C_6H_6–EtOAc 9 1) It gave green colour with $FeCl_3$, appeared yellow in visible light and was characterized as **2** by UV, ^{1}H NMR, IR and MS UV λ_{max}^{MeOH} nm 220, 250, 280, 310 ^{1}H NMR (DMSO-d_6, 80 MHz), δ1 62, 1 72 (*s*, *s*, 3H each, $=CMe_2$), 3 2 (*br*, 2H, CH_2), 3 92 (*s*, 3H, OMe), 5 08 (*br*, 1H, =CH), 6 58 (*d*, J = 8 8 Hz, 1H, H-6), 6 64 (*s*, 1H, H-4), 7.24 (*d*, J = 8 8 Hz, 1H, H-7), 10 91 (*br*, 1H, 5-OH), 11 02 (*br*, 1H, 1-OH), 11 03 (*br*, 1H, 8-OH) MS *m/z* (rel int) 343 (17 7), 342 (83 3), 327 (61 1), 326 (31 7), 299 (90 4), 287 (100), 286 (16 2), 285 (13 9), 274 (17 3), 271 (50 9)

Compound **2**, on acetylation with Ac_2O–pyridine and repeated cryst from $CHCl_3$–EtOH afforded the acetate **3** (30 mg), mp 172–175° UV $\lambda_{max}^{CHCl_3}$ nm 310, 360 ^{1}H NMR ($CDCl_3$, 60 MHz) δ1 69, 1 75 (*s*, *s*, 3H each, $=CMe_2$), 2 41 (*s*, 3H, OAc), 2 45 (*s*, 3H, OAc), 3 30 (*br*, 2H, CH_2), 3 93 (*s*, 3H, OMe), 5 10 (*br t*, =CH), 6 33 (*s*, 1H, H-4), 6 97 (*d*, 1H, J = 8 8 Hz, H-6) and 7 43 (*d*, 1H, J = 8 8 Hz, H-7); MS *m/z* (rel int) 426 (9), 384 (30 6), 341 (41 4), 326 (23 4), 298 (32 4), 286 (30 6), 56 (100)

The mother liquor of **2** on acetylation with Ac_2O–pyridine yielded **3** and **4** which were separated on PTLC silica-gel, C_6H_6–$CHCl_3$ 1 1) Compound **3** was identical with the acetate of **2** while **4** (25 mg), mp 162°, R_f 0 40 (silica gel, C_6H_6–$CHCl_3$ 1 1) showed UV $\lambda_{max}^{CHCl_3}$ nm 310, 360, ^{1}H NMR ($CDCl_3$, 60 MHz) δ1 70, 1 80 (*s*, *s*, 3H each, $=CMe_2$), 2.48 (*s*, 3H, OAc), 3 39 (*d*, J = 6 Hz, CH_2), 3 98 (*s*, 3H, OMe), 5 20 (*br t*, =CH), 6 43 (*s*, 1H, H-4), 7 40 (*d*, 1H, J = 9 Hz, H-8), 7 48 (*d*, 1H, J = 3 Hz, H-5), 8.38 (*d*, 1H, J = 9, 3 Hz, H-7) MS *m/z* (rel int) 368 (M^+, 88 7), 353 (34), 325 (80), 313 (100), 283 (50) and 271 (51) The deacetylation of a small quantity (15 mg) of **4** with EtOH–HCl yielded the parent compound **5** (10 mg), mp above 240°, MS *m/z* (rel int) 326 (M^+, 52 5), 311 (14), 271 (100) and 241 (10)

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REFERENCES

1 Sani, B P and Rao, P L N (1966) *Indian J Chem* **4**, 27
2. Sani, B P and Rao, P L N (1969) *Indian J Chem* **7**, 680
3. Rao, P L N and Verma, S C L (1951) *J Sci Ind Res (India)* **10B**, 184
4 Sultanbawa, M U S (1980) *Tetrahedron* **36**, 1465
5 Monache, G D, Monache, F D, Waterman, P G, Crichton, E G and Lima, R A D (1984) *Phytochemistry* **23**, 1757
6 Sen, A K, Sarkar, K. K, Majumdar, P C and Banerji, N (1986) *Indian J Chem* **25**, 1157
7 Govindachari, T R, Kalyanaraman, S P, Muthukumarswamy, N and Pai, B R (1971) *Tetrahedron* **27**, 3919
8 Perkin, A C (1913) *J Chem Soc* **103**, 657
9 Jafferson, A, Quillimann, A J and Scheinman, F (1970) *Aust J Chem* **23**, 2539
10 Parveen, M and Khan, N U (1987) *Chem Ind* 418
11 Carpenter, J, Lockslay, H D and Scheinmann, F (1969) *J Chem Soc (C)*, 486

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TWO XANTHONE GLYCOSIDES FROM *GENTIANA LUTEA*

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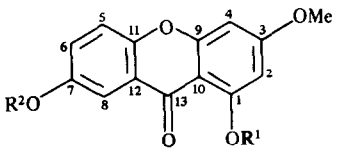
Key Word Index—*Gentiana lutea*, Gentianaceae; gentian, xanthone glycoside, 7-hydroxy-3-methoxy-1-*O*-primeverosylxanthone, 1-hydroxy-3-methoxy-7-*O*-primeverosylxanthone

Abstract—Two new xanthone glycosides have been isolated from the root of *Gentiana lutea* The structures were determined as 7-hydroxy-3-methoxy-1-*O*-primeverosylxanthone and 1-hydroxy-3-methoxy-7-*O*-primeverosylxanthone on the basis of spectroscopic evidence

INTRODUCTION

The dried root of *Gentiana lutea* (gentian) is one of the most important crude drugs used as bitter stomachic and sedative It is known to contain bitter glycosides, such as gentiopicroside [1–3] and amarogentin [4], and xanthone glycosides, gentioside [5, 6] and mangiferin [6] The gentian, used in Japan, is mainly imported from Europe. In past decades, it has been also cultivated in Hokkaido (Japan). The gentian imported from Europe has a yellowish brown internal colour, but that produced in Hokkaido has a white internal colour It is considered



1 R^1 = primeverose, R^2 = H
2 R^1 = H, R^2 = primeverose
Gentisin (**3**) R^1 = R^2 = H

that the difference between them is due to the drying process. In order to clarify the difference, we studied the components of the gentian produced in Hokkaido, and isolated two new xanthone glycosides. The present paper describes the isolation and structure determination of these xanthone glycosides.

RESULTS AND DISCUSSION

A methanol extract of the dried roots was separated into chloroform, *n*-butanol and water-soluble portions. Preparative liquid chromatography of the *n*-butanol-soluble portion on reversed phase column with water containing increasing amounts of methyl cyanide afforded two new xanthone glycosides (**1**, **2**) along with known xanthone glycoside, gentioside [5, 6].

Compound **1**, obtained as colourless needles, had a molecular formula $C_{25}H_{28}O_{14}$. IR absorption established the presence of hydroxyl group (3250 cm^{-1}), conjugated carbonyl (1640 cm^{-1}) and double bonds (1600 cm^{-1}). The UV spectrum showed the characteristic absorption of xanthone chromophore at 238, 251, 304 and 360 nm.

Acid hydrolysis of **1** afforded D-glucose, D-xylose and the aglycone (**3**), yellow needles, mp 268–269°, $C_{14}H_{10}O_5$. The UV spectrum of **3** showed maxima at 235, 257, 305 and 368 nm, a bathochromic shift with aluminium trichloride and the absence of shift with sodium acetate characteristic of the xanthone chromophore. The ^{1}H NMR spectrum (Table 1) showed signals due to five aromatic protons and a methoxyl goup located at 3-position. Signals of a pair of 1H doublets (J = 2 Hz) at δ 6.38 and 6.61 were assignable to the protons located at 2- and 4-positions. Three aromatic protons at δ 7 51 (1H, *d*, J = 9 Hz), 7.31 (1H, *dd*, J = 3 and 9 Hz) and 7 43 (1H, *d*, J = 3 Hz) were assignable to the protons located at 5-, 6- and 8-positions, respectively. Based on these spectral data and ^{13}C NMR spectral data (Table 2), **3** was identified as gentisin (1,7-dihydroxy-3-methoxyxanthone) [5, 10–12].

The ^{1}H NMR spectrum (Table 1) of **1** showed signals at δ 3 87 (3H, *s*, –OMe), 6.67 (1H, *d*, J = 2 Hz, 4-H), 7.35 (1H, *d*, J = 2 Hz, 2-H), 7.45 (1H, *d*, J = 9 Hz, 5-H), 7 59 (1H, *dd*, J = 3 and 9 Hz, 6-H) and 8 17 (1H, *d*, J = 3 Hz, 8-H) due to gentisin moiety. It also showed the signals at δ 4 94 (1H, *d*, J = 7 Hz) and 3 5–4.5 (12H, *m*) assignable to a sugar residue. The ^{13}C NMR spectrum showed the 11 carbon signals due to the sugar residue. The chemical shift of C-6 carbon of glucose moiety, shifted downfield to δ 65.6, indicated the existence of primeverosyl residue in **1** [12] It also showed (Table 2) the signal due to C-1 carbon of gentisin moiety at δ 159.2 ppm shifted upfield (–3 7 ppm) as compared with that of **3**. The signal due to a carbonyl carbon (C-13) also shifted upfield (–5.6 ppm) to δ 174.4 ppm suggested no chelation with the hydroxyl group at 1-position [12] These data clearly showed that the primeverosyl residue in **1** was attached to the C-1 hydroxyl group of gentisin (**3**). The result was supported by the absence of UV bathochromic shift [13] of **1** with $AlCl_3$. Thus, the structure of **1** was determined as 7-hydroxy-3-methoxy-1-*O*-primeverosylxanthone.

Compound (**2**), obtained as colourless needles, had a molecular formula $C_{25}H_{28}O_{14}$. The IR and UV spectra indicated the presence of xanthone chromophore as same as **1** Acid hydrolysis of **2** afforded D-glucose, D-xylose and gentisin (**3**). The ^{1}H NMR spectrum (Table 1) of **2** showed signals due to five aromatic protons and methoxyl protons of gentisin moiety. Furthermore, an anomeric proton at δ 5 62 (1H, *d*, J = 6 Hz) and multiplet signals at δ 4 0–5 0 (12H, *m*) were assignable to the sugar residue The ^{13}C NMR chemical shifts of 11 carbon signals, due to the sugar residue revealed the presence of primeverosyl residue in **2** The chemical shifts (Table 2) of aromatic carbons in *ortho* (C-6 and 8) and *para* (C-11) positions of C-7 carbon, shifted downfield to δ 125 6 (+1 0 ppm, C-6), 110.6 (+2.3 ppm, C-8) and 150.7 (+1.5 ppm, C-11) as compared with those of **3**, indicated the primeverosyl residue was attached to the C-7 hydroxyl group of the gentisin moiety This result was supported by the presence of UV bathochromic shift [13] of **2** with aluminium trichloride and the chemical shift of the carbonyl carbon (δ 174.7 ppm), suggesting monochelation with the hydroxyl group at 1-position [12] Consequently, the structure of **2** was determined as 1-hydroxy-3-methoxy-7-*O*-primeverosylxanthone.

HPLC analysis of the yellowish brown gentian imported from Europe showed that it also contain these two new xanthone glycosides and gentioside. These xanthone gly-

Table 1 ^{1}H NMR chemical shifts for compounds **1**–**3** (δ, ppm)

Compounds	2-H	4-H	5-H	6-H	8-H	–OMe
1*	7.35 (1H, *d*, J = 2)	6 67 (1H, *d*, J = 2)	7 45 (1H, *d*, J = 9)	7 59 (1H, *dd*, J = 3, 9)	8.17 (1H, *d*, J = 3)	3.87 (3H, *s*)
2*	6.55 (1H, *d*, J = 2)	6 50 (1H, *d*, J = 2)	7 53 (1H, *d*, J = 9)	7 93 (1H, *dd*, J = 3, 9)	8 20 (1H, *d*, J = 3)	3.82 (3H, *s*)
3†	6.38 (1H, *d*, J = 2)	6.61 (1H, *d*, J = 2)	7 51 (1H, *d*, J = 9)	7 31 (1H, *dd*, J = 3, 9)	7 43 (1H, *d*, J = 3)	3.89 (3H, *s*)

*In pyridine-d_5.
†In DMSO-d_6.

cosides were determined by HPLC, [Europe] **1**, 0.30 mg/g; **2**, 0.91 mg/g, gentioside, 1 51 mg/g, [Hokkaido]: **1**, 1 53 mg/g, **2**, 1 56 mg/g, gentioside, 1 31 mg/g The contents of xanthone glycosides in the yellowish brown gentian are smaller than those in the white one produced in Hokkaido Thus it is supposed that the colourless xanthone glycosides were hydrolysed to yellow xanthones during the drying process to give yellowish brown gentian

EXPERIMENTAL

Mps uncorr ^{1}H and ^{13}C NMR spectra were determined at 100 and 25 MHz, respectively, with TMS as int standard UV spectra were taken in MeOH Mass spectra were determined at 70 eV GC was carried out on a Hitachi model 163 gas chromatograph using a glass column (3mm i d × 2m) packed with 2% OV-1 on 80–100 mesh Chromosorb WAW DMCS at 265°, with N_2 carrier gas at a flow rate of 30 ml/min Prep LC was performed on a Waters' System 500 using a C_{18} cartridge column (5 7 cm i d × 30 cm) HPLC was performed on a Hitachi model 655 liquid chromatograph A Nucleosil $5C_{18}$ column (4 mm i d × 250 mm, Macherey–Nagel) was used for analysis The mobile phase was MeCN–H_2O–AcOH (20 80 1) The flow rate was 1 ml/min The peaks were detected at 280 nm

Extraction and isolation Dried, milled roots (200 g) of *Gentiana lutea*, cultivated at Hokkaido Prefectural Kitami Agricultural Experiment Station, were extracted three times with MeOH (200 ml) at room temp The combined extracts, after evaporation of MeOH under red pres., was dissolved in H_2O (200 ml) The aq soln was successively extracted with $CHCl_3$ (200 ml), EtOAc (200 ml) and *n*-BuOH (200 ml) The *n*-BuOH-soluble portion (7 95 g) was separated by prep LC on an octadecylsilyl bonded column, eluting with H_2O containing increasing amount of MeCN to give fractions containing **1**, **2** and gentioside, respectively Crystallization of these fractions from pyridine–H_2O afforded **1**, 86 1 mg, **2**, 86 4 mg and gentioside, 85 0 mg, showing single peaks by HPLC analysis, respectively Retention time **1**, 6 3 min, **2**, 15 5 min, gentioside 13 1 min

7-hydroxy-3-methoxy-1-O-primeverosylxanthone (**1**) Colourless needles, mp 259–260° (decomp) $[\alpha]_D^{20}$ −81 5° (pyridine, *c* 0 40) Found C, 54 05, H, 5 08 $C_{25}H_{28}O_{14}$ requires C, 54 35, H, 5 11% IR ν_{max}^{Nujol} cm^{-1} 3250, 1640, 1620, 1600, 1570 UV λ_{max}^{MeOH} nm (log ε) 238 (4 54, *sh*), 251 (4 55), 284 (4 08, *sh*), 304 (4 14), 360 (3 83) No change was observed when the spectra were determined on addition of NaOAc or $AlCl_3$ ^{13}C NMR (Table 2, DMSO-d_6) carbon signals of primeverosyl residue at δ 104 0, 102 5, 76 5, 75 9 (2C), 73 3 (2C), 69 7, 69 5, 68 6, 65 6

1-Hydroxy-3-methoxy-7-O-primeverosylxanthone (**2**) Colourless needles, mp 293–294° (decomp) $[\alpha]_D^{20}$ −69 4 (pyridine, *c* 0 36) Found. C, 54 22, H, 5 05 $C_{25}H_{28}O_{14}$ requires C, 54 35, H, 5 11% IR ν_{max}^{Nujol} cm^{-1} 3300, 1650, 1600, 1560 UV λ_{max}^{MeOH} nm (log ε) 235 (4 46), 256 (4 54), 305 (4 17), 360 (3 77) +$AlCl_3$ 227, 269, 327, 412 nm No change was observed when the spectrum was determined on addition of NaOAc ^{13}C NMR (Table 2, DMSO-d_6) carbon signals of primeverosyl residue at δ 104 0, 101 4, 76 4 (2C), 75 9, 73 3 (2C), 69 6 (2C), 68 3, 65 6

Acid hydrolysis of **1** *and* **2** A soln of **1** or **2** in conc HCl was heated at 100° for 15 min The reaction mixture was poured into H_2O and the H_2O insoluble part recrystallized from MeOH to give yellow needles of gentisin (**3**, 1,7-dihydroxy-3-methoxyxanthone) mp 268–269° EIMS *m/z* 258(M^+) Found C, 65 02, H, 3 79 $C_{14}H_{10}O_5$ require C, 65 12, H, 3 90% IR ν_{max}^{Nujol} cm^{-1} 3350, 1650, 1620, 1600, 1580 UV λ_{max}^{MeOH} nm (log ε) 235 (4 46), 257 (4 57), 305 (4 17), 368 (3 81) +$AlCl_3$ 230, 271, 324 and 416 nm

Table 2 ^{13}C NMR chemical shifts for compounds **1**–**3** (δ, ppm, in DMSO-d_6)

Compounds	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C=O (13)	OMe (14)
1	159 2	100 4	164 5	95 4	118 5	123 4	153 8	108 8	158 5	106 5	148 1	122 7	174 4	56 0
2	162.3	96 9	166 4	92 6	119 1	125.6	153 8	110 6	157 2	102.7	150 7	120 2	179 7	56 1
3	162 9	96 7	166.3	92 3	118 1	124.6	154 1	108 3	157 4	102 9	149 2	120 5	180 0	55 9

No change was observed when the spectrum was determined on addition of NaOAc.

The H_2O-soluble part was evapd to dryness for the identification of the sugar The residue was dissolved in 8% HCl–MeOH and refluxed for 16 hr. After evapn of the solvent, the residue was dissolved in dry pyridine and added with hexamethyldisilazane and trimethylchlorosilane The reagent was removed in a stream of N_2 and the residue was dissolved in MeOH GC analysis of the resulting soln showed the existence of methylglycoside TMS ethers of D-glucose (rt 8 8, 9.8 min) and D-xylose(room temp , 3.4, 3 7 min), by comparison with the authentic samples

Gentioside (1-*hydroxy*-7-*methoxy*-3-O-*primeverosylxanthone*) Colourless needles, mp 278–279° (decomp.) $[\alpha]_D^{20}$ −50.0° (pyridine; *c* 0 30) Found C, 54.15; H, 5.07 $C_{25}H_{28}O_{14}$ requires C, 54.35, H, 5 11%. IR ν_{max}^{Nujol} cm^{-1}: 3400, 1640, 1600, 1580 UV λ_{max}^{MeOH} nm (log ε) 234 (4 35), 258 (4 53), 300 (4 03), 368 (3.72) + $AlCl_3$ 231, 273, 318, 419 nm No change was observed when the spectrum was determined on addition of NaOAc. ^1H NMR (pyridine-d_5) δ 3 74 (3H, *s*, −OMe), 4.0–5.0 (12H, *m*), 5.71 (1H, *d*, *J* = 7 Hz), 6.85 (1H, *d*, *J* = 2 Hz, 4-H), 7.15 (1H, *d*, *J* = 2, 2-H), 7.36 (1H, *dd*, *J* = 3 and 9 Hz, 6-H), 7 70 (1H, *d*, *J* = 9 Hz, 5-H), 7 72 (1H, *d*, *J* = 3 Hz, 8-H) ^{13}C NMR (DMSO-d_6): δ 180.0 (C=O), 164 2 (C-3), 162.2 (C-1), 157 4 (C-9), 155.8 (C-7), 150.3 (C-11), 124.9 (C-6), 120 2 (C-12), 119 5 (C-5), 105 3 (C-8), 103.5 (C-10), 98 8 (C-2) 94 6 (C-4), 55.8 (OMe); carbon signals of primeverosyl residue at δ 104 2, 99.9, 76.5, 76.4, 75 6, 73 4, 73.1, 69 7, 69 6, 68 7. 65.6.

Acid hydrolysis of gentioside by the same treatment as **1** or **2**, gave D-glucose, D-xylose and isogentisin. D-glucose and D-xylose were identified by the same method for **1** or **2**.

Isogentisin (1,3-*dihydroxy*-7-*methoxyxanthone*). Yellow needles (MeOH), mp 241–242°, EIMS *m/z* 258 (M^+). Found: C, 64 93, H, 3 98 $C_{14}H_{10}O_5$ requires. C, 65.12; H, 3.90% IR ν_{max}^{Nujol} cm^{-1}: 3350, 1640, 1615, 1600, 1570. UV λ_{max}^{MeOH} nm (log ε). 234 (4.52), 258 (4.60), 308 (4 17), 366 (3 91) + $AlCl_3$. 230, 271, 324, 414 nm. + NaOAc· 230, 259, 268 (sh), 345, 380 (sh) nm ^1H NMR (pyridine-d_5)· δ 3.87 (3H, *s*, −OMe), 6 22 (1H, *d*, *J* = 2 Hz, 2-H), 6 39 (1H, *d*, *J* = 2, 4-H), 7.40 (1H, *dd*, *J* = 3 and 9 Hz, 6-H), 7.59 (1H, *d*, *J* = 9 Hz, 5-H), 7 52 (1H, *d*, *J* = 3 Hz, 8-H). ^{13}C NMR (DMSO-d_6) δ 179 3 (C=O), 165 7 (C-3), 162 9 (C-1), 157 4 (C-9), 155 6 (C-7), 150 6 (C-11), 123 8 (C-6), 120 3 (C-12), 118.7 (C-5), 105 6 (C-8), 102 1 (C-10), 98 1 (C-2), 93.9 (C-4), 55 6 (OMe)

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REFERENCES

1. Canonica, L , Pelizzoni, F , Manitto, P and Jommi, G (1961) *Tetrahedron* **16**, 192
2. Inoue, H , Yoshida, T , Nakamura, Y and Tobita, S (1968) *Tetrahhedron Letters* 4429
3. Inoue, H , Ueda, S and Nakamura, Y (1970) *Chem, Pharm Bull. (Tokyo)* **18**, 1856
4. Inoue, H and Nakamura, Y (1971) *Tetrahedron* **27**, 1951
5. Canonica, L and Pelizzoni, F (1955) *Gazz Chim. Ital* **85**, 1007
6. Bellmann, G and Jacot-Guillarmod, A (1973) *Helv Chim. Acta* **56**, 284
7. Hostettmann, K and Wagner, H (1977) *Phytochemistry* **16**, 821
8. Carpenter, I., Locksley, H D and Sheinmann,, F (1969) *Phytochemistry* **8**, 2013.
9. Roberts, J C (1961) *Chem. Rev* **61**, 591
10. Atkinson, J E , Gupta, P. and Lewis, J. R (1969) *Tetrahedron* **24**, 1507
11. Morimoto, I , Nozaka, T , Watanabe, F., Ishio, M , Hirose, Y and Okitsu, T. (1983) *Mutation Research* **116**, 103.
12. Miura, I , Hostettmann, K. and Nakanishi, K (1981) *Nouv J. Chemie* **2**, 653
13. Lins Mesquita, A A., De Barros Correa, D., Gottlieb, O R and Taveira Magalhaes, M (1968) *Anal. Chim Acta* **42**, 311
14. De Barros Correa, D., Fonseca E Silva, L. G , Gottlieb, O R and Janot Goncalves, S (1970) *Phytochemistry* **9**, 447