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LILIOSIDE C, A GLYCEROL GLUCOSIDE FROM *LILIUM LANCIFOLIUM**

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Key Word Index—*Lilium lancifolium*; Liliaceae; tiger lily; glycerol glucoside; 3-*O*-β-D-glucopyranosyl-*sn*-glycerol; lilioside C; stereochemistry; synthesis.

Abstract—A new glycerol glucoside, lilioside C, has been isolated from the leaves and stems of *Lilium lancifolium*. Its structure including the configuration of the aglycone moiety has been elucidated by chemical and spectroscopic means and by its synthesis.

INTRODUCTION

In a previous paper [1], we reported the isolation of two new glycerol glucosides, lilioside A (**1**) and B (**2**), from *Lilium longiflorum*. They are, except for the widely occurring glycosylglycerides (glyceroglycolipids), the first examples of naturally occurring glycerol glucosides and are the first glycerol glycosides to be isolated from higher plants [1]. Their structures were determined, but the absolute configuration of C-2 of the glycerol part of **1** has not yet been settled.

This paper describes the isolation and structure determination of another new glycerol glucoside, lilioside C, from another *Lilium* species, *L. lancifolium*.

RESULTS AND DISCUSSION

Lilioside C (**4**), a colourless viscous syrup, was isolated from the aqueous 80% methanol extract of the fresh leaves and stems of the plant. Acetylation of **4** afforded a hexa-acetate (**5**), $C_{21}H_{30}O_{14}$, mp 115–116°. Acid hydrolysis of **4** gave glucose and glycerol in equimolar amounts. The IR and ^{1}H NMR spectra, and the TLC and GLC behaviour, of **4** and **5** were very similar to those of lilioside B (**2**) and its hexa-acetate (**3**), respectively, but they were not identical. In addition the crystals of **5** had a lower mp than those of **3**. These facts suggested that **4** was another new glycerol glucoside, namely a positional isomer of **2**, and that the glucose residue was linked to one of the two primary hydroxyl groups of glycerol. This suggestion was supported by a periodate oxidation study, in which **4** consumed *ca* 3 mol of oxidant with the formation of *ca* 1 mol each of formic acid and formaldehyde. Enzymatic hydrolysis of **4** with emulsin (β-glucosidase) also yielded glucose and glycerol in equimolar amounts. In the ^{1}H NMR spectra the anomeric proton signals of **4** and **5** were observed at δ 5.03 (*d*, $J = 7$ Hz) and δ 4.54 (*d*, $J = 7$ Hz), respectively. Therefore, **4** must have a β-glucosidic linkage. At this stage, however, the configuration of the glycerol moiety, which arose by an asymmetric substitution of glycerol, was uncertain.

The syntheses of a pair of diastereoisomers of 1-*O*-β-D-glucopyranosyl- and 3-*O*-β-D-glucopyranosyl-*sn*-glycerol had been reported by Brundish and Baddiley [2], but the mps and optical rotations of their corresponding hexa-acetates [mp 107° (EtOH), $[\alpha]_D - 54.6°$ ($CHCl_3$; *c* 0.5) and mp 144° (EtOH), $[\alpha]_D - 4.4°$ ($CHCl_3$; *c* 0.4) respectively] were all clearly distinct from those of **5** [mp 115–116°(*n*-hexane–Et_2O), $[\alpha]_D^{16} - 14.9°$($CHCl_3$; *c* 0.47)]. On the other hand, the glycerol glycosides so far obtained from glyceroglycolipids of higher plants [3] and micro-organisms [4] had all proved to be 3-glycosyl-*sn*-glycerol. Therefore, we first attempted the synthesis of 3-*O*-β-D-glucopyranosyl-*sn*-glycerol as an authentic sample by a method different from that of Brundish and Baddiley [2]. First we prepared 1,2-di-*O*-benzyl-*sn*-glycerol (**6**) according to Beving *et al.* [5] starting from D-mannitol, and characterized it as its triphenylmethyl ether (**7**), the mp and $[\alpha]_D$ of which were both identical with the reported values. In addition, the $[\alpha]_D$ of **7** was of equal magnitude and opposite sign as compared with that of the enantiomer, 2,3-di-*O*-benzyl-1-*O*-triphenylmethyl-*sn*-glycerol, reported by Wickberg [7]. Glucosidation of **6** with tetra-*O*-acetyl-α-D-glucopyranosyl bromide was carried out in alcohol-free chloroform in the presence of silver oxide, Drierite and a catalytic amount of iodine. This

*Part 2 in the series "Glycerol Glucosides in the *Lilium* Genus". For Part 1, see Kaneda, M., Mizutani, K., Takahashi, Y., Kurono, G. and Nishikawa, Y. (1974) *Tetrahedron Letters* 3937.

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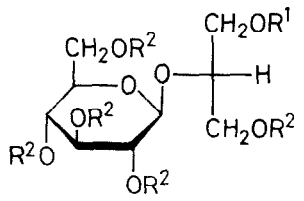
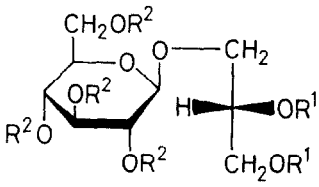
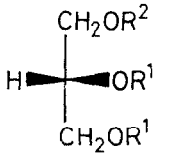


1 R^1=Ac, R^2=H
2 R^1=R^2=H
3 R^1=R^2=Ac



4 R^1=R^2=H
5 R^1=R^2=Ac
8 R^1=CH_2Ph, R^2=Ac
9 R^1=H, R^2=Ac
10 ≡**4**
11 ≡**5**



6 R^1=CH_2Ph, R^2=H
7 R^1=CH_2Ph, R^2=CPh_3

method followed Raynolds' and Evans' modification[6] of the Koenigs-Knorr reaction. The product, 1,2-di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-*sn*-glycerol (**8**), was subsequently debenzylated by catalytic hydrogenation over palladium-black in methanol to give colourless needles of 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-*sn*-glycerol (**9**), which was then deacetylated with sodium methoxide in methanol to afford a colourless viscous syrup of 3-*O*-β-D-glucopyranosyl-*sn*-glycerol (**10**). This glucoside exhibited identical TLC and GLC behaviour with lilioside C (**4**) and yielded glucose and glycerol on enzymatic hydrolysis with emulsin (β-glucosidase). Furthermore the hexa-acetate (**11**) of **10** showed the same mp and $[\alpha]_D$ (including sign) as the hexa-acetate (**5**) of **4**. Thus, the identity of the synthetic and natural glucoside was unequivocally established through the corresponding hexa-acetates, **11** and **5**, by comparison of their TLC, IR and ^{1}H NMR, and by the mmp which showed no depression.

From the above data, the structure of lilioside C (**4**) was established as (2*R*)-3-*O*-β-D-glucopyranosyl-*sn*-glycerol.

It is interesting from a chemotaxonomic viewpoint that glycerol glucosides such as lilioside A, B and C appear to be characteristic constituents of the *Lilium* genus and that the position of glucosidation and the stereochemistry of the glycerol moiety seem to vary according to the species in the genus. These points will be made much clearer by further studies of liliosides isolated from other *Lilium* spp. The results of such studies will be reported in future papers.

EXPERIMENTAL

All mps are uncorr. ^{1}H NMR: 100 MHz, TMS as int. standard, IR: KBr. Analytical TLC: Si gel G or GF 254 (Type 60, Merck), detection by spraying with 10% H_2SO_4 followed by heating. GLC: 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 a cultivated variety collected in Kanazawa city.

Extraction and isolation of **4**. Fresh leaves and stems (1.3 kg) of *L. lancifolium* 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 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 GLC. The fraction eluted with 5% EtOH gave a mixture of **4** and sucrose, which was separated on a Si gel (Wakogel C-200) column. The $CHCl_3$–MeOH (5 : 1 to 4 : 1) eluate afforded pure lilioside C (**4**) (120 mg).

Lilioside C (**4**). Colourless viscous syrup, $[\alpha]_D^{25}$ −31.5°(H_2O; *c* 1.27), 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 3.8–4.6 (many protons), 5.03 (1H, *d*, *J* = 7 Hz, anomeric H); GLC: TMSi derivative, R_t 5.5 min (column temp. 230°).

Hexa-acetyllilioside C (**5**). Treatment of **4** (40 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 **5** (54 mg), mp 115–116°, $[\alpha]_D^{16}$ −14.9° ($CHCl_3$; *c* 0.47). (Found: C, 49.95; H, 6.06. Calc. for $C_{21}H_{30}O_{14}$: C, 49.80; H, 5.97%). IR ν_{max}^{KBr} cm^{-1}: 1750, 1240, 1220; ^{1}H NMR($CDCl_3$): δ 2.01–2.12 (3H × 6, *s*, OAc), 3.62–4.42 (7H, *m*), 4.54 (1H, *d*, *J* = 7 Hz, anomeric H), 4.88–5.30 (4H, *m*).

Acid hydrolysis of **4**. A soln of **4** (2 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. GLC [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_f 16.3 and 17.2 min) and glycerol (R_f 4.1 min).

Enzymatic hydrolysis of **4**. To a soln of **4** (1.9 mg) in H_2O (1 ml) was added emulsin (β-glucosidase from almonds; Sigma) (0.5 mg) and the mixture 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 GLC, which was performed as described above. The results confirmed the composition to be an equimolar mixture of glucose and glycerol.

Periodate oxidation studies of **4**. Lilioside C (**4**) (3.5 mg) was dissolved in 0.015 M aq. $NaIO_4$ soln (10 ml). After allowing the mixture to stand at room temp. in the dark for 24 hr, samples were withdrawn and analysed. The periodate consumption was determined spectrophotometrically [8, 9], formaldehyde produced was determined by the arsenite reduction and chromotropic acid method [10] and formic acid produced was titrated with 0.005 M NaOH soln using phenol red as an indicator [11]. The results were as follows: 2.88 mol. equivalent of periodate was consumed, and 1.08 mol. equivalent of formaldehyde and 1.06 mol. equivalent of formic acid were produced after 24 hr.

1,2-*Di*-O-*benzyl*-sn-*glycerol* (**6**). This compound, prepared from D-mannitol by the method of Beving *et al.* [5], was obtained as a syrup, $[\alpha]_D^{28} - 6.32°$ ($CHCl_3$; *c* 2.5), which was shown to be pure by TLC (Et_2O–C_6H_6, 2:1).

Triphenylmethylation of **6**. A mixture of **6** (0.3 g), TrCl (0.3 g) and pyridine (1 ml) was stirred at room temp. for two nights and treated in the usual manner. Recrystallization from MeOH–EtOH (×2) afforded pale-pink needles of 1,2-di-*O*-benzyl-3-*O*-triphenylmethyl-*sn*-glycerol (**7**) (98 mg), mp 84–85°, $[\alpha]_D^{14} + 9.0°$ ($CHCl_3$; *c* 1.0) [lit. [5] mp 84–84.5°, $[\alpha]_D + 9.2°$ ($CHCl_3$; *c* 1)]; the data for the enantiomer [7] mp 84.5–86° $[\alpha]_D^{18} - 9°$ ($CHCl_3$; *c* 2.5). (Found: C, 83.68; H, 6.71. Calc. for $C_{36}H_{34}O_3$: C, 84.01; H, 6.66%.)

1,2-*Di*-O-*benzyl*-3-O-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-sn-*glycerol* (**8**). To a soln of 1,2-di-*O*-benzyl-*sn*-glycerol (**6**) (2.5 g) in dry, alcohol-free $CHCl_3$ (25 ml) were added Ag_2O (4.5 g) and Drierite (11.5 g). After stirring for 1 hr, I_2 (0.6 g) was added and then a soln of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (4.5 g) in dry, alcohol-free $CHCl_3$ (23 ml) was added dropwise to the stirred reaction mixture over a period of 1 hr. After stirring for a further 24 hr with the exclusion of moisture and light, the reaction mixture was filtered and the residue was washed with $CHCl_3$. The filtrate and washings were combined and washed with H_2O, dried over Na_2SO_4, and evaporated *in vacuo*. The residual yellow syrup was chromatographed on a Si gel (Wakogel C-200) column (C_6H_6–Me_2CO, 15:1) to afford a colourless syrup of **8** (4.1 g), $[\alpha]_D^{20} - 15.5°$ ($CHCl_3$; *c* 1), which showed one spot on TLC (C_6H_6–Me_2CO, 7:1).

Debenzylation of **8**. **8** (3 g) was hydrogenated with Pd-black (150 mg) in MeOH (35 ml) for 9.5 hr. Filtration and evaporation gave a colourless syrup, which crystallized from *n*-hexane–Et_2O–Me_2CO as colourless needles of 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-*sn*-glycerol (**9**) (1.9 g), mp 113–115°, $[\alpha]_D^{20} - 14.2°$ ($CHCl_3$; *c* 1.06). IR ν_{max}^{KBr} cm^{-1}: 3400–3500 (*br*, OH), 1760, 1230 (ester). (Found: C, 47.96; H, 6.35. Calc. for $C_{17}H_{26}O_{12}$: C, 48.34; H, 6.20%.)

3-O-β-D-*Glucopyranosyl*-sn-*glycerol* (**10**). To a soln of **9** (1 g) in dry MeOH (10 ml) was added 0.2 M NaOMe (3 ml) and the mixture stirred at room temp. for 3 hr. The Na^+ was removed by stirring with Dowex 50W-X8 (H^+ form) until the soln was neutral. The resin was filtered and the filtrate evaporated to yield a colourless viscous syrup of **10** (0.52 g), which was shown to be chromatographically pure (TLC and GLC) and exhibited identical behaviour with lilioside C (**4**) on TLC, GLC and on enzymatic hydrolysis by emulsin.

3-O-β-D-*Glucopyranosyl*-sn-*glycerol hexa-acetate* (**11**). Acetylation of **10** (10 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 **11** (11 mg), mp 115–116.5°, $[\alpha]_D^{28} - 14.0°$ ($CHCl_3$; *c* 1.0). (Found: C, 49.65: H, 6.00. Calc. for $C_{21}H_{30}O_{14}$: C, 49.80; H, 5.97%.) This compound was shown to be identical with the hexa-acetate (**5**) of lilioside C(**4**), by mmp, TLC (C_6H_6–Me_2CO; 5:1), ^1H NMR ($CDCl_3$) and IR (KBr).

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