{12}\cdot H_2O$ requires: C, 45.07; H, 6.15%).

(ii) *From the root stocks and roots.* The root stocks and roots (0.89 kg) of the plants were extracted with boiling MeOH (1.6 l. × 4). After concn of the combined extracts *in vacuo*, the residue was diluted with H_2O (0.8 l.) and extracted with EtOAc (0.6 l. × 4). The H_2O and EtOAc layers were concd *in vacuo* to give residues 138.0 and 31.6 g, respectively. The residue of the H_2O layer, which contained a small amount of swertiamarin (**5**) and gentiopicroside (**6**), was worked up in a similar way as in the case of the aerial parts to afford the tetraacetate (**8**) (0.55 g, colourless needles, mp 176–178°) of swertiamarin (**5**) and that (3.42 g, colourless needles, mp 135–137°) of gentiopicroside (**6**). The residue of the EtOAc layer has not been examined yet.

Acetylation of eustomoside (**1**). Eustomoside (**1**) (575 mg) was acetylated (Ac_2O–Py) and the product was recrystallized from EtOH to give eustomoside tetraacetate (**7**) (670 mg) as colourless needles, mp 201–203°. $[\alpha]_D^{20}$ −101.8° ($CHCl_3$, c = 1.01); UV λ_{max}^{MeOH} nm (log ε): 233.0 (3.96); IR ν_{max}^{KBr} cm^{-1}: 3550, 3150, 1750, 1712, 1620; ^1H NMR: see Table 1; ^{13}C NMR: see Table 2. (Found: C, 51.35; H, 5.47. $C_{24}H_{30}O_{15}$ requires: C, 51.61; H, 5.41%).

Epoxidation of swertiamarin tetraacetate (**8**) *with* m-*chloroperbenzoic acid. m*-Chloroperbenzoic acid (1.59 g) was added to a soln of **8** (1.54 g) in dry C_6H_6 (30 ml). After stirring for 20 hr at 50–55°, the reaction mixture was poured into iced H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was washed successively with 2 N aq. NaOH and H_2O, dried and evapd. The residue (1.71 g) was submitted to PLC (20 plates, 4 developments) with $CHCl_3$–EtOAc–C_6H_6 (1:2:1) as eluent. Of the two major bands,

the more mobile one was scraped off and extracted with MeOH–$CHCl_3$ (1:9). After concn of the extract, the residue (0.95 g) was recrystallized from EtOH to yield 8-epi-eustomoside tetraacetate (**9**) (0.76 g) as colourless needles, mp 228–229°. $[\alpha]_D^{20}$ −95.4° ($CHCl_3$, c = 1.01); UV λ_{max}^{MeOH} nm (log ε): 233.5 (3.95); IR ν_{max}^{KBr} cm^{-1}: 3560, 3160, 1755, 1708, 1630; ^1H NMR ($CDCl_3$): δ 2.33–3.05 (3H, *m*, 8-H, 10-H), 3.77 (1H, *br s*, 5-OH), 5.82 (1H, *d*, J = 1.5 Hz, 1-H), 7.53 (1H, *s*, 3-H). (Found: C, 51.45; H, 5.46. $C_{24}H_{30}O_{15}$ requires: C, 51.61; H, 5.41%). The less mobile band, on the same work-up as mentioned above, afforded colourless needles (114 mg), mp 201–203°. $[\alpha]_D^{20}$ −102.8° (c = 1.00, $CHCl_3$). (Found: C, 51.71; H, 5.66. Calc. for $C_{24}H_{30}O_{15}$: C, 51.61; H, 5.41%). This compound was identical with the tetraacetate (**7**) of eustomoside (**1**) isolated from the plant.

Acetylation of eustoside (**2**). Eustoside (**2**) (40 mg) was acetylated in the usual way and the product was recrystallized from EtOH to furnish eustoside pentaacetate (**10**) (42 mg) as colourless needles, mp 158–160°. $[\alpha]_D^{20}$ −81.0° ($CHCl_3$, c = 1.00); UV λ_{max}^{MeOH} nm (log ε): 233.5 (3.93); IR ν_{max}^{KBr} cm^{-1}: 3530, 1755, 1712, 1628; ^1H NMR: see Table 1; ^{13}C NMR: see Table 2. (Found: C, 49.09; H, 5.24; Cl, 5.59. $C_{26}H_{33}O_{16}Cl$ requires: C, 49.03; H, 5.22; Cl, 5.57%). These crystals, on recrystallization from MeOH, gave rise to colourless plates, mp 130–132°. Both crystal forms are mutually convertible upon exchanging the above described solvents for recrystallization.

Treatment of eustomoside tetraacetate (**7**) *with conc* HCl. Conc HCl (0.8 ml) was added dropwise to a stirred soln of **7** (1.00 g) in EtOAc (100 ml) at room temp. After stirring for a further 10 min, the soln was washed successively with 10% aq. NaOH and H_2O, dried and evapd. The residue was recrystallized from EtOH yielding eustoside tetraacetate (**11**) (0.83 g) as colourless plates, mp 161–162° (decomp.). $[\alpha]_D^{20}$ −79.1° ($CHCl_3$, c = 1.01); UV λ_{max}^{MeOH} nm (log ε): 235.0 (3.92); IR ν_{max}^{KBr} cm^{-1}: 3530, 3500, 1750, 1703, 1635; ^1H NMR ($CDCl_3$): δ 2.65 (1H, *dd*, J = 6.0, 1.0 Hz, 9-H), 2.95 (1H, *d*, J = 6.0 Hz, 8-OH), 3.86 (1H, *br s*, 5-OH), 5.81 (1H, *d*, J = 1.0 Hz, 1-H), 7.45 (1H, *br s*, 3-H). (Found: C, 47.19; H, 5.29; Cl, 5.94. $C_{24}H_{31}O_{15}Cl{\cdot}H_2O$ requires: C, 47.03; H, 5.43; Cl, 5.78%).

Acetylation of eustoside tetraacetate (**11**). Substance (**11**) (208 mg) was acetylated and the product was recrystallized from EtOH to give colourless needles (182 mg), mp 158–160°. $[\alpha]_D^{19}$ −81.2° ($CHCl_3$, c = 0.99). (Found: C, 49.06; H, 5.36; Cl, 5.51. Calc. for $C_{26}H_{33}O_{16}Cl$: C, 49.03; H, 5.22; Cl, 5.57%). This compound was identical in all respects with the pentaacetate (**10**) of eustoside (**2**) isolated from the plant.

Treatment of eustoside tetraacetate (**11**) *with* BF_3. BF_3-etherate (1.0 ml) was added dropwise to a stirred soln of eustoside tetraacetate (**11**) (3.00 g) in dry THF (100 ml) at 0–5°. After stirring for a further 5 hr at room temp., the reaction mixture was poured into iced H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried and concd *in vacuo* to give a residue, which was subjected to chromatography on a Si gel (80 g) column with Et_2O–$CHCl_3$ as eluent with an increasing Et_2O content. After concn of the eluate with Et_2O–$CHCl_3$ (1:1), the residue was recrystallized from EtOH–Et_2O yielding the tricyclic compound (**12**) (1.06 g) as colourless needles, mp 182–183°. $[\alpha]_D^{20}$ −87.3° ($CHCl_3$, c = 1.02); UV λ_{max}^{MeOH} nm (log ε): 226.5 (3.70); IR ν_{max}^{KBr} cm^{-1}: 1760, 1740, 1710; ^1H NMR (C_5D_5N): δ 3.35 (1H, *dd*, J = 11.0, 9.0 Hz, 10-H), 3.59 (1H, *dd*, J = 11.0, 5.0 Hz, 10-H), 4.22 (1H, *m*, 8-H), 5.78 (1H, *d*, J = 2.5 Hz, 1-H), 6.23 (1H, *s*, 3-H). ^1H NMR (100 MHz, $CDCl_3$): see Table 1; ^{13}C NMR: see Table 2. (Found: C, 49.81; H, 5.22; Cl, 6.42. $C_{24}H_{29}O_{14}Cl$ requires: C, 49.96; H, 5.07; Cl, 6.14%).

Treatment of 8-epi-eustomoside tetraacetate (**9**) *with conc* HCl. Conc HCl (1.6 ml) was added dropwise to a stirred soln of **9** (1.35 g) in EtOAc (200 ml) at room temp. After stirring for a further 10 min, the soln was washed successively with 1 N aq. NaOH and H_2O, dried and concd *in vacuo*. The residue was chromatographed on a Si gel (40 g) column with Et_2O–$CHCl_3$ as eluent with an increasing Et_2O content. The residue of the eluate with Et_2O–$CHCl_3$ (1:1) was recrystallized from EtOH yielding 8-epi-eustoside tetraacetate (**13**) (0.43 g) as colourless needles, mp 205–205.5° (decomp.). $[\alpha]_D^{26}$ −97.8° (dioxane, c = 0.50); UV λ_{max}^{MeOH} nm (log ε): 237.0 (3.95); IR ν_{max}^{KBr} cm^{-1}: 3550, 3480, 1760, 1710, 1630; ^1H NMR (100 MHz, C_5D_5N): δ 2.85 (1H, *dd*, J = 5.0, 1.0 Hz, 9-H), 3.93 (2H, *m*, 10-H), 6.48 (1H, *d*, J = 1.0 Hz, 1-H), 7.86 (1H, *s*, 3-H). (Found: C, 48.71; H, 5.37; Cl, 6.08. $C_{24}H_{31}O_{15}Cl$ requires: C, 48.45; H, 5.25; Cl, 5.96%).

Treatment of 8-epi-eustoside tetraacetate (**13**) *with* BF_3. BF_3-etherate (0.15 ml) was added dropwise to a soln of 8-epi-eustoside tetraacetate (**13**) (352 mg) in dry THF (30 ml) with stirring at 0–5°. After stirring for a further 3 hr at room temp., the reaction mixture was worked up in the same way as in the preparation of **12** to afford another tricyclic compound (**14**) (82 mg) as colourless needles, mp 198–200°. $[\alpha]_D^{26}$ −91.8° ($CHCl_3$, c = 1.00); UV λ_{max}^{MeOH} nm (log ε): 218.5 (3.61); IR ν_{max}^{KBr} cm^{-1}: 1755, 1720, 1220; ^1H NMR (100 MHz, C_5D_5N): δ 2.68 (2H, *m*, 6-H), 3.03 (1H, *d*, J = 2.5 Hz, 9-H), 3.37 (2H, *br s*, 10-H), 5.98 (1H, *d*, J = 2.5 Hz, 1-H), 6.24 (1H, *s*, 3-H); ^1H NMR ($CDCl_3$): see Table 1; ^{13}C NMR: see Table 2. (Found: C, 50.24; H, 5.26; Cl, 6.19. $C_{24}H_{29}O_{14}Cl$ requires: C, 49.96; H, 5.07; Cl, 6.14%).

Acetylation of eustomorusside (**3**). Eustomorusside (**3**) (40 mg) was acetylated and the product was recrystallized from EtOH–Et_2O to give eustomorusside hexaacetate (**15**) (43 mg) as colourless plates, mp 142–144°. $[\alpha]_D^{23}$ −67.9° ($CHCl_3$, c = 1.00); UV λ_{max}^{MeOH} nm (log ε): 233.0 (3.97); IR ν_{max}^{KBr} cm^{-1}: 3525, 1750, 1710, 1630; ^1H NMR: see Table 1; ^{13}C NMR: see Table 2. (Found: C, 51.09; H, 5.50. $C_{28}H_{36}O_{18}$ requires: C, 50.91; H, 5.49%).

Oxidation of swertiamarin tetraacetate (**8**) *with* OsO_4. A soln of OsO_4 (266 mg) in dry C_6H_6 (5 ml) was added to a stirred soln of **8** (500 mg) in dry C_6H_6 (15 ml) at 0–5° over a period of 10 min. After stirring for a further 1 hr at room temp., dry Et_2O (50 ml) was added and the resulting brown ppt. was collected and dissolved in $CHCl_3$ (20 ml). A soln of mannitol (2.00 g) and KOH (0.20 g) in H_2O (20 ml) was added to this soln and the mixture was stirred vigorously at room temp. for 5 hr. The $CHCl_3$ layer was separated, washed with H_2O, dried and evapd to give a residue, which was submitted to PLC (10 plates, 8 developments) with $CHCl_3$–EtOAc (1:3) as eluent. Of the two major bands, the more mobile one was extracted with MeOH–$CHCl_3$ (1:9). After concn of the extract, the residue was recrystallized from EtOH–Et_2O yielding eustomorusside tetraacetate (**16**) (92 mg) as colourless needles, mp 166.5–168°. $[\alpha]_D^{24}$ −78.7° ($CHCl_3$, c = 1.04); UV λ_{max}^{MeOH} nm (log ε): 235.5 (3.94); IR ν_{max}^{KBr} cm^{-1}: 3550, 1760, 1710, 1625, 1230; ^1H NMR ($CDCl_3$): δ 2.58 (1H, *m*, 9-H), 3.63 (2H, *m*, 10-H), 3.87 (1H, *br s*, 5-OH), 5.83 (1H, *br s*, 1-H), 7.47 (1H, *s*, 3-H). (Found: C, 49.78; H, 5.79. $C_{24}H_{32}O_{16}$ requires: C, 50.00; H, 5.59%). An analogous treatment of the less mobile band afforded 8-epi-eustomorusside tetraacetate (**17**) (22 mg) as colourless plates, mp 187.5–189.5° $[\alpha]_D^{23}$ −107.2° ($CHCl_3$, c = 0.99); UV λ_{max}^{MeOH} nm (log ε): 236.5 (3.94); IR ν_{max}^{KBr} cm^{-1}: 3550, 1760, 1730, 1700, 1630; ^1H NMR (C_5D_5N): δ 2.92 (1H, *br d*, J = 5.0 Hz, 9-H), 3.95–4.17 (1H, *m*, 10-H), 6.67 (1H, *br s*, 1-H), 7.92 (1H, *s*, 3-H). (Found: C, 50.28; H, 5.51. $C_{24}H_{32}O_{16}$ requires: C, 50.00; H, 5.59%).

Acetylation of eustomorusside tetraacetate (**16**). Substance (**16**) (33 mg) was acetylated and the product was recrystallized from EtOH–Et_2O to give colourless needles (34 mg), mp 142–144°. $[\alpha]_D^{22}$ −68.6° ($CHCl_3$, c = 1.02). (Found: C, 51.02; H, 5.63. Calc. for $C_{28}H_{36}O_{18}$: C, 50.91; H, 5.49%). This compound was

identical with the hexaacetate (**15**) of eustomorusside (**3**) isolated from the plant.

Acetylation of 8-epi-eustomorusside tetraacetate (**17**). Substance (**17**) (51 mg) was acetylated and the product was recrystallized from EtOH–Et_2O to afford 8-epi-eustomorusside hexaacetate (**18**) (26 mg) as colourless needles, mp 128–130°. $[\alpha]_D^{26}$ −137.0° ($CHCl_3$, c = 0.40); UV λ_{max}^{MeOH} nm (log ε): 235.5 (3.89); IR ν_{max}^{KBr} cm^{-1}: 3550, 1750, 1710, 1625; ^1H NMR (100 MHz, $CDCl_3$): δ 2.60 (1H, *br d*, J = 4.0 Hz, 9-H), 3.84 (1H, *br s*, 5-OH), 5.86 (1H, *br s*, 1-H), 7.47 (1H, *s*, 3-H). (Found: C, 50.62; H, 5.76. $C_{28}H_{36}O_{18}$ requires: C, 50.91; H, 5.49%).

Conversion of eustomorusside tetraacetate (**16**) *into eustoside tetraacetate* (**11**). A soln of triphenylphosphine (60 mg) in CCl_4 (3.0 ml) was refluxed for 30 min under N_2 and cooled. Then, a soln of **16** (110 mg) in DMF (1.0 ml) was added to this soln and the mixture was refluxed with stirring for 30 min. The reaction mixture was poured into iced H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried and evapd *in vacuo*. The residue was subjected to PLC (3 plates, 3 developments) with $CHCl_3$–EtOAc (1:1) as eluent. The fast mobile major band was scraped off and extracted with MeOH–$CHCl_3$ (1:9). After concn of the extract *in vacuo*, the residue was recrystallized from EtOH–Et_2O furnishing **11** (35 mg) as colourless needles, mp 161–162° (decomp.). $[\alpha]_D^{23}$ −79.1° ($CHCl_3$, c = 1.00). (Found: C, 47.16; H, 5.20; Cl, 5.94. Calc. for $C_{24}H_{31}O_{15}Cl \cdot H_2O$: C, 47.03; H, 5.43; Cl, 5.78%). This substance was identical with the tetraacetate (**11**) of eustoside (**2**) isolated from the plant.

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