Phytochemistry, Vol. 23, No. 4, pp. 795–798, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

LILIOSIDES D AND E, TWO GLYCEROL GLUCOSIDES FROM *LILIUM JAPONICUM**†

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(*Revised received* 15 *July* 1983)

Key Word Index—*Lilium japonicum*; Liliaceae; glycerol glucosides; 1-*O*-β-D-glucopyranosyl-*sn*-glycerol; 3-*O*-acetyl-1-*O*-β-D-glucopyranosyl-*sn*-glycerol; lilioside D; lilioside E; structure elucidation; stereochemistry; synthesis; ^{13}C NMR.

Abstract—Two new glycerol glucosides, liliosides D and E, have been isolated from the leaves and stems of *Lilium japonicum*. Their structures have been elucidated by chemical, spectroscopic and synthetic methods.

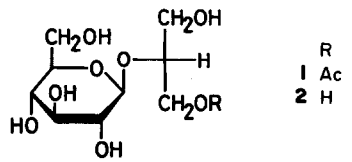
INTRODUCTION

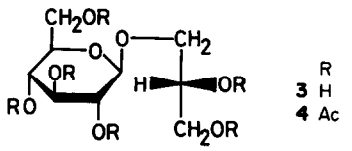
In previous papers [1, 2], we reported the isolation and structure elucidation of three new glycerol glucosides, liliosides A (**1**) and B (**2**) from *Lilium longiflorum*, and lilioside C (**3**) from *L. lancifolium*. These glycerol glucosides seem to be characteristic constituents of the *Lilium* genus and their structures appear to vary depending on the species. In a continuation of this chemotaxonomic study, we have now isolated two new glycerol glucosides, liliosides D (**5**) and E (**6**), from another *Lilium* species, *L. japonicum* L., and have proved them to be 1-*O*-β-D-glucopyranosyl-*sn*-glycerol and 3-*O*-acetyl-1-*O*-β-D-glucopyranosyl-*sn*-glycerol, respectively.

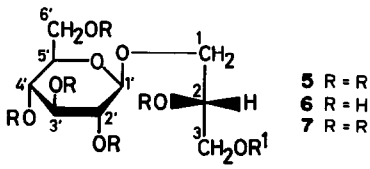
RESULTS AND DISCUSSION

From the aqueous 80% methanol extract of fresh leaves and stems, two new glycerol glucosides, liliosides D (**5**) and E (**6**), were isolated in about 0.01 and 0.001% yield (fresh plant weight).

Lilioside D (**5**) was obtained as a colourless viscous syrup, $[\alpha]_D^{15}$ −30.1° (H_2O; *c* 1.23), and exhibited almost the same TLC and GC behaviour as lilioside C (**3**) [2], but was definitely different from lilioside B (**2**) [1] on TLC and GC. Acetylation of **5** with acetic anhydride and pyridine gave a hexa-acetate (**7**), $C_{21}H_{30}O_{14}$, mp 107–108° (*n*-hexane–ether), $[\alpha]_D^{25}$ −35.3° ($CHCl_3$; *c* 0.71). Acid hydrolysis and enzymatic degradation with emulsin (β-glucosidase) of **5** afforded glucose and glycerol in equimolar amounts as well as liliosides B (**2**) and C (**3**). The IR and ^1H NMR spectra of **5** and its hexa-acetate (**7**) were very similar to those of lilioside C (**3**) and its hexa-acetate (**4**), respectively, but crystalline **7** had a lower melting point than **4** and showed a definite melting point depression with **4**. In the ^1H NMR spectra of **5** and **7**, the anomeric protons were observed at δ 4.88 (*d*, *J* = 7 Hz) and δ 4.54 (*d*, *J* = 7 Hz), respectively. From this and the result of the enzymatic hydrolysis, **5** must have a β-glucosidic linkage.







The above findings suggested that lilioside D (**5**) might differ from lilioside C (**3**) in the configuration of C-2 of the glycerol moiety. As the structure of **3** had been established as (2*R*)-3-*O*-β-D-glucopyranosyl-*sn*-glycerol [2], **5** should be (2*S*)-1-*O*-β-D-glucopyranosyl-*sn*-glycerol.

Authentic 1-*O*-β-D-glucopyranosyl-*sn*-glycerol had been synthesized by Brundish and Baddiley [3] using the Koenigs–Knorr reaction of 2,3-di-*O*-benzyl-*sn*-glycerol and acetobromoglucose and subsequent deacetylation and debenzylation. The reported melting point of its hexa-acetate was the same as that of **7**, but its rotation, $[\alpha]_D$ −54.6° ($CHCl_3$; *c* 0.4), differed from that of **7**, $[\alpha]_D^{25}$ −35.1° ($CHCl_3$; *c* 0.71). Unfortunately, an authentic

*Part 3 in the series "Glycerol Glucosides in the *Lilium* genus". For Part 2, see Kaneda, M., Mizutani, K. and Tanaka, K. (1982) *Phytochemistry* **21**, 891.

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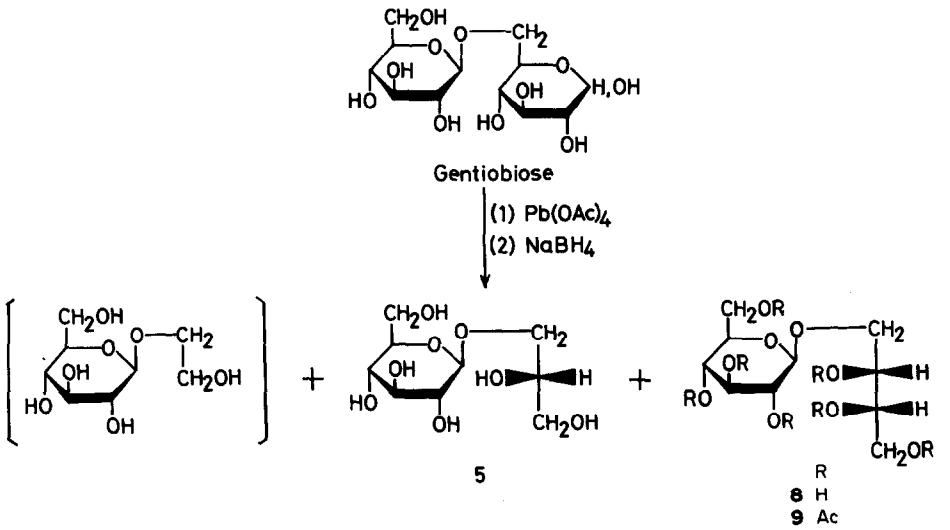

Scheme 1.

sample of the synthetic material could not be obtained. Therefore, in order to confirm the structure of lilioside D, we attempted the synthesis of **5** by a method different from that of Brundish and Baddiley [3], namely, by oxidative degradation of gentiobiose with lead tetra-acetate. This method is a modification of that described by Charlson and Perlin [4].

β-Gentiobiose was degraded with about two molar equivalents of lead tetra-acetate. The resulting mixture of three aldehydes was reduced with sodium borohydride to yield the three corresponding polyalcohols. From this mixture, 1-*O*-β-D-glucopyranosyl-*sn*-glycerol (**5**) and 4-*O*-β-D-glucopyranosyl-D-erythritol (**8**) were separated by silica gel column chromatography, but a third product, which was presumed to be 1-*O*-β-D-glucopyranosyl ethylene glycol, was not further identified. The glycerol glucoside (**5**) obtained in this fashion exhibited identical TLC and GC behaviour with lilioside D and afforded equimolar amounts of glucose and glycerol on enzymatic hydrolysis with emulsin. Furthermore, the hexa-acetate of the synthetic material had the same melting point and $[\alpha]_D$ (including sign) as hexa-acetyllilioside D. The identity of the synthetic and the natural glucoside was thus unambiguously established through the corresponding hexa-acetates, by comparison of their TLC, IR and ^{1}H NMR, and by the mixed melting point which showed no depression. Hence, the structure of lilioside D has been established as (2*S*)-1-*O*-β-D-glucopyranosyl-*sn*-glycerol.

Lilioside E (**6**) was isolated as a colourless viscous film, $[\alpha]_D^{20}$ −23.0° (H_2O; *c* 0.43). Its IR spectrum showed the presence of an ester carbonyl group (1725, 1250 cm^{-1}). Its ^{1}H NMR spectrum exhibited a signal of one acetoxyl group at δ 1.96 (3H, *s*), but was otherwise very similar to that of lilioside D (**5**). Acetylation of **6** with acetic anhydride and pyridine afforded penta-acetyllilioside E, mp 107–108° (*n*-hexane–ether), which was proved to be identical with hexa-acetyllilioside D (**7**) by comparison of TLC, ^{1}H NMR, IR and mmp. Hence, lilioside E (**6**) was a monoacetate of lilioside D (**5**). Furthermore, enzymatic hydrolysis of **6** with emulsin (β-glucosidase) gave glucose, glycerol monoacetate and a small amount of glycerol. Thus, the acetyl group of **6** must be in the glycerol moiety. In order to determine its position, we compared the ^{13}C NMR spectra of **5** and **6**. The assignment of the carbon signals of the two compounds were achieved, as shown in Table 1, on the basis of their multiplicities in the off-resonance spectra and the reported values of methyl β-D-glucopyranoside [5] and several glycerol glycosides [6, 7]. On going from **5** to **6**, the C-3 signal was displaced downfield by 3.2 ppm and that of C-2 was shifted upfield by 2.4 ppm, while other carbon signals remained almost unchanged. On consideration of the acetylation shift [8], such changes in the chemical shifts of C-3 and C-2 can only be explained if the primary hydroxyl group at C-3 of **6** is acetylated. This thus establishes the structure of lilioside E (**6**) as (2*S*)-3-*O*-acetyl-1-*O*-β-D-glucopyranosyl-*sn*-glycerol.

Glycerol glucosides such as liliosides A, B, C, D and E thus seem to be characteristic constituents of the *Lilium* genus with the position of glucosidation and the stereo-

Table 1. ^{13}C NMR data for **5** and **6** [25.15 MHz, CD_3OD–H_2O (1:1), TMS as int. standard]

C	5	6	Δδ
1	71.7 (*t*)*	71.8 (*t*)	
2	71.7 (*d*)	69.3 (*d*)	−2.4
3	63.4 (*t*)	66.6 (*t*)	+3.2
1′	103.9 (*d*)	104.2 (*d*)	
2′	74.3 (*d*)	74.7 (*d*)	
3′	76.9 (*d*)	77.4 (*d*)	
4′	70.8 (*d*)	71.1 (*d*)	
5′	76.9 (*d*)	77.4 (*d*)	
6′	61.8 (*t*)	62.2 (*t*)	
OCOMe		20.9 (*q*)	
OCO		174.1 (*s*)	

*Abbreviations given in parentheses denote the multiplicities in the off-resonance spectra.

chemistry of the glycerol moiety varying with the species. Liliosides A (**1**) and B (**2**) in *L. longiflorum* [1] are glucosidated at C-2 of glycerol, lilioside C (**3**) in *L. lancifolium* [2] is glucosidated at C-3 of *sn*-glycerol, whereas liliosides D (**5**) and E (**6**) in *L. japonicum* are glucosidated at C-1 of *sn*-glycerol.

EXPERIMENTAL

Mps uncorr.; ^{1}H NMR: 100 MHz, TMS as int. standard; ^{13}C NMR: 25.15 MHz in CD_3OD–H_2O (1:1), employing the FT mode, TMS as int. standard; IR: KBr; TLC: silica gel G or GF 254 (Type 60, Merck), detected by spraying with 10% H_2SO_4 followed by heating; GC: FID, glass column (2 m × 4 mm i.d.) packed with 1.5% OV-1 on Shimalite W(201D), N_2 50 ml/min. The plant material was collected in the suburbs of Kanazawa City and identified by Dr. H. Kimura, Faculty of Pharmaceutical Sciences, Kanazawa University, where herbarium specimens of the plant are deposited.

Extraction and isolation of **5** *and* **6**. Fresh leaves and stems (3.4 kg) of *L. japonicum* were cut into pieces and extracted with aq. 80% MeOH at room temp. for 5 days. The 80% MeOH extract was evaporated to dryness under red. pres. and low temp. (40°), dissolved in H_2O and washed with $CHCl_3$. The H_2O layer was concd to a 10% soln and placed on an active charcoal (Wako, for chromatography) column, which was eluted first with H_2O to remove monosaccharides and then with 2.5, 5 and 10% aq. EtOH successively. Each fraction was examined by TLC and GC. The fraction eluted with 5% EtOH afforded a mixture of lilioside D (**5**) and sucrose, which was separated on a silica gel (Wakogel C-200) column. The $CHCl_3$–MeOH (5:1 to 4:1) eluate gave pure lilioside D (**5**) (360 mg). The 10% EtOH fraction of the above active charcoal CC was further purified by CC on silica gel. The $CHCl_3$–MeOH (4:1) eluate yielded pure lilioside E (**6**) (40 mg).

Lilioside D (**5**). Colourless viscous syrup, $[\alpha]_D^{15}$ −30.1° (H_2O; *c* 1.23), R_f 0.43 (TLC, $CHCl_3$–MeOH, 2:1). IR ν_{max}^{KBr} cm^{-1}: 3380 (*br*); ^{1}H NMR (C_5D_5N): δ 3.6–4.6 (many protons), 4.88 (1H, *d*, *J* = 7 Hz, anomeric H); GC: TMSi derivative, R_t 5.5 min (column temp. 230°).

Hexa-acetyllilioside D (**7**). Treatment of **5** (31 mg) with Ac_2O (0.5 ml) and pyridine (0.5 ml) at room temp. overnight, followed by the usual work-up, afforded a product which was crystallized from *n*-hexane–Et_2O to give colourless needles of **7** (39 mg), mp 107–108°, $[\alpha]_D^{25}$ −35.3° ($CHCl_3$; *c* 0.71). (Found: C, 49.67; H, 5.81. Calc. for $C_{21}H_{30}O_{14}$: C, 49.80; H, 5.97%.) IR ν_{max}^{KBr} cm^{-1}: 1750, 1230; ^{1}H NMR ($CDCl_3$): δ 2.00–2.11 (3H × 6, *s*, OAc), 3.60–4.40 (7H, *m*), 4.54 (1H, *d*, *J* = 7 Hz, anomeric H), 4.88–5.30 (4H, *m*).

Acid hydrolysis of **5**. A soln of **5** (1.5 mg) in 5% H_2SO_4 was heated at 95° for 6 hr. The reaction mixture was neutralized by stirring with Dowex 1-X8 (HCO_3^- form), and the resin was removed by filtration and washed with H_2O. The filtrate and washings were combined, evaporated *in vacuo* to dryness, and the hydrolysis products converted to TMSi derivatives. GC [120° for 7 min, from 120 to 250° (10°/min), 250° to the end of the run] showed the presence of equimolar amounts of glucose (R_t 16.3 and 17.2 min) and glycerol (R_t 4.1 min).

Enzymatic hydrolysis of **5**. To a soln of **5** (2 mg) in H_2O (1 ml) was added emulsin (β-glucosidase from almonds; Sigma) (0.5 mg) and the mixture was allowed to stand at room temp. overnight. After evaporation of the solvent *in vacuo*, the residual hydrolysate was converted to the TMSi derivatives and examined by GC, which was performed as described above for the acid hydrolysis product of **5**. The results confirmed the composition to be an equimolar mixture of glucose and glycerol.

Lead tetra-acetate oxidation of β-gentiobiose. β-Gentiobiose (100 mg) was dissolved in H_2O (1 ml) and the soln was diluted with HOAc (10 ml). To that soln, $Pb(OAc)_4$ (280 mg, 2.16 mol equivalent) was added and the mixture was stirred at room temp. until the soln gave a negative starch–iodide test. After treating with a soln of oxalic acid in HOAc, the reaction mixture was filtered with Hyflo super cel and the filtrate was evaporated *in vacuo*. The residue was dissolved in H_2O and the soln was stirred successively with Dowex 50-X8 (H^+ form) and Dowex 1-X8 (OH^- form) to remove the remaining ion. The resin was filtered and the filtrate was evaporated *in vacuo* to give as a colourless syrup the mixture of aldehydes.

1-O-β-D-*Glucopyranosyl*-sn-*glycerol* (**5**) *and* 4-O-β-D-*glucopyranosyl*-D-*erythritol* (**8**). The mixture of aldehydes was dissolved in H_2O and treated with an excess of $NaBH_4$ for 12 hr. After decomposing the excess $NaBH_4$ with HOAc, the sodium was removed by stirring with Dowex 50W-X8 (H^+ form). The resin was filtered and the solvent evaporated *in vacuo*. To the residual syrup was added a small amount of MeOH which was then evaporated *in vacuo*. Boric acid was removed by repeating this addition and distillation of MeOH. The resulting syrup showed three spots on TLC ($CHCl_3$–MeOH, 7:3; R_f 0.41, 0.35 and 0.26). This mixture was chromatographed over silica gel (Merck, silica gel 60 extra pure; $CHCl_3$–MeOH, 4:1). The fractions containing the first-eluted compound were combined and evaporated to give a transparent film (10 mg), which was presumed to be 1-*O*-β-D-glucopyranosyl ethylene glycol and was not further characterized. The fractions containing the second-eluted compound were combined and evaporated to yield colourless syrup of the presumed 1-*O*-β-D-glucopyranosyl-*sn*-glycerol (**5**) (18 mg), which exhibited the same TLC and GC behaviour as lilioside D. The fractions containing the last-eluted compound gave, on evaporation of the solvent, a colourless syrup of presumed 4-*O*-β-D-glucopyranosyl-D-erythritol (**8**) (27 mg), $[\alpha]_D^{24}$ −20.5° (H_2O; *c* 0.2). This compound (**8**) afforded glucose and erythritol on acid hydrolysis and also on enzymatic hydrolysis with emulsin performed as described above for **5**.

1-O-β-D-*Glucopyranosyl*-sn-*glycerol hexa-acetate* (**7**). The synthetic glycerol glucoside (**5**) (10 mg) obtained above was acetylated with Ac_2O (0.5 ml) and pyridine (0.5 ml) at room temp. overnight. Usual work-up gave a product which was crystallized from *n*-hexane–Et_2O to yield colourless needles of the hexa-acetate (**7**) (12 mg), mp 107–108°, $[\alpha]_D^{16}$ −35.1° ($CHCl_3$; *c* 0.37). This compound was proved to be identical with hexa-acetyllilioside D by mmp and comparison of TLC, IR and ^{1}H NMR. (Found: C, 49.79; H, 5.84. Calc. for $C_{21}H_{30}O_{14}$: C, 49.80; H, 5.97%.)

4-O-β-D-*Glucopyranosyl*-D-*erythritol hepta-acetate* (**9**). Acetylation of **8** (10 mg) with Ac_2O (0.5 ml) and pyridine (0.5 ml) at room temp. overnight, followed by the usual work-up, yielded a colourless syrup, which was crystallized from *n*-hexane–Et_2O to give colourless needles of **9** (13 mg), mp 116–116.5°, $[\alpha]_D^{21}$ −12.1° ($CHCl_3$; *c* 0.58). (Found: C, 49.76; H, 5.87. Calc. for $C_{24}H_{34}O_{16}$: C, 49.82; H, 5.92%.) ^{1}H NMR ($CDCl_3$): δ 2.01–2.09 (3H × 7, *s*, OAc), 3.50–4.42 (7H, *m*), 4.53 (1H, *d*, *J* = 7 Hz, anomeric H), 4.90–5.35 (5H, *m*).

Lilioside E (**6**). Colourless viscous film, $[\alpha]_D^{20}$ −23.0° (H_2O; *c* 0.43), R_f 0.59 (TLC, $CHCl_3$–MeOH, 2:1). IR ν_{max}^{KBr} cm^{-1}: 3380, 1725, 1250; ^{1}H NMR (C_5D_5N): δ 1.96 (3H, *s*, OAc), 3.75–4.60 (many protons), 4.88 (1H, *d*, *J* = 7 Hz, anomeric H); GC: TMSi derivative, R_t 6.3 min (column temp. 230°).

Enzymatic hydrolysis of lilioside E (**6**). Lilioside E (**6**) (2 mg) was hydrolysed in H_2O (1 ml) with emulsin (0.6 mg) as described above for lilioside D (**5**). The resulting hydrolysate was dried and converted to the TMSi derivatives. GC, performed as described above for the hydrolysate of **5**, gave peaks of glucose (R_t 16.3 and

17.2 min), monoacetylglycerol (R_t 5.8 min) and glycerol (R_t 4.1 min).

Penta-acetyllilioside E (7). Acetylation of **6** (10 mg) with Ac_2O (0.5 ml) and pyridine (0.5 ml) at room temp. overnight, followed by the usual work-up gave a product, which was crystallized from *n*-hexane–Et_2O to afford colourless needles of penta-acetyllilioside E (12 mg), mp 107–108°. This compound was shown to be identical with hexa-acetyllilioside D (7) by mmp, TLC (C_6H_6–Me_2CO, 5:1). ^1H NMR ($CDCl_3$) and IR (KBr).

Acknowledgements—We wish to express our gratitude to Professor J. Baddiley, University of Newcastle, U.K. for valuable suggestions. We are also grateful to Dr. H. Kimura of the Faculty of Pharmaceutical Sciences, Kanazawa University, for identification of the plant material, and to Dr. Y. Nishikawa and Professor Y. Tsuda of the same Faculty of Kanazawa University for helpful discussions and encouragement. Thanks are also due to Mr. Y. Itatani and the members of the Central Analytical Laboratory of the same Faculty of Kanazawa University for ^1H and ^{13}C NMR spectral measurement and elemental analyses. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan, which is gratefully acknowledged.

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