A Method to Identify the Absolute Configuration of Rhamnose, Lyxose, and 2,6-Dideoxy Sugars, Cymarose, Oleandrose, Diginose, and Digitoxose, Using a Chiral High-Performance Liquid Chromatography (HPLC) Column

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D-Diginose and L-diginose (2,6-dideoxy-3-*O*-methyl-*lyxo*-hexose) have been separated as the carbamoyl derivatives of the methyl glycosides by high-performance liquid chromatography (HPLC) using a chiral column (SUMIPAX OA-4100). In the cases of other 2,6-dideoxyhexoses, cymarose, oleandrose, and digitoxose, this column was more suitable for the enantiomeric separation than the column previously used (SUMIPAX OA-1000). The enantiomeric mixtures of cymarose obtained from *Cynanchum wilfordi* glycosides were analyzed by using the SUMIPAX OA-4100 column.

Further, the enantiomers of rhamnose and lyxose have been separated as the carbamoyl derivatives of the acetonides by means of the chiral column.

Keywords chiral HPLC column; absolute configuration; 2,6-dideoxy sugar; diginose; cymarose; oleandrose; digitoxose; rhamnose; lyxose; 3,5-dinitrophenylcarbamate

Some sugars occur in nature as both D- and L-series. We have already reported the structures of glycosides which contain D- and L-cymaroses (2,6-dideoxy-3-*O*-methyl-*ribo*-hexose) (**1**) in the sugar chains, isolated from *Cynanchum wilfordi*[1] and *C. africanum*[2] (Asclepiadaceae). Then, we developed a method to separate D- and L-series of 2,6-dideoxy sugars using a chiral high-performance liquid chromatography (HPLC) column (SUMIPAX OA-1000[3]) with a small amount of sample. In the course of the study, enantiomeric **1**,[4] oleandrose (2,6-dideoxy-3-*O*-methyl-*arabino*-hexose) (**2**),[5] and digitoxose (2,6-dideoxy-*ribo*-hexose) (**3**)[6] were optically resolved as the 3,5-dinitrophenylcarbamates of methyl cymarosides, 3,5-dinitrophenylcarbamates of methyl oleandrosides, and 1-*O*-(3,5-dinitrophenylcarbamoyl)-3,4-*O*-isopropylidene-α-digitoxopyranose, respectively, by the column. The technique has been applied to determine the chirality of 1—2 mg of **1**, **2**, and **3** obtained from less than 20 mg of glycoside,[2c] and should be very useful for the structural elucidation of glycosides containing the 2,6-dideoxy sugars. In the case of diginose (**4**), the peaks of the enantiomers of 3,5-dinitrophenylcarbamates of methyl diginosides were not separated enough on the HPLC to determine the absolute configuration of **4** with the column, but the application of another chiral column (SUMIPAX OA-4100[7]) made it possible to optically resolve the enantiomers. In this paper we wish to report the optical resolution of **4** and common sugars, rhamnose (**5**) and lyxose (**6**), by using the chiral column. In addition, we report a reinvestigation of **1**, **2**, and **3** and analyses of enantiomeric mixtures of **1** obtained from *C. wilfordi* glycosides[1a] using the chiral column.

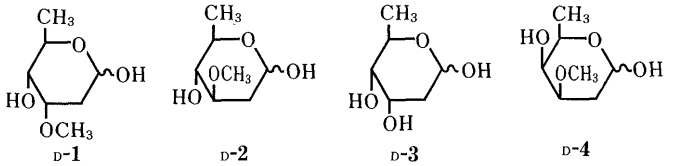

Chart 1

Results and Discussion

The chiral site of the packing material of SUMIPAX OA-4100[7] is a urea derivative of (*R*)-1-(α-naphthyl)-ethylamine, a π-electron donor, and (*S*)-valine chemically bonded to aminopropyl silica gel. So, for the optical resolution experiment, sugars were led to the 3,5-dinitrophenylcarbamoyl derivatives as π-electron acceptors, and consequently high sensitivity was obtained by the use of an ultraviolet (UV) detector.

So far as we know, D-**4** has hitherto been isolated only from plants.[8] On the other hand, L-**4** has been found in both plants[1,9] and metabolites[10] of micro-organisms. For the experiment in this paper, D-**4** derived from odoroside B,[8b] $[\alpha]_D$ +59.6°, and L-**4** from the crude glycoside of *C. wilfordi*,[1] $[\alpha]_D$ −60.6°, were used. Both enantiomers of **4** were converted to 3,5-dinitrophenylcarbamates of their methyl glycosides, as were **1**[4] and **2**.[5] Methylglycosylation of D-**4** usually gives methyl α- (**4a**) and β-diginopyranosides (**4b**) and methyl α- (**4c**) and β-diginofuranosides (**4d**) in the

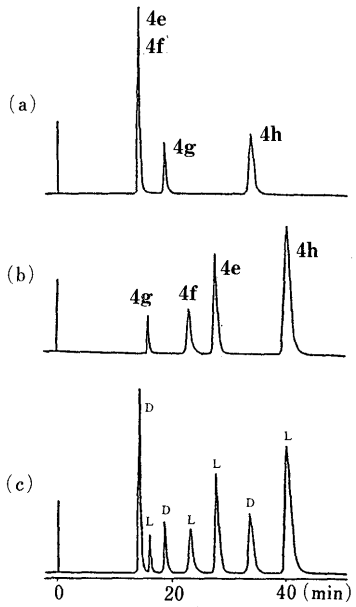

Fig. 1. HPLC Analyses of 3,5-Dinitrophenylcarbamates Derived from Methyl D- (a) and L-Diginosides (b), and the Enantiomeric Mixture (c)

Conditions: column, SUMIPAX OA-4100 (5 μ, 4 mm i.d. × 25 cm); mobile phase, hexane–ethanol (40 : 1); flow rate, 1.0 ml/min; detector, UV (254 nm).

ratio of *ca.* 3 : 1 : 1 : 3, based on the intensity of the anomeric proton signals in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum. Each mixture of methyl D- and L-diginosides (D- and L-**4a**—**d**) was allowed to react with 3,5-dinitrophenyl isocyanate in dry toluene in the presence of dry pyridine to afford mixtures of carbamates (D- and L-**4e**—**h**, respectively). Both mixtures (the mixture of D-**4e**—**h** and that of L-**4e**—**h**) and the enantiomeric mixture (the mixture of D- and L-**4e**—**h**) were analyzed by HPLC using the SUMIPAX OA-4100 column (Fig. 1). The mixture of carbamates derived from the D-enantiomer showed three peaks and that from the L-enantiomer showed four peaks. When **4** was an enantiomeric mixture, the seven peaks were completely separated from each other. Therefore, both enantiomers of **4** are optically distinguishable by the chiral column as the carbamoyl derivatives of the methyl glycosides. All the peaks were identified on the basis of the retention time of each carbamate (D- and L-**4e**—**h**). However, using the SUMIPAX OA-1000 column, peaks of both enantiomers overlapped each other, and hence the absolute configuration of **4** could not be determined by the analysis.

For the HPLC analysis, **5** was converted to 2,3-*O*-isopropylidene-α-rhamnofuranose (**5a**) with 2,2-dimethoxypropane, and this was allowed to react with 3,5-dinitrophenyl isocyanate in dry toluene in the presence of dry pyridine. Using two equivalents of the isocyanate, the 1,5-di-*O*-carbamate (**5c**) was obtained, and using one equivalent of the isocyanate, only the 5-*O*-carbamate (**5b**) was obtained. 2,3-*O*-Isopropylidene-α-lyxofuranose (**6a**) afforded the 5-*O*-carbamate (**6b**) and the 1,5-di-*O*-carbamate (**6c**) under the same conditions. Both enantiomeric pairs (**5b**, **5c**, **6b**, and **6c**) were analyzed by HPLC using the SUMIPAX OA-4100 column (Fig. 2). An enantiomeric mixture of **5b** and **5c** was optically resolved clearly. In the case of **6**, the retention time of **6c** was different for the D- and L-series but no difference was observed for **6b**. The method described in this paper makes it possible to determine the ratio of D- and L-enantiomers of **5** and **6** on the basis of the intensity of the peaks, if the sample is an enantiomeric mixture.

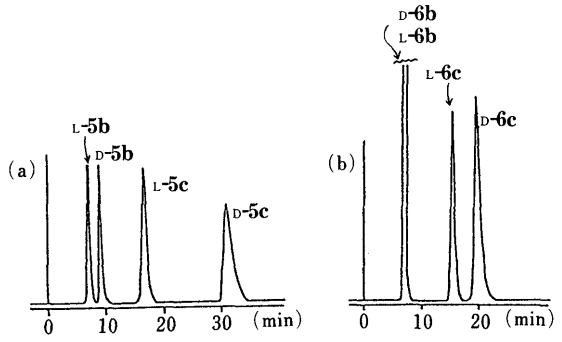

Fig. 2. HPLC Analyses of Enantiomeric Pairs: (a) 5-*O*-(3,5-Dinitrophenyl)carbamoyl-2,3-*O*-isopropylidene-α-rhamnofuranose (**5b**) and 1,5-Di-*O*-(3,5-dinitrophenyl)carbamoyl-2,3-*O*-isopropylidene-α-rhamnofuranose (**5c**) and (b) 5-*O*-(3,5-Dinitrophenyl)carbamoyl-2,3-*O*-isopropylidene-α-lyxofuranose (**6b**) and 1,5-Di-*O*-(3,5-dinitrophenyl)carbamoyl-2,3-*O*-isopropylidene-α-lyxofuranose (**6c**)

Conditions: column, SUMIPAX OA-4100 (5 μ, 4 mm i.d. × 25 cm); mobile phase, hexane–ethanol (3 : 1); flow rate, 1.0 ml/min; detector, UV (254 nm).

The reinvestigation of the carbamoyl derivatives of **1**—**3** using the SUMIPAX OA-4100 column was carried out as follows. On the methylglycosylation of **1**, methyl α- (**1c**), and β-cymarofuranosides (**1d**) are formed much more than methyl α- (**1a**) and β-cymaropyranosides (**1b**) (**1a** : **1b** : **1c** : **1d** = *ca.* 1 : 3 : 10 : 5). Using the SUMIPAX OA-4100 column, the retention times of the carbamoyl derivatives of **1a** and **1b** (**1e** and **1f**) were different for the D- and L-series on the HPLC, but the peaks of the enantiomers of the carbamates of **1c** and **1d**, which were produced much more than **1a** and **1b** (**1g** and **1h**), overlapped.[4] On the other hand, using the SUMIPAX OA-4100 column, both enantiomers of **1g** were optically resolved completely in addition to **1e** and **1f**, and the retention time of **1h** was a little different for the D- and L-series (Fig. 3). As for **2**, using the SUMIPAX OA-1000 column, enantiomers of the carbamoyl derivatives of methyl β-oleandropyranoside (**2b**) and methyl β-oleandrofuranoside (**2d**) (**2f** and **2h**) were optically resolved distinctly, but enantiomers of those of methyl α-oleandropyranoside (**2a**) and methyl α-oleandrofuranoside (**2c**) (**2e** and **2g**) were poorly resolved.[5] However, using the SUMIPAX OA-4100 column, the eight peaks were separated perfectly (Fig. 3). In the case of **3**, enantiomers of 1-*O*-(3,5-dinitrophenylcarbamoyl)-3,4-*O*-isopropylidene-α-digitoxopyranose (**3a**) were optically resolved completely within 8 min by the SUMIPAX OA-4100 column (Fig. 3), though, using the SUMIPAX OA-1000 column, the peaks of D-**3a** and L-**3a** were not separated perfectly.[6]

The relative quantities of D-**1** and L-**1** which exist in each sugar chain of wilfoside C3N (**7**), C1N (**8**), and C2N (**9**) isolated from *C. wilfordi*[1a] were 2/0 (D/L), 2/1, and 1/1, respectively, on the basis of the specific rotation. Each **1** from **7**, **8**, and **9** was converted into the carbamoyl derivatives and analyzed by HPLC with SUMIPAX OA-4100 (Fig. 4). Each chromatogram revealed the relative quantities of D-**1** and L-**1** on the basis of the intensity of the

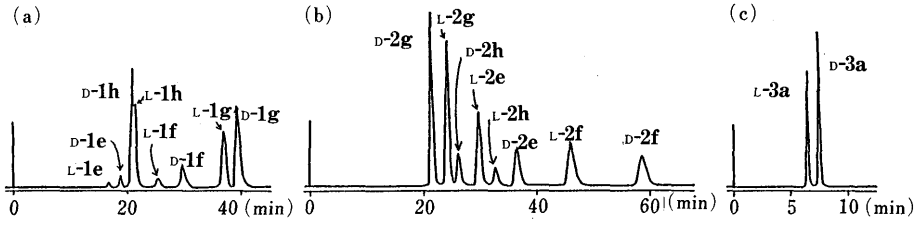

Fig. 3. HPLC Analyses of Enantiomeric Mixtures of 3,5-Dinitrophenylcarbamates Derived from Methyl Cymarosides (a), Methyl Oleandrosides (b), and 3,4-*O*-Isopropylidene-α-digitoxopyranose (c)

Conditions: column, SUMIPAX OA-4100 (5 μ, 4 mm i.d. × 25 cm); mobile phase, (a) hexane–ethanol (40 : 1), (b) hexane–1,2-dichloroethane (55 : 5 : 1), (c) hexane–ethanol (10 : 1); flow rate, 1.0 ml/min; detector, UV (254 nm).

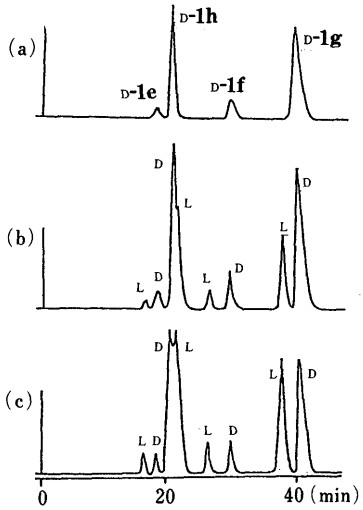

Fig. 4. HPLC Analyses of 3,5-Dinitrophenylcarbamates Derived from Methyl Glycosides of Cymarose Obtained from *Cynanchum wilfordi* Glycosides: (a) Wilfoside C3N, (b) Wilfoside C1N, and (c) Wilfoside C2N

Conditions: column, SUMIPAX OA-4100 (5 μ, 4 mm i.d. × 25 cm); mobile phase, hexane–ethanol (40 : 1); flow rate, 1.0 ml/min; detector, UV (254 nm).

peaks. The results of HPLC analyses of the samples agreed with the results based on the specific rotation.

Conclusion

The absolute configurations of **4**, **5**, and **6** were distinguished unequivocally after simple derivatization by HPLC analysis with a chiral column (SUMIPAX OA-4100) without measuring the optical rotation.

Hydrogen bonding association and charge–transfer interaction exist between the sugar derivatives and the column packing material.[7] The dicarbamates (**5c** and **6c**) were more tightly adsorbed on the packing material than the monocarbamates (**5b** and **6b**), and the retention times of the dicarbamates were longer than those of the monocarbamates. The D-series was adsorbed more strongly than the L-series except for **6b**, which suggested that the L-series was sterically hindered from association with the packing material.

The peaks of the samples derived from **1**, **2**, **3**, and **4** were each separated by this column much better than by the SUMIPAX OA-1000 column.[4-6] The chiral site of the packing material of SUMIPAX OA-1000[3] is (*S*)-1-(α-naphthyl)ethylamine bonded to silica gel, containing one asymmetric center. Both diastereomeric hydrogen bonding association and charge–transfer complexation contributed to the separation of enantiomers by HPLC using SUMIPAX OA-1000[3] and OA-4100,[7] and the excellent enantioselectivity of SUMIPAX OA-4100 was due to the two chiral constituents of the packing material.

The method described in this paper can also estimate the relative quantities of D- and L-isomers of **1**, **2**, **3**, **4**, **5**, and **6** on the basis of the intensity of the HPLC peaks when the sample is an enantiomeric mixture. It is very effective for the structural elucidation of small amounts of natural products containing **1**, **2**, **3**, **4**, **5**, or **6**.

Experimental

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter at room temperature. UV spectra were obtained with a Shimadzu UV-220 spectrometer. Infrared (IR) spectra were recorded on a JASCO A-102 spectrometer. ^{1}H-NMR spectra were run on a JEOL GX-270 (270.05 MHz) or GX-500 (500.00 MHz) machine in $CDCl_3$ or CD_3COCD_3 with tetramethylsilane (TMS) as an internal standard. Field-desorption mass spectra (FD-MS) was measured with a JEOL JMS-01SG-2. HPLC was conducted with a Waters 204 compact model, using a column of SUMIPAX OA-4100 (5 μ, 4 mm i.d. × 25 cm) (Sumitomo Chemical Co., Ltd.). Thin layer chromatography (TLC) was performed on Merck precoated plates (Kiesel gel F_{254}) with the following solvent systems: Rf_1 MeOH–$CHCl_3$ (5 : 95, v/v), Rf_2 hexane–ethyl acetate (1 : 3, v/v), Rf_3 hexane–ethyl acetate (5 : 4, v/v), Rf_4 $CHCl_3$–acetone (10 : 1, v/v), Rf_5 MeOH–$CHCl_3$ (1 : 9, v/v), and Rf_6 acetone–hexane (1 : 1, v/v). Column chromatography was carried out on Wakogel C-200 (200 mesh). D-Rhamnose was prepared from D-mannose,[11] and L-rhamnose, D- and L-lyxoses were commercial products.

Methylglycosylation of L-Diginose (L-4) Isolation of L-**4** was reported in the previous paper.[1a] A solution of L-**4** (233.5 mg) in MeOH (10 ml) was allowed to react with 1% H_2SO_4–MeOH (10 ml) at room temperature for 20 min, then H_2O (10 ml) was added and the reaction mixture was neutralized with saturated $Ba(OH)_2$. The precipitates were filtered off and the filtrate was evaporated to give a mixture of methyl L-diginosides. The product was chromatographed on silica gel with 0.5% MeOH–$CHCl_3$ to give methyl β-L-diginopyranoside (L-**4b**, 16.8 mg), methyl α-L-diginofuranoside (L-**4c**, 33.8 mg), and a mixture of methyl α-L-diginopyranoside (L-**4a**) and methyl β-L-diginofuranoside (L-**4d**). The mixture was rechromatographed on silica gel with ethyl acetate–hexane (2 : 5, v/v) to afford L-**4a** (50.5 mg) and L-**4d** (52.0 mg). These methyl L-diginosides were obtained as colorless syrups.

Methyl α-L-Diginopyranoside (L-4a) Rf_1 0.66 and Rf_2 0.36. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.32 (3H, d, J=6.6 Hz, 6-Me), 1.84 (1H, ddd, J=13.1, 11.6, 3.4 Hz, 2-CH_{ax}), 1.90 (1H, dddd, J=13.1, 5.5, 1.3, 1 Hz, 2-CH_{eq}), 3.33 (3H, s, 1-OMe), 3.39 (3H, s, 3-OMe), 3.62 (1H, ddd, J=11.6, 5.5, 3.1 Hz, 3-CH), 3.78 (1H, dt, J=3.1, 1 Hz, 4-CH), 3.83 (1H, dq, J=1, 6.6 Hz, 5-CH), 4.82 (1H, dd, J=3.4, 1.3 Hz, 1-CH).

Methyl β-L-Diginopyranoside (L-4b) Rf_1 0.56 and Rf_2 0.33. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.37 (3H, d, J=6.4 Hz, 6-Me), 1.67 (1H, ddd, J=12.5, 11.9, 9.8 Hz, 2-CH_{ax}), 2.02 (1H, ddd, J=12.5, 4.9, 2.1 Hz, 2-CH_{eq}), 3.35 (1H, ddd, J=11.9, 4.9, 3.1 Hz, 3-CH), 3.41 (3H, s, 3-OMe), 3.46 (1H, dq, J=2, 6.4 Hz, 5-CH), 3.50 (3H, s, 1-OMe), 3.71 (1H, dd, J=3.1, 2 Hz, 4-CH), 4.32 (1H, dd, J=9.8, 2.1 Hz, 1-CH).

Methyl α-L-Diginofuranoside (L-4c) Rf_1 0.87 and Rf_2 0.44. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.23 (3H, d, J=6.2 Hz, 6-Me), 2.11 (1H, ddd, J=13.9, 5.5, 4.8 Hz, 2-CH), 2.26 (1H, ddd, J=13.9, 7.0, 2.2 Hz, 2-CH), 3.31 (3H, s, 3-OMe), 3.41 (3H, s, 1-OMe), 3.72 (1H, dq, J=4.8, 6.2 Hz, 5-CH), 3.92 (1H, dd, J=4.8, 2.9 Hz, 4-CH), 4.02 (1H, ddd, J=7.0, 4.8, 2.9 Hz, 3-CH), 5.12 (1H, dd, J=5.5, 2.2 Hz, 1-CH).

Methyl β-L-Diginofuranoside (L-4d) Rf_1 0.70 and Rf_2 0.33. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.27 (3H, d, J=6.2 Hz, 6-Me), 2.00 (1H, ddd, J=14.3, 2.6, 1.1 Hz, 2-CH), 2.15 (1H, ddd, J=14.3, 7.7, 5.5 Hz, 2-CH), 3.34 (3H, s, 3-OMe), 3.39 (3H, s, 1-OMe), 3.74 (1H, ddd, J=7.7, 4.0, 2.6 Hz, 3-CH), 3.78 (1H, heptet, J=6.2 Hz, 5-CH), 3.86 (1H, dd, J=6.2, 4.0 Hz, 4-CH), 5.05 (1H, dd, J=5.5, 1.1 Hz, 1-CH).

Carbamoylation of a Mixture of Methyl L-Diginosides A solution of a mixture of methyl L-diginosides (7.2 mg) in dry toluene (0.5 ml) was allowed to react with 3,5-dinitrophenyl isocyanate (*ca.* 10 mg) in the presence of dry pyridine (0.05 ml) at 60 °C for 30 min, and then the solvent was evaporated off. The product was purified by column chromatography on silica gel using hexane–ethyl acetate (5 : 1, v/v) to give a carbamoyl mixture of methyl L-diginosides (12.0 mg).

Carbamoylation of a Mixture of Methyl L-Diginosides Carbamoylation of L-**4a** (10.9 mg), L-**4b** (11.2 mg), L-**4c** (13.3 mg), and L-**4d** (8.4 mg) was carried out under the same conditions as applied for the mixture of methyl L-diginosides. The products were chromatographed on silica gel using hexane–ethyl acetate (5 : 1, v/v) for L-**4e** and L-**4f** and (6 : 1, v/v) for L-**4g** and L-**4h** to give the carbamates L-**4e** (18.0 mg), L-**4f** (15.9 mg), L-**4g** (13.3 mg), and L-**4h** (18.1 mg), respectively, each of which crystallized as needles from hexane–ethyl acetate.

Methyl α-L-Diginopyranoside 3,5-Dinitrophenylcarbamate (L-4e) Rf_3 0.42 and Rf_4 0.37. mp 210—213 °C. $[\alpha]_D^{20}$ +79.6° (c=1.00, $CHCl_3$). *Anal.* Calcd for $C_{15}H_{19}N_3O_9$: C, 46.75; H, 4.97; N, 10.91. Found: C, 46.48; H, 5.00; N, 11.06. FD-MS m/z: 385 (M^+). UV λ_{max}^{EtOH} nm (log ε): 225 (4.29), 250 (4.03), 337 (3.30). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 1740, 1605, 1550, 1530, 1460, 1440, 1345, 1240, 1110, 1040, 800—700. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.26 (3H, d, J=6.6 Hz, 6-Me), 1.88 (1H, ddd, J=12.8, 11.7, 3.7 Hz, 2-CH_{ax}), 2.00 (1H, ddd, J=12.8, 5.5, 1 Hz, 2-CH_{eq}), 3.37 (3H, s, 1-OMe),

3.40 (3H, s, 3-OMe), 3.75 (1H, ddd, J=11.7, 5.5, 2.9 Hz, 3-CH), 4.02 (1H, dq, J=1, 6.6 Hz, 5-CH), 4.88 (1H, dd, J=3.7, 1 Hz, 1-CH), 5.26 (1H, dd, J=2.9, 1 Hz, 4-CH), 8.66 (2H, d, J=1.8 Hz, *o*-aromatic H), 8.72 (1H, t, J=1.8 Hz, *p*-aromatic H).

Methyl β-L-Diginopyranoside 3,5-Dinitrophenylcarbamate (L-4f) Rf_3 0.33 and Rf_4 0.33. mp 198—200 °C. $[\alpha]_D^{20}$ −19.4° (c=0.71, $CHCl_3$). *Anal.* Calcd for $C_{15}H_{19}N_3O_9$: C, 46.75; H, 4.97; N, 10.91. Found: C, 46.81; H, 5.14; N, 10.78. FD-MS m/z: 385 (M^+). UV λ_{max}^{EtOH} nm (log ε): 225 (4.21), 245 (3.96), 337 (3.19). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 1740, 1605, 1550, 1530, 1460, 1440, 1345, 1240, 1110, 1060, 800—700. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.33 (3H, d, J=6.6 Hz, 6-Me), 1.76 (1H, dt, J=9.9, 12.5 Hz, 2-CH_{ax}), 2.12 (1H, ddd, J=12.5, 3.3, 2.2 Hz, 2-CH_{eq}), 3.42 (3H, s, 3-OMe), 3.47 (1H, ddd, J=12.5, 4.8, 3.3 Hz, 3-CH), 3.55 (3H, s, 1-OMe), 3.65 (1H, dq, J=1.1, 6.6 Hz, 5-CH), 4.44 (1H, dd, J=9.9, 2.2 Hz, 1-CH), 5.19 (1H, dd, J=4.8, 1.1 Hz, 4-CH), 8.64 (2H, d, J=1.8 Hz, *o*-aromatic H), 8.72 (1H, t, J=1.8 Hz, *p*-aromatic H).

Methyl α-L-Diginofuranoside 3,5-Dinitrophenylcarbamate (L-4g) Rf_3 0.43 and Rf_4 0.32. mp 120—122 °C, $[\alpha]_D^{20}$ +28.0° (c=0.40, $CHCl_3$). *Anal.* Calcd for $C_{15}H_{19}N_3O_9$: C, 46.75; H, 4.97; N, 10.91. Found: C, 46.71; H, 5.04; N, 10.71. FD-MS m/z: 385 (M^+). UV λ_{max}^{EtOH} nm (log ε): 225 (4.34), 247 (4.09), 340 (3.32). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 1740, 1605, 1550, 1530, 1460, 1440, 1345, 1240, 1110, 1040, 800—700. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.38 (3H, d, J=6.2 Hz, 6-Me), 2.06 (1H, ddd, J=13.2, 6.6, 5.1 Hz, 2-CH), 2.30 (1H, ddd, J=13.2, 6.2, 1.8 Hz, 2-CH), 3.34 (3H, s, 3-OMe), 3.36 (3H, s, 1-OMe), 3.93 (1H, t, J=6.2 Hz, 4-CH), 3.96 (1H, dt, J=6.6, 6.2 Hz, 3-CH), 5.02 (1H, heptet, J=6.2 Hz, 5-CH), 5.09 (1H, dd, J=5.1, 1.8 Hz, 1-CH), 8.66 (2H, d, J=1.8 Hz, *o*-aromatic H), 8.70 (1H, t, J=1.8 Hz, *p*-aromatic H).

Methyl β-L-Diginofuranoside 3,5-Dinitrophenylcarbamate (L-4h) Rf_3 0.37 and Rf_4 0.45. mp 126—128 °C. $[\alpha]_D^{20}$ −54.9° (c=0.70, $CHCl_3$). *Anal.* Calcd for $C_{15}H_{19}N_3O_9$: C, 46.75; H, 4.97; N, 10.91. Found: C, 46.83; H, 5.06; N, 10.72. FD-MS m/z: 385 (M^+). UV λ_{max}^{EtOH} nm (log ε): 225 (4.11), 247 (3.87), 337 (3.28). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 1740, 1605, 1550, 1530, 1460, 1440, 1345, 1240, 1110, 1040, 800—700. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.42 (3H, d, J=6.2 Hz, 6-Me), 2.09 (1H, ddd, J=14.3, 2.6, 1.5 Hz, 2-CH), 2.20 (1H, ddd, J=14.3, 7.7, 5.1 Hz, 2-CH), 3.36 (3H, s, 3-OMe), 3.39 (3H, s, 1-OMe), 3.74 (1H, ddd, J=7.7, 4.4, 2.6 Hz, 3-CH), 4.11 (1H, dd, J=6.2, 4.4 Hz, 4-CH), 5.08 (1H, heptet, J=6.2 Hz, 5-CH), 5.10 (1H, dd, J=5.1, 1.5 Hz, 1-CH), 8.67 (2H, d, J=1.8 Hz, *o*-aromatic H), 8.71 (1H, t, J=1.8 Hz, *p*-aromatic H).

Acidic Hydrolysis of Odoroside B A solution of odoroside B (300 mg) in MeOH (60 ml) was reacted with 0.2 N H_2SO_4 (20 ml) at 60 °C for 30 min, then H_2O (60 ml) was added and the mixture was concentrated to 80 ml. The solution was kept at 60 °C for a further 30 min, and extracted with ether (80 ml). The ether layer was washed with saturated $NaHCO_3$ (20 ml × 3) and saturated NaCl (20 ml × 3), and the solvent was evaporated off to give uzarigenin (191.5 mg). The aqueous layer was neutralized with saturated $Ba(OH)_2$. The precipitates were filtered off and the filtrate was evaporated to give a syrup, which was chromatographed on silica gel with H_2O–MeOH–$CHCl_3$ (1 : 3 : 15, v/v, lower layer) to afford D-diginose (D-**4**, 54.9 mg), mp 90—91 °C, $[\alpha]_D^{17}$ +59.6° (c=1.48, H_2O).

Methylglycosylation and Carbamoylation of D-Diginose (D-4) Methylglycosylation of D-diginose (D-**4**, 54.9 mg) yielded a mixture of methyl D-diginosides (41.4 mg), and 26.5 mg of this mixture was separated into methyl α-D-diginopyranoside (D-**4a**, 10.4 mg), methyl β-D-diginopyranoside (D-**4b**, 33.5 mg), methyl α-D-diginofuranoside (D-**4c**, 3.9 mg), and methyl β-D-diginofuranoside (D-**4d**, 4.9 mg) by column chromatography. Carbamoylations of these methyl D-diginosides gave the carbamates D-**4e** (17.4 mg), $[\alpha]_D^{20}$ −78.3° (c=1.05, $CHCl_3$); D-**4f** (7.1 mg), $[\alpha]_D^{20}$ +20.0° (c=1.70, $CHCl_3$); D-**4g** (4.0 mg), $[\alpha]_D^{20}$ −29.3° (c=1.05, $CHCl_3$) and D-**4h** (7.0 mg), $[\alpha]_D^{20}$ +58.8° (c=1.01, $CHCl_3$); respectively. The mixture of methyl D-diginosides (14.9 mg) was carbamoylated to give a mixture (28.0 mg) of D-**4e**, D-**4f**, D-**4g**, and D-**4h**. The procedures of methylglycosylation, column chromatography, and carbamoylation were performed in the same way as for L-**4**.

2,3-*O*-Isopropylidene-α-D-rhamnofuranose (D-5a) A mixture of D-**5** (48.3 mg) 2,2-dimethoxypropane (0.5 ml), *N*,*N*-dimethylformamide (0.1 ml), and a small amount of *p*-toluenesulfonic acid was stirred at room te perature for 2 h. The mixture was neutralized with 2 N potassium carbonate solution, and extracted with ether (0.5 ml × 2). The aqueous layer was concentrated to dryness, and the residue was purified by column chromatography on silica gel using MeOH–$CHCl_3$ (7 : 93, v/v) to afford a colorless syrup (D-**5a**) (42.6 mg). Rf_5 0.33, Rf_6 0.47. $[\alpha]_D^{20}$ +21.4° (c=1.00, $CHCl_3$). FD-MS m/z: 205 [$(M+H)^+$, 23%], 189 (100%). IR ν_{max}^{Nujol} cm^{-1}: 3420. ^{1}H-NMR (270 MHz, CD_3COCD_3) δ: 1.20 (3H, d, J=6.2 Hz, 6-Me), 1.28, 1.37 (each 3H, s, CMe_2), 3.55 (1H, d, J=5.5 Hz, 5-COH), 3.79 (1H, dd, J=8.4, 3.7 Hz, 4-CH), 3.97 (1H, ddq, J=8.4, 5.5, 6.2 Hz, 5-CH), 4.53 (1H, d, J=5.9 Hz, 2-CH), 4.83 (1H, dd, J=5.9, 3.7 Hz, 3-CH), 5.22 (1H, d, J=3.7 Hz, 1-CH), 5.24 (1H, d, J=3.7 Hz, 1-COH).

5-*O*-(3,5-Dinitrophenyl)carbamoyl-2,3-*O*-isopropylidene-α-D-rhamnofuranose (D-5b) A mixture of D-**5a** (10.7 mg, 0.046 mmol), 3,5-dinitrophenyl isocyanate (9.6 mg, 0.046 mmol), dry toluene (0.5 ml), and dry pyridine (0.05 ml) was kept at 60 °C for 75 min. Then, the solvent was evaporated off, and the residue was purified by preparative TLC using MeOH–$CHCl_3$ (0.5 : 99.5, v/v) to yield a colorless syrup (D-**5b**) (12.4 mg, 0.029 mmol). Rf_5 0.47, Rf_6 0.06. $[\alpha]_D^{20}$ +30.7° (c=1.03, $CHCl_3$). FD-MS m/z: 413 (M^+, 27%), 398 (100%), UV λ_{max}^{EtOH} nm (log ε): 226 (5.00), 249 (4.74), 344 (3.88). IR ν_{max}^{Nujol} cm^{-1}: 3420, 1745, 1710, 1600, 1550, 1530, 1345. ^{1}H-NMR (270 MHz, CD_3COCD_3) δ: 1.26, 1.39 (each 3H, s, CMe_2), 1.40 (3H, d, J=6.2 Hz, 6-Me), 4.12 (1H, dd, J=7.7, 3.7 Hz, 4-CH), 4.58 (1H, d, J=5.9 Hz, 2-CH), 4.83 (1H, dd, J=5.9, 3.7 Hz, 3-CH), 5.15 (1H, dq, J=7.7, 6.2 Hz, 5-CH), 5.30 (1H, d, J=3.7 Hz, 1-CH), 5.47 (1H, d, J=3.7 Hz, 1-COH), 8.59 (1H, t, J=2.2 Hz, *p*-aromatic H), 8.89 (2H, d, J=2.2 Hz, *o*-aromatic H), 9.62 (1H, s, NH).

1,5-Di-*O*-(3,5-dinitrophenyl)carbamoyl-2,3-*O*-isopropylidene-α-D-rhamnofuranose (D-5c) Carbamoylation of D-**5a** (8.7 mg, 0.037 mmol) was carried out with 3,5-dinitrophenyl isocyanate (15.4 mg, 0.074 mmol) in the same way as described above to afford a colorless syrup (D-**5c**) (11.6 mg, 0.028 mmol). Rf_5 0.62, Rf_6 0.67. $[\alpha]_D^{20}$ +53.3° (c=1.04, $CHCl_3$). FD-MS m/z: 623 [$(M+H)^+$, 5%], 607 (22%), 398 (100%). UV λ_{max}^{EtOH} nm (log ε): 226 (4.88), 249 (4.60), 343 (3.81). IR ν_{max}^{Nujol} cm^{-1}: 3420, 1745, 1710, 1600, 1550, 1530, 1345. ^{1}H-NMR (270 MHz, CD_3COCD_3) δ: 1.31 (3H, s, CMe_2), 1.44 (3H, d, J=6.6 Hz, 6-Me), 1.47 (3H, s, CMe_2), 4.27 (1H, dd, J=6.6, 3.8 Hz, 4-CH), 4.92 (1H, d, J=6.0 Hz, 2-CH), 5.02 (1H, dd, J=6.0, 3.8 Hz, 3-CH), 5.24 (1H, quintet, J=6.6 Hz, 5-CH), 6.22 (1H, s, 1-CH), 8.59, 8.62 (each 1H, t, J=2.2 Hz, *p*-aromatic H), 8.87 (4H, d, J=2.2 Hz, *o*-aromatic H), 9.65, 9.79 (each 1H, s, NH).

2,3-*O*-Isopropylidene-α-L-rhamnofuranose (L-5a), 5-*O*-(3,5-Dinitrophenyl)carbamoyl-2,3-*O*-isopropylidene-α-L-rhamnofuranose (L-5b), and 1,5-Di-*O*-(3,5-dinitrophenyl)carbamoyl-2,3-*O*-isopropylidene-α-L-rhamnofuranose (L-5c) Under the same conditions as used for the D-isomers, L-**5a** (190.5 mg), $[\alpha]_D^{20}$ −19.7° (c=1.01, $CHCl_3$), derived from L-**5** (204.5 mg). Compound L-**5b** (16.2 mg, 0.039 mmol), $[\alpha]_D^{20}$ −28.0° (c=1.05, $CHCl_3$), was obtained from L-**5a** (12.8 mg, 0.055 mmol) by treatment with 3,5-dinitrophenyl isocyanate (11.5 mg, 0.055 mmol). Compound L-**5c** (19.1 mg, 0.030 mmol), $[\alpha]_D^{20}$ −55.6° (c=1.00, $CHCl_3$), was obtained from L-**5a** (10.4 mg, 0.045 mmol) by derivation with 3,5-dinitrophenyl isocyanate (18.8 mg, 0.090 mmol).

2,3-*O*-Isopropylidene-α-D-lyxofuranose (D-6a) A colorless syrup D-**6a** (140.3 mg) was prepared from D-**2** (205.1 mg) in the same way as D-**5**. Rf_5 0.23, Rf_6 0.30. $[\alpha]_D^{20}$ +59.6° (c=1.10, $CHCl_3$). FD-MS m/z: 191 [$(M+H)^+$, 16%], 175 (100%). IR ν_{max}^{Nujol} cm^{-1}: 3420. ^{1}H-NMR (270 MHz, CD_3COCD_3) δ: 1.27, 1.36 (each 3H, s, CMe_2), 3.59 (1H, dd, J=6.6, 5.5 Hz, 5-COH), 3.70, 3.80 (each 1H, ddd, J=11.4, 6.6, 5.5 Hz, 5-CH), 4.16 (1H, ddd, J=6.6, 5.5, 3.7 Hz, 4-CH), 4.53 (1H, d, J=5.9 Hz, 2-CH), 4.78 (1H, dd, J=5.9, 3.7 Hz, 3-CH), 5.21 (1H, d, J=3.7 Hz, 1-COH), 5.23 (1H, d, J=3.7 Hz, 1-CH).

5-*O*-(3,5-Dinitrophenyl)carbamoyl-2,3-*O*-isopropylidene-α-D-lyxofuranose (D-6b) Carbamoylation of D-**6a** (5.1 mg, 0.023 mmol) was achieved with 3,5-dinitrophenyl isocyanate (4.8 mg, 0.023 mmol) in the same way as described for D-**5a** to afford D-**6b** (3.9 mg, 0.0098 mmol) as a colorless syrup. Rf_5 0.43, Rf_6 0.57. $[\alpha]_D^{20}$ +49.0° (c=0.39, $CHCl_3$). FD-MS m/z: 400 [$(M+H)^+$, 13%], 384 (100%). UV λ_{max}^{EtOH} nm (log ε): 226 (4.74), 248 (4.47), 343 (3.64). IR ν_{max}^{Nujol} cm^{-1}: 3420, 1745, 1710, 1600, 1550, 1530, 1345. ^{1}H-NMR (270 MHz, CD_3COCD_3) δ: 1.30, 1.41 (each 3H, s, CMe_2), 4.32—4.55 (3H), 4.60 (1H, d, J=5.9 Hz, 2-CH), 4.89 (1H, dd, J=5.9, 3.3 Hz, 3-CH), 5.31 (1H, d, J=4.0 Hz, 1-CH), 5.46 (1H, d, J=4.0 Hz, 1-COH), 8.59 (1H, t, J=2.2 Hz, *p*-aromatic H), 8.89 (2H, d, J=2.2 Hz, *o*-aromatic H).

1,5-Di-*O*-(3,5-dinitrophenyl)carbamoyl-2,3-*O*-isopropylidene-α-D-lyxofuranose (D-6c) Compound D-**6c** (4.9 mg 0.0081 mmol) was obtained from D-**6a** (6.1 mg, 0.027 mmol) by derivation with 3,5-dinitrophenyl isocyanate (11.5 mg, 0.054 mmol) as a colorless syrup. Rf_5 0.57, Rf_6 0.65. $[\alpha]_D^{20}$ +32.0° (c=0.49, $CHCl_3$). FD-MS m/z: 608 (M^+, 10%), 593 (13%), 384 (100%). UV λ_{max}^{EtOH} nm (log ε): 226 (4.64), 249 (4.37), 343 (3.52). IR ν_{max}^{Nujol} cm^{-1}: 3420, 1745, 1710, 1600, 1550, 1530, 1345. ^{1}H-NMR (270 MHz, CD_3COCD_3) δ: 1.35, 1.48 (each 3H, s, CMe_2), 4.35 (1H, dd, J=11.7, 8.1 Hz, 5-CH), 4.48 (1H, ddd, J=8.1, 3.7, 3.0 Hz, 4-CH), 4.62 (1H, dd, J=11.7, 3.0 Hz, 5-CH), 4.95 (1H, d, J=5.9 Hz, 2-CH), 5.07 (1H, dd, J=5.9, 3.7 Hz, 3-CH), 6.23 (1H, s, 1-CH), 8.58, 8.62 (each 1H, t, J=2.2 Hz, *p*-

aromatic H), 8.87, 8.88 (each 2H, d, $J=2.2$ Hz, *o*-aromatic H), 9.75, 9.81 (each 1H, s, NH).

2,3-*O*-Isopropylidene-α-L-lyxofuranose (L-6a), 5-*O*-(3,5-Dinitrophenyl)-carbamoyl-2,3-*O*-isopropylidene-α-L-lyxofuranose (L-6b), and 1,5-Di-*O*-(3,5-dinitrophenyl)carbamoyl-2,3-*O*-isopropylidene-α-L-lyxofuranose (L-6c) Under the same conditions as used for the D-enantiomers, **L-6a** (191.8 mg), $[\alpha]_D^{20}$ −62.4° ($c=1.11$, $CHCl_3$), was obtained from **L-6** (221.0 mg). Compound **L-6b** (3.7 mg, 0.0093 mmol), $[\alpha]_D^{20}$ −52.3° ($c=0.39$, $CHCl_3$), was obtained from **L-6a** (4.0 mg, 0.018 mmol) by treatment with 3,5-dinitrophenyl isocyanate (3.8 mg, 0.018 mmol). Compound **L-6c** (4.8 mg, 0.0079 mmol), $[\alpha]_D^{20}$ −29.9° ($c=0.48$, $CHCl_3$), was obtained from **L-6a** (5.0 mg, 0.023 mmol) by derivation with 3,5-dinitrophenyl isocyanate (9.6 mg, 0.045 mmol).

Methylglycosylation and Carbamoylation of 1 from 7, 8, and 9 A solution of **1** (1.6 mg) from **7** in MeOH (2 ml) was stirred in the presence of Amberlite IR-120 (H^+) at room temperature for 30 min. The Amberlite was filtered off and the filtrate was evaporated to give a mixture of methyl cymarosides. The product was carbamoylated in the same way as described above. Further 2.0 mg of **1** from **8** and 1.8 mg of **1** from **9** were treated in the same way.

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