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^{13}C-Nuclear Magnetic Resonance (NMR) Spectra of *O*-Acylglucoses. Additivity of Shift Parameters and Its Application to Structure Elucidations[1]

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CMR spectra of all positional isomers of mono-*O*-myristoyl-α- and β-D-glucopyranoses in pyridine-d_5 are reported and discussed in connection with the anomalies of 1α- and 1β-*O*-acyl derivatives. Calculation of the chemical shifts of some dimyristates using the acylation shifts obtained from monomyristate (Table III) gave values in agreement with the observed values for each compound, confirming the additivity of the acylation shift parameters.

The effects of the solvent and the nature of the acyl moiety on the acylation shifts also examined using twenty samples, and it was concluded that the acylation shift parameters are independent of the solvent(s) and the kind(s) of acyl group(s), so that the above shift values obtained for the myristoyl group can be regarded as a universal set of additive parameters for usual di- and tri-acyl derivatives. This additivity rule of parameters was successfully applied to the structure elucidation of two naturally occurring acylglucose derivatives, tuliposide-A and spirarin. The previously reported data for various glucosyl γ-nitropropanoates in acetone-d_6 are compared with the values calculated from the above parameters, again showing fair agreement.

Keywords——^{13}C-NMR; solvent effect; effect of acyl group; additivity rule; steric interaction effect; 1-*O*-acylglucoses; hemiacetal group; tuliposide-A; spirarin; acylation shift parameters

In the previous paper[3] we reported a systematic study of ^{13}C-NMR (CMR) acylation shifts induced by myristoylation on each hydroxyl group of methyl α- and β-D-glucopyranosides, showing that the shifts on each carbon atom in the glucose moiety are additive parameters. Anomalies in the shifts of 2-*O*-acyl-β-D-glucosides, particularly at C-2, were noted, and their origin was discussed. In this paper we deal with the CMR spectra of acylglucoses, which usually exist (in solutions) as a mixture of α- and β-anomers, hence giving more complex spectra than those of acylglucosides. Systematic study of this subject is desirable since there are various naturally occurring and synthetic acylglucose derivatives which require identification. These are sometimes obtained as mixtures and structure determinations by chemical means are usually laborious.

First, we will deal with the CMR spectra of all positionally isomeric mono-*O*-myristoyl-α- and β-D-glucopyranoses, myristoyl being chosen as an acyl group for comparison with data obtained for the corresponding glucosides.[3] The discussion will then be extended to di-*O*-myristates, and finally to acylglucoses carrying various acyl group(s). Solvent effects on the acylation shifts are also discussed.

One of the most important conclusions in the present study is that the acylation shifts are independent of solvents and kind(s) of acyl groups (as long as the inductive effects of acyl chains do not differ very much), so that the shift values obtained for the myristoyl group can conveniently be adopted as a universal set of parameters.

1) Part IV of "Utilization of Sugars in Organic Synthesis." Part III: Y. Tsuda, T. Nunozawa, K. Nitta, and Y. Yamamoto, *Chem. Pharm. Bull.*, **28**, 920 (1980).
2) Location: *13-1 Takara-machi, Kanazawa 920, Japan.*
3) K. Yoshimoto, Y. Itatani, K. Shibata, and Y. Tsuda, *Chem. Pharm. Bull.*, **28**, 208 (1980).

Experimental

Materials——All *O*-myristoylglucoses, except **6α**, were reported previously.[4] They are in the $^4C_1$ pyranose conformation, on the basis of ^{1}H-NMR data.

2- and 4-*O*-Myristate (**2** and **4**) were mutarotated mixtures of anomers in approximately equal amounts when dissolved in pyridine-d_5 (py-d_5). 3- and 6-*O*-Myristate (**3** and **5**), though they are α-anomers in the crystalline form, anomarized to give 5:4—1:1 anomeric mixtures on standing in py-d_5 at room temp. for 30 hr. 2,3- and 4,6-Di-*O*-myristate (**7** and **8**) were α-anomers in fresh py-d_5 solution.

1α,6-Di-*O*-myristate (**6α**) was prepared by acylation of **1α** with myristoyl chloride in pyridine–CH_2Cl_2, as colorless needles, mp 67—70°. IR (ν_{max}^{KBr} cm^{-1}): 1715, 1740. ^{1}H-NMR (py-d_5) δ: 6.85 (1H, d, J=3.8 Hz, C^1–H).

6-*O*-Cinnamate (**10**) was prepared by direct acylation of glucose with cinnamoyl chloride in pyridine, as colorless needles, mp 144—147° (from acetone–benzene). IR (ν_{max}^{KBr} cm^{-1}): 1700, 1635. ^{1}H-NMR (py-d_5) δ: 5.89 [d, J=3.8 Hz, C^1-H (α-anomer)], 5.34 [d, J=7.5 Hz, C^1-H (β-anomer)]. It was a mutarotated mixture of anomers in py-d_5.

Measurements of NMR Spectra——Natural abundance ^{1}H noise-decoupled ^{13}C FT NMR (at 25.0 MHz) spectra were recorded on a JEOL FX-100 FT NMR spectrometer using 5 mm spinning tubes at 24.5°. Samples were dissolved, in most cases, in py-d_5. Tetramethylsilane (Me_4Si) served as an internal reference (δ0). Concentrations were about 0.1—0.3 mmol/ml. FT NMR measurement conditions were as follows: spectral width, 6024 Hz; pulse flipping angle, 45°; acquisition time, 0.6799s; number of data points, 8192. Accuracies of δ value were thus about ±0.1 ppm.

Factors Which May Influence the ^{13}C Chemical Shifts——Concentration: The NMR spectra were measured at various concentrations of 2,3-*O*-isopropylidene-D-glycerol[5] (from 0.257 to 5.194 mmol/ml) and 1,2-propyleneglycol (from 0.355 to 6.880 mmol/ml), both in acetone-d_6 solution, at 24.5°. In both cases δ_C of each signal changed within the range of ±0.3 ppm.

Temperature: The spectra of 2,3-*O*-isopropylidene-D-glycerol in acetone-d_6 at 24.5° and 60° were compared. The difference of each signal at the two temperatures was within the range of 0.2 ppm. The literature data[6] on various glycosides in py-d_5 at 30° and 100° also indicate that the signal deviations between these two temperatures are at most ±0.4 ppm.

Table I. ^{13}C Chemical Shifts (δ_C) of Glucopyranoses in Various Solvents (at 24.5°)[a]

Solvent	Carbon number					
	C-1	C-2	C-3	C-4	C-5	C-6
α-Anomer						
py-d_5[b]	94.0	74.3	75.2	72.4	73.5	63.1
Methanol-d_4	93.9	73.9	74.9	72.0	73.0	62.8
D_2O[c]	93.3	72.7	74.0	70.9	72.7	62.1
	(93.3)[d]	(72.8)	(74.2)	(70.9)	(72.5)	(62.3)
Acetone-d_6[e]	92.9	72.8	73.9	70.9	72.2	61.9
β-Anomer						
py-d_5[b]	98.7	76.7	78.5	71.9	78.3	62.9
Methanol-d_4	98.2	76.3	78.1	71.8	78.0	62.8
D_2O[c]	97.2	75.4	77.1	70.9	77.0	61.9
	(97.2)[d]	(75.5)	(77.1)	(70.9)	(77.1)	(62.3)
Acetone-d_6[e]	97.0	75.3	77.0	70.7	76.8	61.9

a) For signal assignment in D_2O, see A.S. Perlin and H.J. Koch (see below).
b) Assignments at C-4 and C-6 are based on the effect of orientational difference of the anomeric hydroxyl group (see Table IV).
c) Internal reference with dioxane (67.81 ppm).
d) Parenthetical data are those of A.S. Perlin and H.J. Koch, *Can. J. Chem.*, **48**, 2596 (1970).
e) Extrapolated data from 1:4 to 4:1 mixtures of acetone-d_6 and H_2O; P.E. Pfeffer and K.M. Valentine, *Carbohydr. Res.*, **73**, 1 (1979).

4) K. Yoshimoto, K. Tahara, S. Suzuki, K. Sasaki, Y. Nishikawa, and Y. Tsuda, *Chem. Pharm. Bull.*, **27**, 2661 (1979).
5) E. Baer and H.O.L. Fischer, *J. Biol. Chem.*, **128**, 463 (1939).
6) S. Seo, Y. Tomita, K. Tori, and Y. Yoshimura, *J. Am. Chem. Soc.*, **100**, 3331 (1978).

Internal *vs.* External References: Some literature data[7] are available on the ^{13}C chemical shifts of glucose and its derivatives relative to external Me_4Si. In acetone-d_6 solution, the correction of the shift value between external and internal reference is +0.53 ppm, as determined by measurement of external Me_4Si from internal Me_4Si at 24.5°.

TABLE II. ^{13}C Chemical Shifts (δ_C) of Mono-*O*-myristoyl-D-glucopyranoses in Pyridine-d_5

Compd.	Carbon number					
	C-1	C-2	C-3	C-4	C-5	C-6
1-*O*- **1α**	93.6	72.3	75.2	71.3	76.8	62.4
1-*O*- **1β**	95.8	74.1	78.4	71.0	79.3	62.1
2-*O*- **2α**	91.1	75.9[a]	72.2[a]	72.7	73.5	63.0
2-*O*- **2β**	96.6	77.0[a]	76.4[a]	72.3	78.7	62.8
3-*O*- **3α**	94.0	72.6	77.5	70.1	73.6	62.6
3-*O*- **3β**	98.8	74.9	79.5	69.9	78.4	62.5
4-*O*- **4α**	93.9	74.6	72.7	73.4	71.5	62.7
4-*O*- **4β**	98.9	77.0	76.0	72.9	76.3	62.5
6-*O*- **5α**	94.1	74.3	75.2	72.3	70.8	65.1
6-*O*- **5β**	98.8	76.8	78.3	71.7	75.2	65.1

a) Assignments were confirmed by ^1H selective hetero-spin decoupling. For ^1H signal assignment, see refs. 3), 4), and 8).

TABLE III. Acylation Shift Parameters for D-Glucopyranoses and Methyl D-Glucopyranosides (in Parentheses)

Compd.	Carbon number					
	C-1	C-2	C-3	C-4	C-5	C-6
1-*O*-Acyl α	−0.4[a]	−2.0	0	−1.1	+3.3[a]	−0.7
1-*O*-Acyl β	−2.9[a]	−2.6	−0.1	−0.9	+1.0[a]	−0.8
2-*O*-Acyl α	−2.9(−3.3)	+1.6(+1.1)	−3.0(−3.1)	+0.3(0)	0 (+0.1)	−0.1(−0.3)
2-*O*-Acyl β	−2.1(−2.8)	+0.3[a](−0.3)	−2.1(−2.3)	+0.4(+0.2)	+0.4(+0.3)	−0.1(−0.2)
3-*O*-Acyl α	0 (−0.1)	−1.7(−1.8)	+2.3(+1.9)	−2.3(−2.4)	+0.1(0)	−0.5(−0.6)
3-*O*-Acyl β	+0.1(−0.1)	−1.8(−2.0)	+1.0(+0.6)	−2.0(−2.1)	+0.1(−0.1)	−0.4(−0.5)
4-*O*-Acyl α	−0.1(−0.1)	+0.3(+0.2)	−2.5(−2.6)	+1.0(+0.9)	−2.0(−2.1)	−0.4(−0.5)
4-*O*-Acyl β	+0.2(0)	+0.3(+0.2)	−2.5(−2.7)	+1.0(+0.9)	−2.0(−2.2)	−0.4(−0.3)
6-*O*-Acyl α	+0.1(+0.1)	0 (0)	0 (0)	−0.1(−0.1)	−2.7(−2.9)	+2.0(+1.8)
6-*O*-Acyl β	+0.1(+0.1)	+0.1(−0.1)	−0.2(−0.1)	−0.2(−0.1)	−3.1(−3.1)	+2.2(+1.9)

a) Anomalies of these shifts are discussed in the text.

TABLE IV. Steric Interaction Effects of Anomeric Hydroxyl and Methoxyl Functions (in Parentheses)

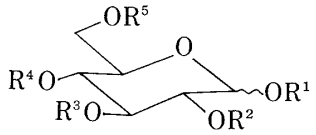
Compd.	Δδ (α-Anomer—β-Anomer) Carbon number					
	C-1 (α)	C-2 (β)	C-3 (γ)	C-4 (δ)	C-5 (γ)	C-6 (δ)
D-Glucose	−4.7(−4.2)	−2.4(−1.3)	−3.3(−3.1)	+0.5(+0.5)	−4.8(−4.3)	+0.2(+0.2)
2-*O*-Acyl	−5.5(−4.7)	−1.1(+0.1)[a]	−4.2(−3.9)	+0.4(+0.3)	−5.2(−4.5)	+0.2(+0.1)
3-*O*-Acyl	−4.8(−4.2)	−2.3(−1.1)	−2.0(−1.8)[a]	+0.2(+0.2)	−4.8(−4.2)	+0.1(+0.1)
4-*O*-Acyl	−5.0(−4.3)	−2.4(−1.3)	−3.3(−3.0)	+0.5(+0.5)	−4.8(−4.2)	+0.2(0)
6-*O*-Acyl	−4.7(−4.2)	−2.5(−1.2)	−3.1(−3.0)	+0.6(+0.5)	−4.4(−4.1)	0 (+0.1)

a) These values are anomalous compared with the others.

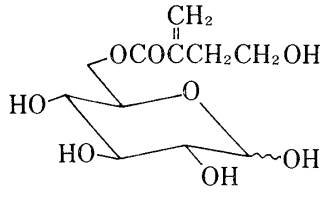
7) P.E. Pfeffer and K.M. Valentine, *Carbohydr. Res.*, **73**, 1 (1979).
8) P.E. Pfeffer, G.G. Moore, P.D. Hoagland, and E.S. Rothman, "Synthetic Methods for Carbohydrates," ed. by H.S. El Khadem, ACS, Symposium, 39, p. 155, 1976.

Assignments of ^{13}C-NMR Signals——Differentiation of the signals of α- and β-anomers was done by comparison of the signal intensities of the spectra measured immediately after dissolving the material and after 30 hr. The signals were assigned as described in the previous paper,[3] using known chemical shift rules and literature data on glucose, methyl *O*-acylglucosides, and analogous compounds. Assignments were confirmed, if necessary and if possible, by the ^{1}H selective hetero-spin decoupling technique.

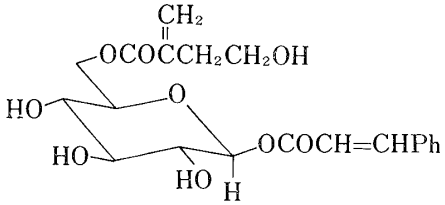
For mono-*O*-myristate, if ambiguity arose, the assignments were chosen so as to produce the best fit between observed and calculated values when the shift parameters thereof were applied to di-*O*-acyl derivatives, and to produce a reasonable steric compression effect on each carbon atom.



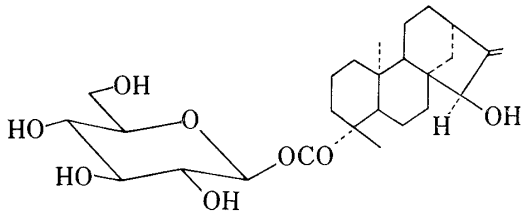
Compd.	R^1	R^2	R^3	R^4	R^5
1	$CH_3(CH_2)_{12}CO$	H	H	H	H
2	H	$CH_3(CH_2)_{12}CO$	H	H	H
3	H	H	$CH_3(CH_2)_{12}CO$	H	H
4	H	H	H	$CH_3(CH_2)_{12}CO$	H
5	H	H	H	H	$CH_3(CH_2)_{12}CO$
6	$CH_3(CH_2)_{12}CO$	H	H	H	$CH_3(CH_2)_{12}CO$
7	H	$CH_3(CH_2)_{12}CO$	$CH_3(CH_2)_{12}CO$	H	H
8	H	H	H	$CH_3(CH_2)_{12}CO$	$CH_3(CH_2)_{12}CO$
9	$CH_3(CH_2)_{16}CO$	H	H	H	H
10	H	H	H	H	PhCH=CHCO
11	H	$CH_3(CH_2)_{14}CO$	H	H	H
12	CH_3CO	CH_3CO	CH_3CO	CH_3CO	CH_3CO
13	PhCH=CHCO	H	H	H	H
14	H	$CH_3(CH_2)_{12}CO$	H	H	$CH_3(CH_2)_{12}CO$
16	H	H	CH_3CO	H	H
17	$CH_3(CH_2)_{14}CO$	H	H	H	H
18	$NO_2CH_2CH_2CO$	H	H	H	$NO_2CH_2CH_2CO$
19	H	$NO_2CH_2CH_2CO$	H	H	$NO_2CH_2CH_2CO$
20	H	H	H	$NO_2CH_2CH_2CO$	$NO_2CH_2CH_2CO$
21	$NO_2CH_2CH_2CO$	$NO_2CH_2CH_2CO$	H	H	$NO_2CH_2CH_2CO$
22	$NO_2CH_2CH_2CO$	H	$NO_2CH_2CH_2CO$	H	$NO_2CH_2CH_2CO$
23	$NO_2CH_2CH_2CO$	H	H	$NO_2CH_2CH_2CO$	$NO_2CH_2CH_2CO$
24	H	$NO_2CH_2CH_2CO$	$NO_2CH_2CH_2CO$	H	$NO_2CH_2CH_2CO$



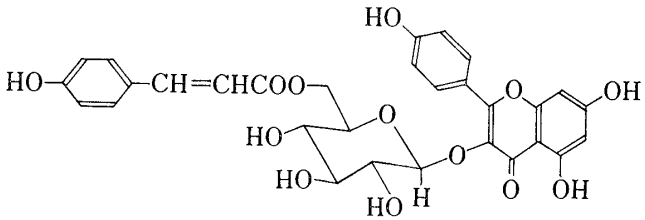
tuliposide-A (**27**α and **27**β)



spirarin (**28**)



paniculoside-I (**15**β)



tiliroside (**29**)

Chart 1

For example, the signal assignment of **1α** is as follows. Pfeffer *et al.*[8] made the following assignments for 1α-*O*-palmitate (**17α**) in $CDCl_3$–CD_3OD: C-1 (92.2), C-2 (73.9), C-3 (74.2), C-4 (69.7), C-5 (71.0), and C-6 (61.4).[9] Following his assignment, the signals of **1α** in py-d_5 should be C-1 (93.6), C-2 (75.2), C-3 (76.8), C-4 (71.3), C-5 (72.3), and C-6 (62.4). However, the chemical shifts of **6α** evaluated by application of the acylation shift values showed marked deviation from the observed data: $\Delta\delta_{max}$ (the largest difference between calculated and observed values) 1.8 ppm, and $\Delta\delta_{av}$ (average value of the differences) 0.9 ppm. Revision of the assignments at C-2, C-3, and C-5 as in Table II and application of the new parameters (Table III) reduced the deviations for **6α** to $\Delta\delta_{max}$ 0.5 and $\Delta\delta_{av}$ 0.23 ppm. This revision also produced an acceptable fit between observed and calculated chemical shifts for **21α** (details are given later).

Results and Discussion

The ^{13}C chemical shifts of D-glucose in various solvents are assembled in Table I, and those of all positionally isomeric mono-*O*-myristoyl-α- and β-D-glucopyranoses in py-d_5 are assembled in Table II. Acylation shifts induced by myristoylation on a particular carbon atom of glucose were derived for each isomer as follows: $\Delta\delta_{Cn}=\delta_{Cn}(\text{acylglucose})-\delta_{Cn}(\text{glucose})$. The resulting shift values are assembled in Table III. The corresponding shift values in methyl *O*-acyl-D-glucopyranosides[3] are shown in parentheses for comparison, and are broadly compatible with those in the corresponding acylglucoses. Except in the case of 1-*O*-acyl derivatives, which gave unusual acylation shifts and will be discussed below, the acylation shift trends of *O*-myristoyl-D-glucopyranoses are the same as those of *O*-myristoyl-D-glucopyranosides: C_α shifted downfield and C_β upfield, whereas C_γ and C_δ showed only small, sometimes negligible shifts. However, the shift values are variable depending on the particular series of anomers and on the position of the acyl group. For each acylation shift, the considerations applicable to methyl *O*-myristoyl-D-glucosides[3] are also applicable to *O*-myristoyl-D-glucoses. As methyl 2-*O*-acyl-β-D-glucopyranoside, **2β** showed a remarkable anomaly in the shift at C-2 (C_α). This anomaly is not as large as that of the glucoside but is still significant, and may arise, as discussed for methyl 2-*O*-acyl-β-D-glucopyranoside, from conformational change of the ester group in **2β** relative to the other acyl derivatives.

Steric interaction effects due to orientational change of the anomeric hydroxyl group from β (equatorial) to α (axial) (Table IV) are larger in every vertical column than those of the corresponding methoxyl group (in parentheses). This may be due to solvation of the hydroxyl group, thus increasing its bulkiness relative to the methoxyl group.

Acylation Shifts of 1-*O*-Acyl-D-glucopyranoses

1α- and 1β-*O*-Acyl-D-glucopyranoses showed different acylation shifts depending on the stereochemistry of the acyloxy group. As already observed in CMR of naturally occurring acylglucosides of di- and triterpene-acids,[10,11] the shifts are completely different from those of other acylglucoses: they are particularly unusual at C-1 (C_α) (upfield shift in contrast to an expected downfield shift, −0.4 and −2.9 ppm for α- and β-anomers, respectively) and C-5 (marked downfield shift, +3.3 and +1.0 ppm for α- and β-anomers, respectively). Pfeffer *et al.*[7] assumed that the anomaly at C-1 is caused by the special stereochemical relationship between the pyranose-ring oxygen and the acyloxy group: the ring oxygen atom perturbs

9) Measurement of the same compound **17α** in the same solvent at the same temperature with our spectrometer gave the values 92.8, 74.3, 75.1, 70.5, 71.6, and 61.9 ppm. Since the concentration factor is practically negligible (see "Experimental"), the significant differences (0.5—0.9 ppm) between the two set of values may be a result of instrumental factors.

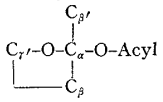
10) *a*) T. Konishi, A. Tada, J. Shoji, R. Kasai, and O. Tanaka, *Chem. Pharm. Bull.*, **26**, 668 (1978); *b*) H. Ishii, K. Tori, T. Tozyo, and Y. Yoshimura, *Chem. Pharm. Bull.*, **26**, 674 (1978); *c*) S. Amagaya, T. Takeda, and Y. Ogihara, *J.C.S. Perkin I*, **1979**, 2044.

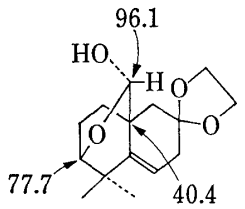
11) *a*) K. Yamasaki, H. Kohda, T. Kobayashi, R. Kasai, and O. Tanaka, *Tetrahedron Lett.*, **1976**, 1005; *b*) I. Sakamoto, K. Yamasaki, and O. Tanaka, *Chem. Pharm. Bull.*, **25**, 844 (1977); *c*) N. Kaneda, R. Kasai, K. Yamasaki, and O. Tanaka, *Chem. Pharm. Bull.*, **25**, 2466 (1977); *d*) K. Yamasaki, H. Kohda, T. Kobayashi, N. Kaneda, R. Kasai, O. Tanaka, and K. Nishi, *Chem. Pharm. Bull.*, **25**, 2895 (1977); *e*) I. Sakamoto, K. Yamasaki, and O. Tanaka, *Chem. Pharm. Bull.*, **25**, 3437 (1977).

Table V. Acylation Shifts at the Hemiacetal Moiety

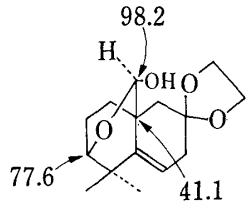
Compd.	Hemicacetal moiety: Alcohol	Conformation: Alcohol	Conformation: Ring	Anomerization	Acyl	Acylation shift ($\Delta\delta$): C_α	C_β	$C_{\beta'}$	$C_{\gamma'}$[b]	Solvent[a]
D-Glucopyranose (**1β**)	*sec.*	*eq.*	Chair	Easy	myr.	−2.9	−2.6	—	+1.0	py-d_5
D-Glucopyranose (**1α**)	*sec.*	*ax.*	Chair	Easy	myr.	−0.4	−2.0	—	+3.3	py-d_5
Lactol (**25a**)	*sec.*	*ax.*	Boat	Easy	Ac	−0.2	−1.2	—	+0.4	$CDCl_3$
Tazettine (**26**)	*tert.*	*ax.*	Half-chair	No	Ac	+2.9	+0.4	+0.8	−2.3	$CDCl_3$

a) The independence of the acylation shifts with respect to the kinds of acyl group and solvent is discussed in the text.
b) $C_{\alpha,\beta,\beta',\gamma'}$ denote

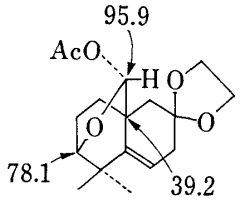




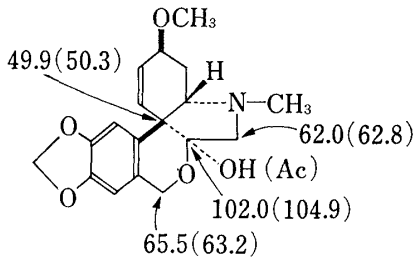
25a



25b



25a-acetate



26 and its acetate (in parentheses)

Chart 2

the carbonyl group at the C-1 esters, forcing it into a shielding orientation with respect to C-1.

To test whether this anomaly at C-1 is specific for an anomeric carbon of carbohydrates or whether it is an inherent property of a hemiacetal moiety, the acylation shifts of **25** and **26** were examined, the results being summarized in Table V.

The lactol[12] (**25**) is forced to adopt a boat conformation. Its secondary hemiacetal moiety undergoes easy anomerization in $CDCl_3$, like that of glucose. Acetylation of one of the isomers (**25a**), in which the hydroxyl group is axial, produced upfield acylation shifts at both the C_α and C_β positions, resembling those in 1-*O*-acylglucoses, but the magnitudes were smaller than those of either **1α** or **1β**.

In contrast, in tazettine (**26**)[13] C_α and C_β both shifted downfield on acetylation at the hemiacetal group, the carbon of which is tertiary. This position is known to be non-epimerizable, the ring having a half-chair conformation.

12) This compound was synthesized in our laboratory by T. Yamashita. Details will be published shortly.
13) For a review of work on this Amaryllidaceae alkaloid, see Manske, "The Alkaloids," XI, 1969, p. 307, XV, 1975, p. 83.

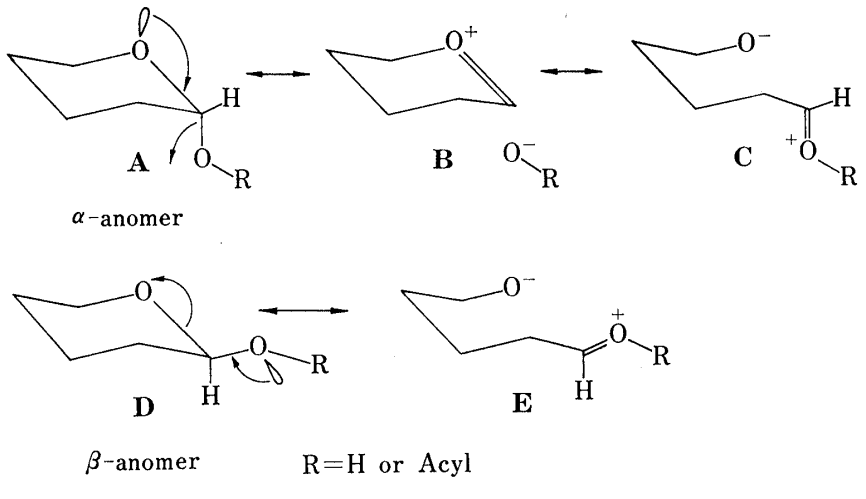


Chart 3

These results suggest that the acylation shift at a hemiacetal moiety depends largely upon the nature of the hemiacetal carbon (whether it is secondary or tertiary and whether it is epimerizable or not).

Accordingly, the anomaly at C-1 of 1-*O*-acylglucoses may be explained as follows. A hemiacetal carbon, if it is anomerizable, is inherently deshielded from the ordinarily expected position by the anomeric and *exo*-anomeric effect (contribution of canonical structures **A**—**E**, where R=H).[14] This deshielding effect can be as much as *ca.* 3 ppm, since on methylation at the anomeric hydroxyl group the C-1 signal shifts downfield 7 ppm, while methylation on the other hydroxyl group causes a 10 ppm downfield shift at the carbon on which substitution occurred.[15] Acylation at the anomeric hydroxyl group apparently suppresses the contribution of the structures **C** and **E** (where R=acyl), hence producing an upfield shift of the C-1 carbon resonance. This effect should be larger in the β-anomer than in the α-anomer, as expected from the greater contribution of the *exo*-anomeric effect to the β-anomer (structure **E** with R=H). The sum of this upfield shift due to suppression of the *exo*-anomeric effect and the downfield shift on acylation, which may be 1–2 ppm for both anomers, corresponds to the observed shift of C-1 signal, *i. e.*, —0.4 and —2.9 ppm for α- and β-anomers, respectively, Of course, branching at the hemiacetal carbon must be taken into account, since the acylation shifts (for an acetyl group) at C_α of ethanol, isopropanol, and *t*-butanol are 2.5, 3.5, and 11.1 ppm.[16]

The anomaly in the acylation shift at C-5 in 1-*O*-acyl derivatives is larger in the 1α- than in the 1β-isomer. In contrast to the expected small shift, this is the largest acylation shift in the 1α-isomer. Steric compression effect due to the axial *O*-myristoyl group cannot explain this anomaly, since it would produce upfield shifts in the 1α-isomer at C_γ and $C_{\gamma'}$. The anomaly at C-5 may be attributed to an increase in the contribution of the structure **B** (R=acyl) in the 1α-*O*-acyl isomer. An antiparallel arrangement of one of the unshared electron pair on the oxygen with the 1α-acyloxy group would produce C^1-*O*-acyl bond elongation, increasing the positive charge on the ring oxygen; this would produce a downfield shift of the resonance at the neighboring carbon (C-5) on acylation at C^1–OH. The contribution of this effect must therefore be larger in the 1α-*O*-acyl than the 1β-*O*-acyl isomer. The conformation of the 1α-*O*-acyl group may be another factor, since the ester carbonyl of the 1α-isomer would not be at a symmetrical position with respect to C-3 and C-5 due to the perturbation of the ring oxygen, leading to different acylation shifts at C-3 and C-5.

14) R.U. Lemieux and S. Koto, *Tetrahedron*, **30**, 1933 (1974).
15) *a*) T. Usui, N. Yamaoka, K. Matsuda, K. Tuzimura, H. Sugiyama, and S. Seo, *J.C.S. Perkin I*, 2425 (1973); *b*) D.E. Dorman, S.J. Angyal, and J.D. Roberts, *J. Amer. Chem. Soc.*, **92**, 1351 (1970).
16) S.W. Pelletier, Z. Djarmati, and C. Pape, *Tetrahedron*, **32**, 995 (1976).

Table VI. Observed and Calculated (in Parentheses) ^{13}C Chemical Shifts of Di-*O*-myristoyl-D-glucopyranoses in Pyridine-d_5[a]

Compd.	Carbon number C-1	C-2	C-3	C-4	C-5	C-6	$\Delta\delta_{max}$
1α,6- **6α**	93.3 (93.7)	72.2 (72.3)	75.0 (75.2)	71.1 (71.2)	73.6 (74.1)	64.3 (64.4)	0.5
1β,6- **6β**	95.6 (95.9)	73.9 (74.2)	78.2 (78.2)	71.0 (70.8)	76.2 (76.2)	64.0 (64.3)	0.5
2,3- **7α**	90.9 (91.1)	73.4[c] (74.2)	73.9[c] (74.5)	69.9 (70.4)	73.6 (73.7)	62.3 (62.5)	0.8
2,6[b]- **14α**	91.1 (91.2)	75.6 (75.9)	72.1 (72.2)	72.4 (72.6)	70.6 (70.8)	64.8 (65.0)	0.3
2,6-**1** **4β**	96.6 (96.7)	76.7 (77.1)	76.1 (76.2)	72.0 (72.1)	75.4 (75.6)	64.6 (65.0)	0.4
4,6- **8α**	94.1 (94.0)	74.6[c] (74.6)	72.6 (72.7)	72.7[c] (72.4)	73.6 (73.7)	63.9 (64.7)	0.8

a) $\Delta\delta_{av.}=0.25$, SD=0.20, SE=0.03 for 36 signals.
b) This was prepared by acyl migration of **6α** in pyridine-d_5. Details will be published later.
c) Assignments were confirmed by ^1H selective hetero-spin decoupling.

Table VII. Observed and Calculated (in Parentheses) ^{13}C Chemical Shifts of Various *O*-Acylglucoses in Various Solvents

Compd.		Carbon number C-1	C-2	C-3	C-4	C-5	C-6	$\Delta\delta_{max}$
Palmitate[a]	**17α**	93.3 (93.5)	72.2 (71.9)	74.8 (74.9)	71.0 (70.9)	76.0 (76.3)	62.3 (62.1)	0.3
Palmitate[a]	**11α**	91.2 (91.1)	75.4 (75.5)	72.0 (71.9)	72.2 (72.3)	72.9 (73.0)	62.8 (62.7)	0.1
Palmitate[a]	**11β**	96.4 (96.1)	76.5 (76.6)	76.2 (76.0)	71.8 (72.2)	78.2 (78.4)	62.6 (62.7)	0.4
Stearate[b]	**9α**	93.5 (93.6)	72.3 (72.3)	75.1 (75.2)	71.3 (71.3)	76.8 (76.8)	62.4 (62.4)	0.1
Cinnamate[c]	**13β**	96.2 (95.8)	74.0 (74.1)	78.4 (78.4)	70.9 (71.0)	79.4 (79.3)	62.2 (62.1)	0.4
Cinnamate[b]	**10α**	94.0 (94.1)	74.2 (74.3)	75.2 (75.2)	72.2 (72.3)	70.7 (70.8)	65.0 (65.1)	0.1
Cinnamate[b]	**10β**	98.8 (98.8)	76.6 (76.8)	78.3 (78.3)	71.6 (71.7)	75.2 (75.2)	65.0 (65.1)	0.2
Acetate[d]	**16α**	93.2 (93.3)	71.0 (71.0)	76.6 (76.3)	69.0 (68.6)	72.4 (72.8)	61.8 (61.6)	0.4
Acetate[d]	**16β**	97.0 (97.3)	73.6 (73.6)	78.6 (78.1)	69.0 (68.9)	76.9 (77.1)	61.5 (61.5)	0.5
Paniculoside-I[e]	**15β**	95.7 (95.8)	74.0 (74.1)	79.0 (78.4)	71.1 (71.0)	79.0 (79.3)	62.1 (62.1)	0.6
Tuliposide-A[b]	**27α**	94.1 (94.1)	74.3 (74.3)	75.2 (75.2)	72.3 (72.3)	70.8 (70.8)	65.4 (65.1)	0.3
Tuliposide-A[b]	**27β**	98.8 (98.8)	76.6 (76.8)	78.4 (78.3)	71.7 (71.7)	75.2 (75.2)	65.4 (65.1)	0.3
Spirarin[c]	**28β**	96.0 (95.9)	74.1 (74.2)	78.2 (78.2)	71.0 (70.8)	76.2 (76.2)	64.5 (64.3)	0.2
Dimyristate[f]	**8α**	94.1 (93.9)	74.0 (74.2)	72.7 (72.4)	72.4 (72.0)	68.8 (68.3)	64.1 (64.4)	0.5
Dimyristate[f]	**8β**	98.5 (98.5)	76.9 (76.7)	75.9 (75.4)	70.6 (71.0)	72.8 (72.9)	64.2 (64.6)	0.5
Pentaacetate[g]	**12α**	89.5 (90.7)	69.9 (72.5)	70.3 (72.0)	68.5 (70.2)	70.3 (72.2)	62.0 (63.4)	2.6

a) Solvent, methanol-d_4 at 24.5°.
b) Solvent, py-d_5.
c) Solvent, py-d_5. Data were provided by Prof. Y. Tanabe, Hokuriku University.
d) Solvent, D_2O. See ref. 17).
e) Solvent, py-d_5. See ref. 11*a*).
f) Solvent, methanol-d_4 at 60°.
g) Solvent, py-d_5. Assignment in $CDCl_3$, see ref. 17) and C.R. Hutchinson, *J. Org. Chem.*, **39**, 1854 (1974).

17) M.R. Vignon and J.A. Vottero, *Tetrahedron Lett.*, **1976**, 2445.

We therefore conclude that the unusual behavior in the CMR spectra of 1-*O*-acylglucopyranoses reflects the anomeric and *exo*-anomeric effects of carbohydrates (such as glucose). Such anomalies may be more or less general in compounds possessing an anomerizable hemiacetal moiety.

Additivity of the Acylation Shift Parameters

The ^{13}C chemical shift of a particular carbon atom in the glucose moiety of various di-*O*-myristoyl-D-glucopyranoses was calculated by simply adding the shift values of the corresponding mono-*O*-myristates in Table III to the resonance of glucose in py-d_5, as was done for the methyl di-*O*-acyl-D-glucopyranosides.[3] Table VI lists the observed and calculated (in parentheses) resonances of the di-*O*-myristates which we have synthesized so far. The calculated and observed values show satisfactory agreement for most signals. The largest error ($\Delta\delta_{max}$ 0.8 ppm), noted at C-2 in **7α**, and C-6 in **8α**, we consider to be a result of accumulation of ester groups at proximate positions, possibly producing slight conformational change of an ester group.

The above findings indicate that the shift values in Table III can be adopted as additive parameters, like those of the methyl *O*-acylglucopyranosides, for evaluation of the chemical shifts of di-*O*-myristoyl-D-glucopyranoses. More universal parameters relating to the esterified position (such as parameters for C_α, C_β, C_γ, and C_δ) were not available for the reasons described in the preceding section: the parameters are not the same for α- and β-anomers and also vary depending on the positions of acyl groups. As discussed below, however, it appears that the additivity of shift values holds not only for the myristoyl group in py-d_5 but also for various acyl groups in various solvents.

Generalization of Acylation Shift Parameters and Its Application to Structure Elucidations of Uncharacterized Acylglucoses

Effect of Acyl Moiety——As expected, slight changes of acyl group such as myristoyl to stearoyl do not affect the glucose carbon resonances (*cf.* **1α** *vs.* **9α**). Tanaka *et al.*[11] isolated a number of 1β-glucosyl esters of diterpenoid-acids, most of which were bulky *ent*-kauran-19-oates. The CMR signals (only one **15β**[11d] among many examples is indicated in Table VII) of the glucose portion in py-d_5[11] showed surprisingly good coincidence with the corresponding signals of **1β**, suggesting that the bulkiness of the acyl moiety does not greatly influence the acylation shift. 1β-*O*-Cinnamate (**13β**)[18] also gave almost the same signals as **1β** for the glucose carbons in py-d_5.

Comparison of the spectrum (in py-d_5) of 6-*O*-cinnamate (**10**) with that of **5** also showed satisfactory coincidence between them as regards the glucose portion for both the α- and β-anomers.

These and many other examples described below indicate that the acylation shift values in Table III can be adopted as a universal set of parameters for various acyl groups as long as the electronegativity of the acyl group does not differ very much. Acyl groups with strong electronegativity such as carbomethoxy,[19] carbobenzoxy,[3] mono-, di-, and trichloroacetyl,[16] *p*-nitrobenzoyl,[16] and mesyl[19] are known to increase the downfield shift at C_α (the effect of the oxaloyl group is not as large as those of these groups and is broadly similar to that of a usual acyl group).[20] That the parameters are applicable to a cinnamoyl derivative is of particular importance, since there are many cinnamate derivatives in nature, formed from shikimate (*e. g.*, *p*-coumarate···see below).

18) The spectra of 1β-*O*-cinnamate and spirarin were provided by Prof. Y. Tanabe, Hokuriku University.
19) Y. Terui, K. Tori, and N. Tsuji, *Tetrahedron Lett.*, **1976**, 621.
20) K. Yamakaki, R. Kasai, Y. Masaki, M. Okihara, O. Tanaka, H. Oshio, S. Takagi, M. Yamaki, K. Masuda, G. Nonaka, M. Tsuboi, and I. Nishioka, *Tetrahedron Lett.*, **1977**, 1231.

TABLE VIII. Observed and Calculated (in Parentheses) ^{13}C Chemical Shifts of Various *O*-Myristoyl and *O*-γ-Nitropropanoylglucoses in Acetone-d_6

Compd.	Carbon number C-1	C-2	C-3	C-4	C-5	C-6	$\Delta\delta_{max}$	$\Delta\delta_{av.}$
Myristate (with internal reference at 60°)								
1- **17α**	92.8 (93.5)[a]	72.4 (71.8)	75.1 (74.9)	71.6 (70.8)	75.6 (76.5)	62.8 (62.2)	0.9	0.63
1- **17β**	95.3 (95.1)[a]	74.0 (73.7)	78.2 (77.9)	71.6 (70.8)	78.3 (78.8)	62.9 (62.1)	0.8	0.48
2,3- **7α**	91.1 (91.0)[a]	72.9[h] (73.7)	73.4[h] (74.2)	70.4[b] (69.9)	72.7 (73.3)	62.7 (62.3)	0.8	0.53
2,3- **7β**	96.1 (96.0)[a]	74.3 (74.8)	76.6 (76.9)	70.2[b] (70.1)	77.5 (78.3)	62.7 (62.4)	0.8	0.35
4,6- **8α**	93.6 (93.7)[a]	74.0 (74.1)	72.3[c] (72.4)	72.7[c] (72.8)	68.5 (68.5)	63.7 (64.5)	0.8	0.20
4,6- **8β**	98.3 (98.3)[a]	76.7 (76.7)	75.6 (75.3)	72.0 (72.5)	73.0 (72.7)	63.7 (64.7)	1.0	0.35
γ-Nitropropanoate (with external reference)[d]								
1,6- **18β**	95.2 (94.2)	73.0 (72.8)	76.9 (76.7)	70.3 (69.6)	75.1 (74.7)	64.2 (63.3)	1.0	0.57
2,6- **19α**	90.0 (90.0)	75.1[h] (74.4)	71.0 (70.9)	71.2[h] (71.1)	69.5 (69.5)	64.4 (63.8)	0.7	0.25
2,6- **19β**	95.1 (95.0)	76.4 (75.7)	74.8[e] (74.7)	70.1 (70.9)	74.1[e] (74.1)	64.3 (64.0)	0.8	0.33
1,2,6- **21α**	89.2 (89.7)	72.1[h] (72.4)	70.4 (70.9)	69.5[f] (70.0)	71.9[f] (72.8)	63.0 (63.1)	0.9	0.47
1,2,6- **21β**	92.5 (92.1)	73.6[h] (73.0)	74.3 (74.6)	70.0 (70.0)	75.1 (74.3)	63.8 (63.2)	0.8	0.43
1,4,6- **23β**	94.8 (94.4)	73.0[h] (73.1)	74.2 (74.2)	71.4[h] (70.6)	72.5 (72.7)	63.0 (62.9)	0.8	0.27
2,3,6- **24α**	89.7 (90.1)	72.2 (72.7)	73.2 (73.2)	68.5 (68.8)	69.3 (69.6)	63.7 (63.3)	0.5	0.32
4,6- **20α**	92.6 (92.9)	72.7[g] (73.1)	71.3 (71.4)	72.2[g] (71.8)	67.2 (67.5)	63.6 (63.5)	0.4	0.23
4,6- **20β**	97.2 (97.3)	75.3 (75.7)	74.3 (74.3)	71.5 (71.5)	72.1 (71.7)	63.6 (63.7)	0.4	0.17
1,3,6- **22β**	94.3 (94.3)	70.5 (71.0)	77.8 (77.7)	67.7 (67.6)	74.2 (74.8)	63.2 (62.9)	0.6	0.27

a) Calculated with an arbitary correction of +1 ppm; see footnote 21).
b, *c*) Assignments may be reversed.
d) All empirical data are those of P.E. Pfeffer and K.M. Valentine, *Carbohydr. Res.*, **73**, 1 (1979).
e, *f*, *g*) In the cases of these signals, the original assignments were reversed.
h) These assignments were confirmed by ^1H selective hetero-spin decoupling.

We applied the above generalization of the shift parameters to the structure investigation of tuliposide, a bacteriocidal constituent of *Tulipa gesneriana* L. In 1968, Tschesche *et al.*[22] reported the isolation of 1-tuliposide-A [1-*O*-(γ-hydroxy-α-methylenebutyroyl)-β-D-glucopyranose] which, they claimed, rearranges to 6-tuliposide-A [6-*O*-(γ-hydroxy-α-methylenebutyroyl)-α-D-glucopyranose] on standing. Later Slob[23] investigated various plants of *Liliiflorae* by gas chromatography (GC) (as TMS derivatives) and found two peaks for tuliposide-A, which, he considered, corresponded to 1α- and 1β-tuliposide-A. We also isolated tuliposide-A

21) All chemical shifts were calculated with a correction of 1 ppm. This value was the estimated sum of the difference due to internal *vs.* external references (+0.5 ppm) and that arising from instrumental factor (as much as +0.5 ppm .. see footnote 9).
22) *a*) R. Tschesche, F.J. Kammerer, and G. Wulff, *Tetrahedron Lett.*, **1968**, 701; *b*) *Idem*, *Chem. Ber.*, **104**, 2057 (1969).
23) *a*) A. Slob, *Phytochemistry*, **12**, 811 (1973); *b*) A. Slob, B. Jekel, B.D. Jong, and E. Schlatmann, *Phytochemistry*, **14**, 1997 (1975).

from the stigma and ovaries of Japanese tulip and found two peaks of almost equal intensity by GC, in agreement with Slob's observation. In conflict with earlier suggestions, its CMR spectrum in py-d_5 (Table VII) showed that it is an anomeric mixture of 6-*O*-(γ-hydroxy-α-methylenebutyroyl)-D-glucopyranose (**27**α and **27**β), since the signals of glucose moiety were practically identical with those of an anomeric mixture of **5**α and **5**β and were clearly different from those of either **1**α and **1**β.[24]

A further example of the additivity of the generalized parameters is the structure elucidation of spirarin (**28**), which was isolated by Tanabe[18] from *Spiraea thunbergii* and proved to be a di-ester of glucose with cinnamic and γ-hydroxy-α-methylenebutyric acids. If the acylation shift parameters are independent of the nature of the acyl group, the ^{13}C chemical shifts of its glucose moiety should be almost identical with those of the corresponding di-*O*-myristate. Comparisons of the empirical signals of spirarin with the values calculated for various di-*O*-acylglucoses immediately led to the conclusion that it is a 1,6-di-*O*-acyl-β-D-glucopyranose. In fact, the δ_C's of its glucose portion in py-d_5 (Table VII) were almost superimposable on those of **6**β. Differentiation of the acyl groups in terms of the position of substitution was done by comparison of the ester carbonyl resonances, leading to the full structure (**28**).[25]

1,2,3,4,6-Penta-*O*-acetyl-α-D-glucopyranose (**12**α) is an example which shows the limitations of the additivity rule. All of the observed signals (Table VII) showed marked deviations from the calculated resonances (Δ 1.2—2.6 ppm). Presumably the accumulation of acyl groups in the same molecule forced a change of conformation of the acyl groups for steric and electrostatic reasons, causing marked changes in the chemical shifts of the carbons to which they are attached. This indicates that the additivity rule is applicable to compounds in which the conformations of acyl groups do not differ very much from the conformation of the corresponding acyl group in the mono-*O*-acyl derivatives (the rule seems to hold for tri-*O*-acyl derivatives ···see below).

Effect of Solvent——The ^{13}C chemical shifts of glucose and acylglucoses vary depending on the solvent. This solvent effect is not constant on each carbon atom in the glucose moiety, as shown by the resonances of glucose in various solvents (Table I): —1.5 ppm at C-2 and —0.7 ppm at C-1 on changing the solvent from py-d_5 to D_2O. Analogous significant solvent effects are also observed in acylglucoses. However, we found that the acylation shifts ($\Delta\delta_C$) are independent of the solvent. Combination of this finding with the above generalization regarding the acyl moiety greatly expands the applicability of the shift parameters in Table III.

Table VII lists the observed and calculated ^{13}C chemical shifts of various *O*-acylglucoses in various solvents. Resonances calculated on the basis of the above considerations are indicated in parentheses. For example, calculated δ's for the 3-*O*-acetate (**16**) in D_2O, using the parameters in Table III and the δ's for glucose in D_2O (Table I) showed good coincidence with the reported data[17] for both the α- and β-anomers. Similarly, the observed and calculated chemical shifts for 1α-*O*-palmitate (**17**α), 2-*O*-palmitate (**11**α and **11**β), and 4,6-di-*O*-myristate (**8**α and **8**β) in methanol-d_4 showed satisfactory agreement for every compound. The acylation shifts due to the *p*-coumaroyl group in DMSO-d_6 reported for tiliroside (**29**)[26] (+2.1 ppm for C-6 and —3.2 ppm for C-5) are also compatible with the shift parameters for 6-*O*-acyl-β-D-glucopyranoside shown in Table III.

Acetone-d_6 is not a very suitable solvent for measurement of acylation shifts. Glucose is practically insoluble in this solvent, and most acylglucoses are also barely soluble at 24.5°. Six compounds (**17**α, **17**β, **7**α, **7**β, **8**α, and **8**β) measured in this solvent at 60° showed rather large errors in their δ_C's compared to values[21] evaluated from the reported δ_C's of glucose in

24) Further details of our study on the structures of tuliposides will be reported in a separate paper.
25) Y. Tanabe and A. Kita, *Yakugaku Zasshi*, **100**, 355 (1980).
26) M. Kuroyanagi, M. Fukuoka, K. Yoshihira, S. Natori, and K. Yamasaki, *Chem. Pharm. Bull.*, **26**, 3594 (1978).

acetone-d_6 (Table VIII). Although $\Delta\delta_{max}$ values were within 1 ppm, $\Delta\delta_{av}$ values (0.20—0.63 ppm) are larger than in other solvents. Since the temperature factor is small (see "Experimental"), this anomaly in acetone-d_6 may be attributed to conformational perturbation of the ester group due to the carbonyl group of the solvent.

Pfeffer *et al.*[7] reported CMR spectra of various di- and tri-*O*-γ-nitropropanoyl-D-glucopyranoses in acetone-d_6 (with an external reference) and derived additive parameters for the γ-nitropropanoyl group based on assigned signals of seven di- and tri-*O*-acyl derivatives (**18β**, **19α**, **19β**, **21α**, **21β**, **23β**, and **24α**) using linear-regression analysis. Their shift parameters are in good accord with ours, except for the 1-*O*-acyl series, as regards the shift trends, but are different as regards the values. In the case of the 1-*O*-acyl derivatives, the two sets of parameters are completely different in both trends and values. Although their concentration of samples was about 10 times greater than ours, the effect of this may be negligible (see "Experimental"). We consider that some of their assignments may be reversed (indicated by *e*, *f*, *g* in Table VIII), since anomalies in the acylation shifts of some compounds (such as C-2 of 2-*O*-acyl and C-5 of 1-*O*-acyl derivatives) were overlooked in their argument. Re-calculations for **18β**, **19α**, **19β**, **21α**, **21β**, **23β**, and **24α** by applying our parameters to their δ's for glucose in acetone-d_6 (Table I) gave results (parenthetical values in Table VIII) which showed a satisfactory fit with the observed values ($\Delta\delta_{max}$ 1.0, $\Delta\delta_{av}$ 0.4 ppm for 42 signals). Better coincidence between observed and calculated values was obtained for **20α**, **20β**, and **22β** (with $\Delta\delta_{max}$ 0.4, 0.4, 0.6 ppm for **20α**, **20β**, and **22β**, respectively), for which the previous workers[7] gave calculated values with $\Delta\delta_{max}$ 0.7, 0.7, and 0.6 ppm, respectively.

The above results support the validity of our argument. We can therefore predict the δ values of an acylglucose in an arbitary solvent from the shift parameters in Table III, as long as the CMR data for glucose in that solvent are known. This will be extremely useful for the structure determination of uncharacterized *O*-acylglucoses (and *O*-acylglucosides).

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