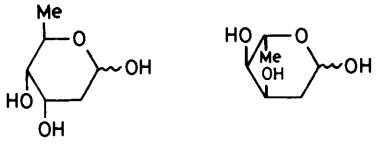
Identification of the Absolute Stereochemistry of D- and L-Digitoxose using a Chiral High-pressure Liquid Chromatography Column

Sachiko Tsukamoto, Koji Hayashi, and Kô Kaneko*
Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo 060, Japan

The absolute stereochemistry of D- and L-digitoxose was determined by h.p.l.c., on a chiral column, of the 1-*O*-(3,5-dinitrophenylcarbamoyl)-3,4-*O*-isopropylidene-α-D- and -L-digitoxopyranose.

We have reported the structures of glycosides isolated from *Cynanchum wilfordi* HEMSLEY[1] and *C. africanum* R. BR.[2] (Asclepiadaceae). The glycosides are unusual in that they contain both D- and L-cymarose in the sugar chain. For the structural elucidation of these glycosides, the ratio of D- to L-cymarose was determined on the basis of the optical rotation of cymarose obtained from the acidic hydrolysate of pure glycosides. However, a considerable quantity of the glycosides was required for the measurement, so we have studied a method of identification of the absolute stereochemistry of sugars using a chiral h.p.l.c. column. The absolute stereochemistry of D- and L-cymarose (2,6-dideoxy-3-*O*-methyl-*ribo*-hexose)[3] and D- and L-oleandrose (2,6-dideoxy-3-*O*-methyl-*arabino*-hexose)[4] has already been determined by h.p.l.c. using a chiral column (SUMIPAX OA-1000).[5] The optically active site of the packing material of the column is (*S*)-1-(α-naphthyl)ethylamine as a π-electron-donor chemically bonded on aminopropylsilica. Therefore, cymarose and oleandrose were converted into the respective methyl 4-*O*-(3,5-dinitrophenylcarbamoyl)glycosides as π-electron-acceptors for the h.p.l.c. analysis. In this paper we describe the results of an h.p.l.c. analysis of D- and L-digitoxose (2,6-dideoxy-*ribo*-hexose) on a chiral column (SUMIPAX OA-1000).



D-(1) L-(1)

Both D- and L-digitoxose, D- and L-(**1**), occur in Nature. The D-enantiomer is well known as a constituent of cardiac glycosides isolated from *Digitalis purpurea*[6] (Scrophulariaceae), *Strophanthus ledienii*[7] (Apocynaceae), *Euonymus atropurpurea*[8] (Celastraceae), *etc.* In addition, both the D- and the L-enantiomer have been found in metabolites of micro-organisms; the D-enantiomer in α-lipomycin[9] (*Streptomyces aureofaciens*) and the L-enantiomer in kijanimicin[10] (*Actinomadura kijaniata*) and tetracarcin A[11] (*Micromonospora chalcea*).

Results and Discussion

By the previously mentioned procedure, cymarose[3] and oleandrose[4] each gave the methyl 4-*O*-carbamoyl-α- and -β-furanoside in addition to the pyranosides. So, in order to prevent the formation of the furanoses, digitoxose (**1**) was converted into the 1-*O*-dinitrophenylcarbamate protected by an isopropylidene group at the vicinal C-3 and C-4 hydroxy groups. For this experiment, D-(**1**) was obtained from the crude glycoside of *C. wilfordi*[1] and L-(**1**) was derived from L-mannose by the procedure previously used.[12] The carbamates for the h.p.l.c. analysis were prepared by the procedure described below.

Treatment of digitoxose (**1**) with 2,2-dimethoxypropane (DMP) in *N*,*N*-dimethylformamide (DMF) containing a trace of toluene-*p*-sulphonic acid (PTSA)[13] gave 3,4-*O*-isopropylidenedigitoxopyranose (**2**) as a syrup. The ^{1}H n.m.r. spectrum of (**2**) showed that both the α- and the β-anomer of (**2**) were present as the chair (*C*) form on the basis of the coupling constants (Figure 1). The ratio of α- to β-anomer of (**2**) was dependent on

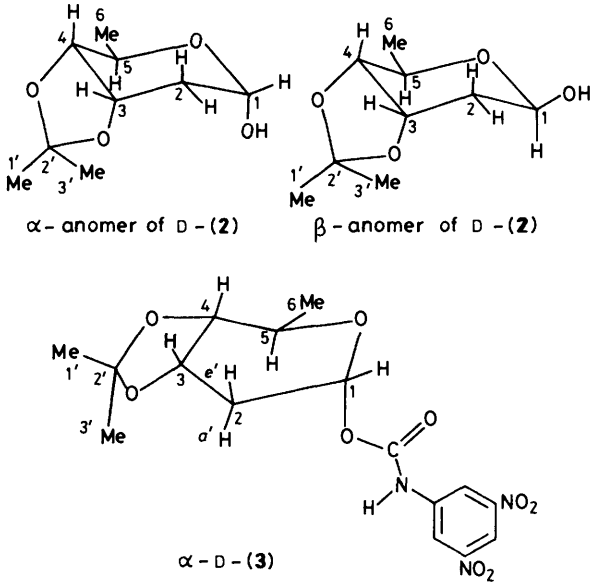


Figure 1. Conformation of α- and β-anomer of D-(**2**) and D-(**3**) in $CDCl_3$

the solvent used; α:β 1:1 in $CDCl_3$, ~1:4 in CD_3OD (Tables 1 and 2). The β-anomer exists preferentially in the more polar solvent, having a lower anomeric effect and being free from steric and dipole–dipole repulsions, which are present in the α-anomer, between the isopropylidene group and the anomeric hydroxy group.

Reaction of (**2**) with 3,5-dinitrophenyl isocyanate in toluene containing pyridine afforded 1-*O*-(3,5-dinitrophenylcarbamoyl)-3,4-*O*-isopropylidene-α-digitoxopyranose (**3**) in low yield, but no side-reaction occurred. In the ^{1}H n.m.r. spectrum of compound (**3**) (Tables 3 and 4) the coupling constants indicated the presence of the twist-boat (*TB*) form depicted in Figure 1 instead of the *C* form. Such a conformation is free from both the anomeric effect and the steric hindrance between the isopropylidene group and the carbamoyl group.

In order to investigate the relationship between the sub-

Table 1. ^{1}H N.m.r. chemical shifts (δ_H) for compound (**2**) in $CDCl_3$, C_5D_5N, CD_3COCD_3, and CD_3OD

	$CDCl_3$		C_5D_5N		CD_3COCD_3		CD_3OD	
Proton[a]	α (1 : 1)	β	α (1 : 2)	β	α (1 : 3)	β	α (1 : 4)	β
1	5.18	5.09	5.52	5.49	5.09	4.95	5.09	4.94
2_{ax}	2.12	1.86	2.26	2.18	1.82	1.78	1.84	1.80
2_{eq}	2.19	2.33	2.43	2.55	2.17	2.14	2.18	2.18
3	4.36	4.42	*ca.* 4.35	4.48	4.26	4.40	4.27	4.42
4	3.71	3.66	3.92	*ca.* 3.75	3.71	3.54	3.73	3.58
5	3.98	4.51	*ca.* 4.35	*ca.* 3.75	3.88	3.44	3.92	3.50
6	1.29	1.28	*c*	*c*	1.17	1.14	1.22	1.21
1'[b]	1.36	1.35	*c*	*c*	1.28	1.28	*c*	1.43
3'[b]	1.53	1.47	*c*	*c*	1.40	1.39	*c*	1.32

[a] In $CDCl_3$ and CD_3COCD_3 OH resonances were exhibited at δ_H 3.83 (α)/3.00(β) and 5.01(α)/5.50(β), respectively. [b] Assignments are interchangeable. [c] Not determined.

Table 2. ^{1}H-{^{1}H} coupling constants (J/Hz) for compound (**2**) in $CDCl_3$, C_5D_5N, CD_3COCD_3, and CD_3OD

	$CDCl_3$		C_5D_5N		CD_3COCD_3		CD_3OD	
J[a]	α	β	α	β	α	β	α	β
$1,2_{ax}$	4.4	9.2	5.9	9.2	5.5	9.2	5.5	9.5
$1,2_{eq}$	3.3	2.6	5.9	2.6	5.5	2.6	5.5	2.6
$2_{ax},2_{eq}$	14.8	14.7	14.3	14.7	14.8	14.7	14.3	14.7
$2_{ax},3$	4.4	4.8	8.1	4.8	8.2	4.8	7.7	4.8
$2_{eq},3$	4.4	2.6	5.9	2.2	5.5	2.6	5.5	2.2
3,4	5.2	4.8	6.6	*b*	6.6	4.8	6.6	4.8
4,5	8.8	9.2	8.8	*b*	8.8	9.2	8.8	9.2
5,6	6.2	6.2	*b*	*b*	6.2	5.9	6.2	5.9

[a] In $CDCl_3$ and CD_3COCD_3 $J_{1\text{-H,1-OH}}$ were 8.1(α)/5.1(β) and 5.5(α)/5.9(β) Hz, respectively. [b] Not determined.

stituent at the C-1 hydroxy group of (**2**) and the conformation, methyl glycoside (**4**) and acetate (**5**) were prepared from compound (**2**). Because of the sensitivity of compound (**2**) toward acids, methylglycosylation of (**2**) was carried out with pyridinium toluene-*p*-sulphonate (PTPS). The ^{1}H n.m.r. spectrum of the product (**4**) showed a pair of signals of equal intensity for the α- and β-anomer in chair form (Tables 3 and 4). Acetylation of compound (**2**) was carried out in the usual way. The ^{1}H n.m.r. spectrum of the product (**5**) showed three signals for the anomeric protons at δ 4.52, 6.06, and 6.00 (*ca.* 2:1:5), suggesting that the product (**5**) was a mixture of three isomers (Tables 3 and 4). The analysis of their coupling patterns indicated that they were two α-anomers in twist-boat (*TB*) form and *C* form, respectively, and one β-anomer in chair form. The anomeric protons of compounds (**3**) and (**5**) in twist-boat (*TB*) form appeared at higher field, resulting from the anisotropic effect by the carbonyl group of each substituent at the C-1 position.

H.p.l.c. analysis using a chiral column (SUMIPAX OA-1000) of each enantiomer of compound (**3**) was performed with hexane-1,2-dichloroethane–ethanol as mobile phase. The retention times of the D- and L-enantiomer were apparently different as illustrated in Figure 2. Therefore, D-(**1**) and L-(**1**) are optically distinguishable on the chiral h.p.l.c. column by means of their 1-*O*-carbamoyl-3,4-*O*-isopropylidene derivatives.

Conclusions

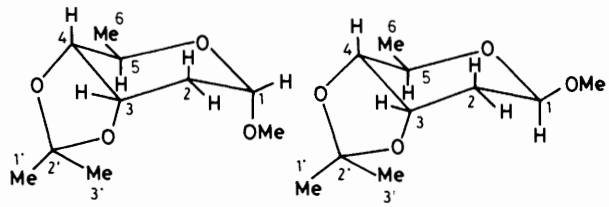
H.p.l.c. analysis with a chiral column makes it possible not only to define the absolute stereochemistry of digitoxose (**1**) unequivocally, without measuring the optical rotation, but also to analyse small amounts of sample by using a u.v. detector. It is suitable for the structural elucidation of small amounts of glycosides and antibiotics containing digitoxose (**1**).

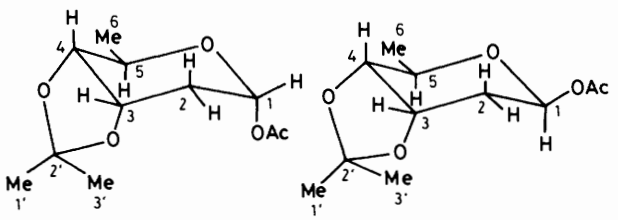
Table 3. ^{1}H N.m.r. chemical shifts (δ_H) for compounds (**3**), (**4**), and (**5**) in $CDCl_3$

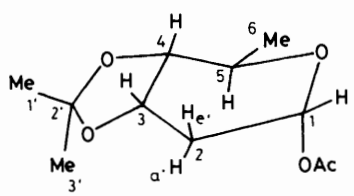
		(**4**)[c]		(**5**)[d]		
Proton	(**3**)[b] α-*TB*	α-*C* (1 :	β-*C* 1)	α-*TB* (2 :	α-*C* 1 :	β-*C* 5)
1	4.53	4.68	4.63	4.52	6.06	6.00
$2_{ax}(e')$	1.84	2.01	1.91	1.77	*e*	2.08
$2_{eq}(a')$	1.76	2.19	2.23	1.65	*e*	2.23
3	3.93	4.26	4.41	3.86	4.29	4.45
4	4.42	3.76	3.66	4.42	*e*	3.70
5	3.91	3.69	3.48	3.85	*e*	3.62
6	1.23	1.29	1.27	1.13	1.31	1.29
1'[a]	1.44	1.34	1.35	1.41	*e*	1.36
3'[a]	1.52	1.49	1.47	1.49	*e*	1.48

[a] Assignments are interchangeable. [b] Compound (**3**) exhibited *o*-and *p*-aromatic protons at δ_H 8.65 and 8.74, respectively. [c] Compound (**4**) exhibited 1-*O*-Me protons at δ_H 3.37(α) and 3.48(β). [d] Compound (**5**) exhibited 1-*O*-Ac protons at δ_H 2.06 (α-*TB*), 2.08(α-*C*), and 2.09(β-*C*). [e] Not determined.

C, chair form; *TB*, twist-boat form.







Experimental

Optical rotations were measured in $CHCl_3$ with a JASCO DIP-4 digital polarimeter at room temperature. U.v. spectra

Table 4. ^1H-{^1H} coupling constants (J/Hz) for compounds (**3**), (**4**), and (**5**) in $CDCl_3$

	(3)[a]	(4)		(5)		
J	*α-TB*	*α-C*	*β-C*	*α-TB*	*α-C*	*β-C*
$1,2_{ax}(e')$	8.1	4.4	8.8	8.4	5.5	8.1
$1,2_{eq}(a')$	3.3	5.1	2.6	3.3	5.5	2.9
$2_{ax}(e'),2_{eq}(a')$	14.4	14.7	14.7	14.3	*b*	14.7
$2_{ax}(e'),3$	2.9	5.8	4.8	2.6	6.3	5.1
$2_{eq}(a'),3$	9.5	5.8	2.6	9.9	6.3	3.7
3,4	9.5	5.8	4.8	9.9	6.3	5.1
4,5	9.5	10.3	9.5	9.9	*b*	9.2
5,6	6.2	6.2	5.9	5.9	5.9	5.9

[a] Coupling constants for aromatic protons were 1.8 Hz. [b] Not determined.

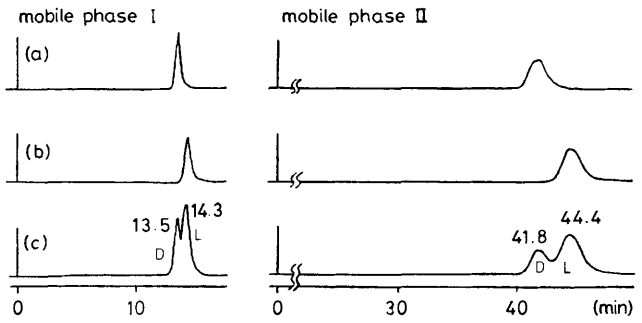


Figure 2. H.p.l.c. analysis of (a) D-(**3**), (b) L-(**3**), and (c) a mixture of D-(**3**) and L-(**3**) (1:1). Conditions: column, SUMIPAX OA-1000 (5μ; 4 mm i.d. × 15 cm); mobile phase, hexane 1,2-dichloroethane–ethanol (I, 150:3:8; II. 150:1:3); flow rate, 1.0 ml min^{-1}; detector, u.v. (254 nm)

were obtained in ethanol with a Shimadzu UV-220 spectrometer, and absorption maxima are given in nm. I.r. spectra were recorded in $CHCl_3$ on a JASCO A-102 spectrometer. ^1H N.m.r. spectra were run on a JEOL GX-270 (270 MHz) spectrometer with tetramethylsilane as internal standard. Field desorption-mass spectroscopy (f.d.–m.s.) was carried out with a JEOL JMS-01SG-2 mass spectrometer. H.p.l.c. was conducted with a Waters 204 compact model, using SUMIPAX OA-1000 (5μ; 4 mm i.d. × 15 cm) (Sumitomo Chemical Co., Ltd.). T.l.c. was performed on Merck precoated plates (Kiesgel gel 60 F_{254}) with AcOEt–hexane (1:2). Column chromatography was carried out on Wakogel C-200 (200 mesh).

3,4-O-*Isopropylidene*-D-*digitoxopyranose* D-(**2**).—A mixture of D-(**1**) (20.3 mg), DMP (50.9 mg), and a trace of PTSA in DMF (1 ml) was stirred at room temperature. After 45 min, t.l.c. showed no starting material remained and the mixture was poured into aqueous potassium carbonate. The syrupy product was extracted with chloroform and the extract was washed with water, and dried ($MgSO_4$). Removal of the solvent under reduced pressure and chromatography of the residue on silica gel [AcOEt–hexane(1:3) as eluant] gave the isopropylidene derivative (**2**) (15.4 mg) as a syrup, R_F 0.31; $[\alpha]_D^{27}$ +58.1° (*c* 1.50 in $CHCl_3$); $\nu_{max.}$ 3 400 cm^{-1}; $\delta_H(CDCl_3)$ in Tables 1 and 2; f.d.–m.s. *m/z* 188 (M^+).

1-O-(3,5-*Dinitrophenylcarbamoyl*)-3,4-O-*isopropylidene*-α-D-*digitoxopyranose* D-(**3**).—A solution of the isopropylidene derivative D-(**2**) (10.7 mg) and 3,5-dinitrophenyl isocyanate (*ca.* 15 mg) in toluene (0.5 ml) containing pyridine (0.05 ml) was heated at 60 °C for 1 h. Removal of the solvent under reduced pressure and chromatography of the residue on silica gel [AcOEt–hexane (1:5) as eluant] gave the 3,5-dinitrophenylcarbamate D-(**3**) (2.8 g) as a syrup, R_F 0.47, $[\alpha]_D^{27}$ −6.4° (*c* 0.22 in $CHCl_3$); $\nu_{max.}$ 3 420, 1 705, 1 600, 1 550, 1 530, 1 460, 1 440, 1 345, 1 240, 1 115, 800—700, and 660 cm^{-1}; $\lambda_{max.}$(EtOH) 226 (log ε 4.48), 247 (4.23), and 340 nm (3.42); $\delta_H(CDCl_3)$ in Tables 3 and 4.

An Anomeric Mixture of Methyl 3,4-O-*Isopropylidene*-D-*digitoxopyranosides* D-(**4**).—A solution of the isopropylidene derivative D-(**2**) (6.0 mg) and PTPS (*ca.* 5 mg) in dichloromethane (1 ml) containing MeOH (0.05 ml) was stirred at room temperature. After 53 h, t.l.c. showed two spots for products. Then, the solution was washed successively with aqueous sodium hydrogen carbonate and water, and dried ($MgSO_4$). Removal of the solvent under reduced pressure and column chromatography of the residue on silica gel [AcOEt–hexane (1:3) as eluant] gave an anomeric mixture of the methyl glycopyranosides D-(**4**) (1.0 mg) as a syrup, which was used for n.m.r. measurements without further purification due to the small amount available, R_F 0.65 and 0.55; $\delta_H(CDCl_3)$ in Tables 3 and 4.

1-O-*Acetyl*-3,4-O-*isopropylidene*-D-*digitoxopyranose* D-(**5**).—A mixture of the isopropylidene compound D-(**2**) (2.2 mg), anhydrous acetic acid (0.4 ml), and pyridine (0.5 ml) was stirred at room temperature. After 35 min, t.l.c. [AcOEt–hexane (1:2)] showed no starting material remained, and the mixture was worked up according to the standard procedure to give the acetate D-(**5**) (2.3 mg), R_F 0.63; $\nu_{max.}$ 1 740 cm^{-1}; $\delta_H(CDCl_3)$ in Tables 3 and 4.

3,4-O-*Isopropylidene*-L-*digitoxopyranose* L-(**2**).—A mixture of L-(**1**) (12.0 mg), DMP (30.6 mg), and a trace of PTSA in DMF (0.5 ml) was stirred at room temperature. After 1 h t.l.c. showed no starting material remained and the mixture was worked up by the same procedure described above to give the isopropylidene derivative L-(**2**) (7.5 mg) as a syrup.

1-O-(3,5-*Dinitrophenylcarbamoyl*)-3,4-O-*isopropylidene*-α-L-*digitoxopyranose* L-(**3**).—A mixture of the isopropylidene derivative L-(**2**) (2.5 mg) and 3,5-dinitrophenyl isocyanate (*ca.* 5 mg) in toluene (0.3 ml) containing pyridine (0.03 ml) was heated at 60 °C. After 30 min, the mixture was worked up by the same procedure described above to give the 3,5-dinitrophenylcarbamate L-(**3**) (0.5 mg) as a syrup.

References

1 S. Tsukamoto, K. Hayashi, and H. Mitsuhashi, *Tetrahedron*, 1985, **41**, 927; S. Tsukamoto, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.*, 1985, **33**, 2294.
2 S. Tsukamoto, K. Hayashi, H. Mitsuhashi, F. O. Snyckers, and T. G. Fourie, *Chem. Pharm. Bull.*, 1985, **33**, 4807; S. Tsukamoto, K. Hayashi, K. Kaneko, H. Mitsuhashi, F. O. Snyckers, and T. G. Fourie, *ibid.*, 1986, **34**, 1337.
3 S. Tsukamoto, K. Hayashi, K. Kaneko, and H. Mitsuhashi, *Chem. Pharm. Bull.*, 1986, **34**, 3130.
4 S. Tsukamoto, K. Hayashi, K. Kaneko, and H. Mitsuhashi, *J. Chem. Soc., Perkin Trans. 1*, 1988, 787.
5 N. Ôi, M. Nagase, and T. Doi, *J. Chromatogr.*, 1983, **257**, 111.
6 T. Reichstein, *Angew. Chem.*, 1951, **63**, 412.
7 H. Lichti, C. Tamm, and T. Reichstein, *Helv. Chim. Acta*, 1956, **39**, 1914.
8 R. Tschesche, S. Wirtz, and G. Snatzke, *Chem. Ber.*, 1955, **88**, 1619.
9 A. Zeek, *Justus Liebigs Ann. Chem.*, 1975, 2079.

10 A. K. Mallams, M. S. Puar, and P. R. Rossman, *J. Am. Chem. Soc.*, 1981, **103**, 3938.
11 F. Tomita, T. Tamaoki, K. Shirahata, M. Kasai, M. Morimoto, S. Ohkubo, K. Mineura, and S. Ishii, *J. Antibiot.*, 1980, **33**, 668.
12 D. M. Clode, D. Horton, and W. Weckerle, *Carbohydr. Res.*, 1976, **49**, 305; J. S. Brimacombe, R. Hanna, M. S. Saeed, and L. C. N. Tucker, *J. Chem. Soc., Perkin Trans. 1*, 1982, 2583.
13 M. E. Evans, F. W. Parrish, and L. Long, Jr., *Carbohydr. Res.*, 1967, **3**, 453.

Received 17*th August* 1987; *Paper* 7/1511