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Chemical and Biochemical Studies on Carbohydrate Esters. I. Preparation and Properties of 1-O-Acyl-β-D-glucopyranose Tetraacetates[1]

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A series of 1-O-acyl-β-D-glucopyranose tetraacetates were prepared by reaction of α-acetobromoglucose with the silver salts of the eleven, normal, saturated fatty acids, *i.e.*, propanoic, butanoic, pentanoic, hexanoic, octanoic, nonanoic, decanoic, dodecanoic, tetradecanoic, hexadecanoic, and octadecanoic acids. Exclusive production of β-anomers was confirmed by measurement of their specific rotations and also by observing their IR and NMR spectra. The characteristic mass spectral features were discussed for these analogs. Gas liquid chromatography was found to be recommendable for the qualitative and quantitative analyses of this type of compounds.

In 1968, Tschesche, *et al.* isolated two constituents, tuliposide-A and tuliposide-B, from tulip (*Tulipa gesneriana* L.), and determined their structures to be (γ-hydroxy-α-methylene-butyryl)- and [(*S*)-β,γ-dihydroxy-α-methylene-butyryl]-β-D-glucopyranoside, respectively.[3] Thus, these compounds belong, structurally, to 1-O-acylglycoses, whose examples have hitherto rarely been found in nature. Both tuliposides are biologically active, showing bacteriotoxic and fungitoxic effects.[3,4] In addition, tuliposide-A has been demonstrated as a precursor of α-methylene-γ-butyrolactone,[5] which is presumed to be the causative principle of an allergic skin disease, known in Holland as "turpenvinger" (tulip finger).[6] Recently, it has also been suggested that tuliposides would become of value to plant systematics.[4a,7]

These findings prompted us to promote the systematic survey work on the distribution of this type of constituents in the plant kingdom. In order to obtain some fundamental informations useful for such investigations, we now prepared a series of fatty esters of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranose, and examined their basic properties by measurements of the melting points, specific rotations, and infrared (IR), nuclear magnetic resonance (NMR), and mass spectra, and also by observing their analytical behaviors in gas liquid and thin-layer chromatography (GLC and TLC). For preparation of these esters, we employed the method described by Wulff,[8] using eleven, normal, saturated fatty acids as starting material, *i.e.*, propanoic, butanoic, pentanoic (valeric), hexanoic (caproic), octanoic (caprylic), nonanoic

1) This work was presented at the 94th Annual Meeting of the Pharmaceutical Society of Japan, Sendai, April, 1974.
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3) *a*) R. Tschesche, F.J. Kammerer, and G. Wulff, *Tetrahedron Letters*, **1968**, 701; *b*) *Idem, Chem. Ber.*, **104**, 2057 (1969).
4) *a*) A. Slob, *Phytochem.*, **12**, 811 (1973); *b*) B.H.H. Bergman, J.C.M. Beijersbergen, J.C. Overeem, and A. Kaars Sijpestein, *Rec. Trav. Chim.*, **86**, 709 (1967).
5) C.R. Huchinson, and E. Leete, *Chem. Comm.*, **1970**, 1189.
6) G.A.W. Verspyck Mijnssen, Thesis, Leiden (1968).
7) *a*) G. Kurono, M. Kaneda, K. Mizutani, Y. Takahashi, and Y. Nishikawa, Abstract of the 37th Meeting of Hokuriku Branch, Pharmaceutical Society of Japan, Toyama, October, 1973; *b*) G. Kurono, K. Mizutani, M. Kaneda, Y. Takahashi, and Y. Nishikawa, Abstract of the 94th Annual Meeting of the Pharmaceutical Society of Japan, Sendai, April, 1974, p. II-251.
8) *a*) G. Wulff, W. Kruger, and G. Rohle, *Chem. Ber.*, **104**, 1387 (1971); *b*) G. Wulff, G. Rohle, and W. Kruger, *Angew. Chem.*, **82**, 480 (1970).

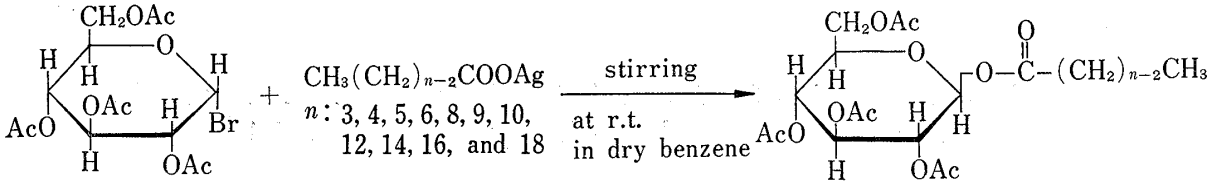

Chart 1

Compd. No.:	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
n :	3	4	5	6	8	9	10	12	14	16	18

(pelargonic), decanoic (capric), dodecanoic (lauric), tetradecanoic (myristic), hexadecanoic (palmitic), and octadecanoic (stearic) acids. As shown in Chart 1, reaction of the silver salts of the respective fatty acids with α-acetobromoglucose (2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide) in dry benzene afforded the corresponding 1-O-acyl-β-D-glucopyranose tetraacetates (compounds I—XI) in good yields. As far as we know, these compounds except the propionoyl derivative (I)[9] have never been reported in the literature previously.

Experimental[10]

For measurements of IR (nujol), NMR (10% $CDCl_3$ solution; internal reference, tetramethyl silane), and mass spectra, a JASCO Model IR-G Spectrophotometer, a JEOL JNM PS-100 Spectrometer (100 MHz), and a JMS-01SG Spectrometer were used, respectively. The specific rotations were estimated with a JASCO Model DIP-SL Automatic Polarimeter. GLC analyses were carried out with a Shimadzu Gas Chromatograph Model GC-4BPF attached with a hydrogen flame ionization detector, using glass columns. Throughout the present study, TLC was performed on Silica gel G layer with a solvent system of ether–hexane (2: 1) (spray reagent, 10% H_2SO_4).

Materials——The fatty acids used were purchased from the commercial sources. They were converted into the corresponding silver salts by the method of Lüttringhaus[11]: yields, 90% or higher. α-Acetobromoglucose was synthesized by the well established procedures[12]: colorless needles, mp 89—90° (lit.,[12] mp 89—90°). β-D-Glucopyranose pentaacetate, mp 132° (lit.,[13] mp 132°), its α-anomer, mp 112—113° (lit.,[13] mp 112—113°), and sucrose octaacetate, mp 89° (lit.,[14] mp 89°) were prepared in the usual manner. Phenyl β-D-glucopyranoside was commercially obtained (Nakarai Chemicals, Ltd.), and it was acetylated with Ac_2O and pyridine to give the tetraacetate, mp 125° (lit.,[15] mp 124—125°).

Preparation of 1-O-Acyl-β-D-glucopyranose Tetraacetates (Compounds I—XI)——The general procedures employed were similar to those reported by Wulff[8] (Chart 1). Dry benzene solution (50 ml) of α-acetobromoglucose (10 mmoles) was added dropwise to the suspension of the silver salt of each fatty acid (12 mmoles) in dry benzene (50 ml), and the whole mixture was stirred at room temperature for several hours. Stirring was continued until the spot of α-acetobromoglucose (*Rf* 0.37) became undetectable by TLC. Then the mixture was filtered for removal of the unreacted silver salt and silver bromide, and the filtrate was evaporated to dryness under a reduced pressure. By TLC examination, the resulting residues were shown to contain, commonly, a small amount of an unidentified material (*Rf* 0.13). Thus the respective products were subjected to silica gel column chromatography, using a mixture of ether–hexane (2: 1) as a developing solvent. The major fractions were recrystallized from a mixture of ether–hexane to give compounds I—XI as colorless needles. Yields, 70 to 80% from α-acetobromoglucose. TLC and GLC analyses guaranteed the purity of these compounds, with one exception of the nonanoyl ester (VI). The latter was shown to contain a trace amount (less than 2%) of octanoyl ester, which was derived from the octanoic acid contained in the nonanoic acid specimen used in the present study.

9) *a*) J. Kocourek, *Collection Czech. Chem. Comm.*, **25**, 2252 (1960); *b*) R.U. Lemieux and C. Brice, *Canad. J. Chem.*, **30**, 295 (1952).
10) All melting points are not corrected.
11) *a*) A. Lüttringhaus, and D. Schade, *Chem. Ber.*, **74**, 1565 (1941); *b*) R. Adams, "Organic Reactions," Vol. IX, ed. by C.V. Wilson, John Willey and Sons, Inc., New York, London, and Sydney, 1957, p. 355.
12) R.U. Lemieux, "Methods in Carbohydrate Chemistry," Vol. II, ed. by R.L. Whistler, M.L. Wolfrom, and J.N. BeMiller, Academic Press Inc., New York, and London, 1963, p. 221.
13) M.L. Wolfrom and A. Thompson, "Methods in Carbohydrate Chemistry," Vol. II, ed. by R.L. Whistler, M.L. Wolfrom and J.N. BeMiller, Academic Press Inc., New York, and London, 1963, p. 211.
14) R.P. Linstead, A. Rutenberg, W.G. Dauben, and W.L. Evans, *J. Am. Chem. Soc.*, **62**, 3260 (1940).
15) B. Helferich and E. Schmitz-Hillebrecht, *Chem. Ber.*, **66**, 380 (1933).

Properties of Compounds I—XI——These compounds are readily soluble in benzene, $CHCl_3$, CCl_4, ether, AcOEt, acetone, MeOH, EtOH, and AcOH, but they show relatively lower solubilities in hexane. Their analytical data, melting points, specific rotations, and the TLC *Rf* values are listed in Table I. The IR, NMR, and mass spectral data are summarized in Table II, and the representative spectra are depicted in Fig. 1, 2, and 3.

GLC Analyses——The relative retention times (rt_R) for compounds I—XI are shown in Table III, together with the details of the operating conditions employed. In these cases, either β-D-glucopyranose pentaacetate or sucrose octaacetate was used as an internal standard (I.S.). The representative gas chromatograms of a synthetic, total mixture of compounds I—XI are reproduced in Fig. 4. The relationship between log rt_R and the number of carbon atoms in acyl groups is shown in Fig. 5. Using β-D-glucopyranose pentaacetate, sucrose octaacetate, or phenyl β-D-glucopyranoside tetraacetate as I.S., the calibration curves for compounds I, V, VIII, and X were prepared by plotting the weight ratios (sample/I.S.) against the peak area ratios (sample/I.S.), which were measured by triangulation (the height x the width at half height) (Fig. 6). The recorder response factors thus determined are shown in Table IV.

Result and Discussion

According to the procedures shown in Chart 1, eleven analogs of 1-O-acylglucose tetraacetates (compounds I—XI) were obtained as colorless needles in good yields. They gave no absorption bands of hydroxyl group in the IR spectra (Fig. 1). The purity of each product was guaranteed by elemental analysis (Table I), and also by TLC and GLC examination, with one exception of nonanoyl ester (VI), in which a trace amount of the octanoyl derivative was found to be present. The impurity was derived from the octanoic acid contained in the nonanoic acid specimen used in the present study.

Table I. Analytical Data of 1-O-Acyl-β-D-glucopyranose Tetraacetates

Compd. No.	mp (°C)	$[\alpha]_D^{26}$ (c=2.0, $CHCl_3$)	Formula	Analysis (%) Calcd. C	Calcd. H	Found C	Found H	TLC[a] *Rf*
I[b]	102—103	+5.3°	$C_{17}H_{24}O_{11}$	50.49	5.98	50.51	5.92	0.26
II	81— 82	+4.4°	$C_{18}H_{26}O_{11}$	51.67	6.26	51.61	6.25	0.29
III	61	+3.8°	$C_{19}H_{28}O_{11}$	52.77	6.53	52.45	6.48	0.32
IV	74— 75	+4.7°	$C_{20}H_{30}O_{11}$	53.80	6.77	53.85	6.77	0.35
V	73— 74	+4.4°	$C_{22}H_{34}O_{11}$	55.69	7.22	55.49	7.21	0.38
VI	49	+4.7°	$C_{23}H_{36}O_{11}$	56.55	7.43	56.28	7.22	0.39
VII	56— 56.5	+4.2°	$C_{24}H_{38}O_{11}$	57.36	7.62	57.65	7.59	0.40
VIII	58— 59	+4.3°	$C_{26}H_{42}O_{11}$	58.86	7.97	59.14	8.19	0.41
IX	65	+4.4°	$C_{28}H_{46}O_{11}$	60.20	8.30	60.06	8.12	0.43
X	72	+3.6°	$C_{30}H_{50}O_{11}$	61.41	8.59	61.31	8.43	0.44
XI	77	+4.0°	$C_{32}H_{54}O_{11}$	62.52	8.85	62.65	8.70	0.45

a) adsorbent, Silica gel G; solvent, ether–hexane (2: 1)
b) *cf.* lit.[9a]; mp 103.5—104.5°, $[\alpha]_D^{20}$ +5.7° (c=4, $CHCl_3$)

In the reaction employed, exclusive production of β-D-anomers was anticipated from the results mentioned by Wulff, who developed extensive investigations on the glycoside synthesis under the similar conditions using various hydroxycarboxylic acids.[8] In fact, β-D-configuration was confirmed for the present products on the basis of the following findings: i) The compounds I—XI showed the positive, low specific rotations (Table I), whose values were in good agreement with that of β-D-glucopyranose pentaacetate ($[\alpha]_D^{20}$ +4° ($CHCl_3$)[13]; *cf.* α-anomer, $[\alpha]_D^{20}$ +102° ($CHCl_3$)[13]). ii) The absorption bands around 905 cm^{-1} in their IR spectra (Table II, and Fig. 1), and the signals of anomeric proton at δ 5.70—5.80 (doublet, J=7.5 Hz) in their NMR spectra (Table II, and Fig. 2) were found to be identical, respectively,

with those of the β-anomer (905 cm^{-1}: δ 5.75 (doublet, J=7.5 Hz)), but not of the α-anomer (840 cm^{-1}: δ 6.35 (doublet, J=3.5 Hz)), of pentaacetyl-D-glucopyranose. iii) The values of melting point and specific rotation of compound I agreed well with those of 1-O-propionoyl-β-D-glucopyranose tetraacetate reported previously[9a] (Table I: *cf.* α-anomer of the latter,[9a] mp 71.3—72.3° and $[\alpha]_D$ +102°).

Table II. Spectral Data of 1-O-Acyl-β-D-glucopyranose Tetraacetates[a]

Compd. No.	IR ν_{max}^{Nujol} cm^{-1}		NMR[b] δ(ppm) Anomeric proton[c]	Mass[d] m/e $CH_3(CH_2)_{n-2}CO^+$
	Ester	β-D-Glucosyl		
I	1735(b)[e]	900	5.72	57
II	1735(b)	900	5.80	71
III	1735(b)	905	5.69	85
IV	1735(b)	905	5.70	99
V	1740(b)	905	5.80	127
VI	1738, 1760	905	5.77	141
VII	1738, 1760	905	5.78	155
VIII	1740, 1760	903	5.74	183
IX	1740, 1760	903	5.80	211
X	1750(b), 1760(sh)[f]	905	5.78	239
XI	1750(b), 1760(sh)	905	5.80	267

a) The representative spectra are shown in Fig. 1, 2, and 3.
b) Other signals observed commonly are as follows. i) acyl moiety —$-CH_2\underline{CH}_3$, 0.88—0.90 (triplet, J=7 Hz) (exceptions: I, 1.14; II, 1.10; III, 0.92); $-COCH_2(\underline{CH}_2)_{n-3}-$, 1.08—1.88 (multiplet) (exceptions: I, undetectable; II, 1.50—2.05); $-COCH_3$ (×4), 1.90—2.30; $-OCOC_2\underline{H}-$, 2.36—2.40 (triplet, J=7 Hz: exception; I, quartet); ii) sugar moiety—C_5–H, 3.85—3.90 (multiplet); C_6–H, 4.23—4.26 (octet); $C_{2,3,4}$–H, 5.00—5.48 (multiplet).
c) doublet, J=7.5 Hz
d) Other peaks detected commonly are shown in the text (see the Result and Discussion part).
e) b : broad
f) sh : shoulder

The general mass spectral features of these compounds were substantially similar each other. Fig. 3 shows the representative spectrum. As may be expected, the fragmentation patterns resemble to that of β-D-glucopyranose pentaacetate. The main pathways for fragmentation of the latter compound have been thoroughly discussed in the reviews.[16] Considering from the fragmentation behavior established for the pentaacetate, it is not surprising that all of the present compounds also showed the peaks at m/e 43, 73 (weak (w)), 98, 103 (w), 109, 115, 140, 145 (w), 157, 169, 200, 242, 331, and 347 (w). One additional series of peaks, so called E-series peaks,[16a] has been reported to occur in the spectrum of glucopyranose pentaacetate (peaks E_1 to E_7 at m/e 317, 257, 215, 197, 155, 137, and 95, respectively). Previous workers have revealed that elimination of the CH_2OAc function from the parent compound followed by the sequential loss of acetic acid and ketene account for these ions. Accordingly, in the case of the present compounds, the peaks belonging to the E-series should shift to m/e M-73, M-133, M-175, M-193, M-235, M-253, and M-295, respectively, as required by the substitution of the C_1-acetyl group for a longer fatty acyl function. In the actual spectra of the present compounds, most of these peaks were visible, though generally in very low intensities. On the other hand, our careful examination failed to detect the peaks attributable to the possible fragments derived from the furanose isomers. Thus, the compounds I—XI have been demonstrated to consist solely of the pyranose derivatives, without containing their furanose isomers. It is well known that the molecular ion of β-D-glucose penta-

16) *a*) N.K. Kochetkov, and O.S. Chizhov, "Methods in Carbohydrate Chemistry," Vol. VI, ed. by R.L. Whistler, and J.N. BeMiller, Academic Press, New York and London, 1972, p. 540; *b*) H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structural Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden-Day, Inc., SanFrancisco, London, and Amsterdam, 1964, p. 203.

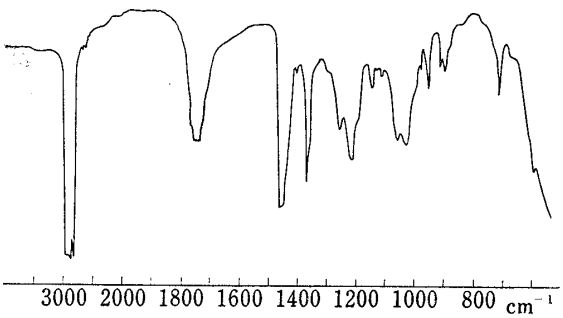

Fig. 1. IR Spectrum (Nujol) of Compound X

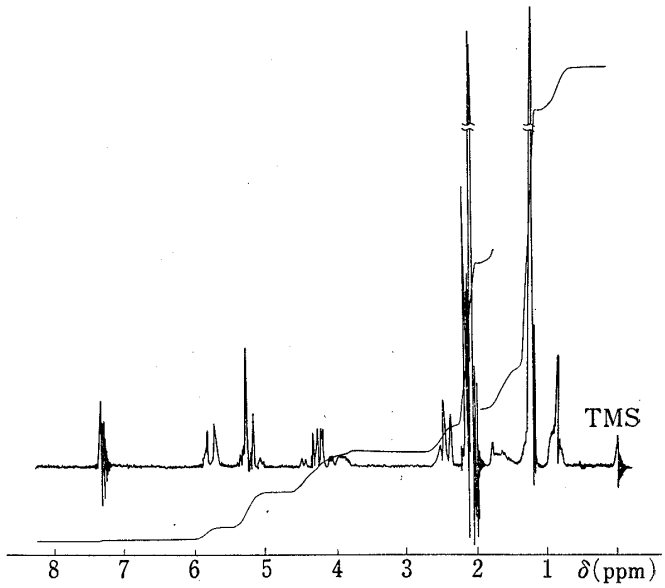

Fig. 2. NMR Spectrum of Compound IX
100 MHz, $CDCl_3$

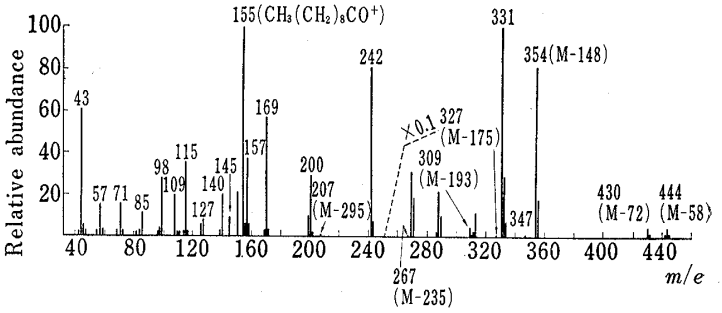

Fig. 3. Mass Spectrum of Compound VII

acetate is undetectable, and the largest recognizable ion is formed by the loss of 43 mass units (CH_3CO^+) from the glycosidic C_1-acetyl group. Similarly, our present compounds produce no molecular ion peaks, but give the intense peaks corresponding to $CH_3(CH_2)_{n-2}CO^+$ fragments (*m/e* M-347) (see Table II and the peak at *m/e* 155 in Fig. 3). The mass shifts in the latter peaks thus offer a convenient means of determining the chain-length of the acyl substituent attached to C_1. Besides the peaks mentioned above, a series, of alkyl ion peaks (*m/e* 57, 71, 85, ------) formed by fission of the acyl moiety, as well as some unidentified peaks (*e.g.*, *m/e* M-58 (w), M-72 (w), and M-148 (relatively strong)), could be observed commonly in the mass spectra of compounds I—XI.

Chromatographic behaviors of the present products were examined by TLC and GLC under various conditions. TLC analyses were carried out on silica gel G layer, using a variety of developing solvent systems. But none of the solvent systems tested gave a good resolution of these compounds. By many workers the mixtures of benzene–acetone or benzene–methanol in appropriate ratios have been recommended for the separation of various sugar esters. Our present compounds showed, however, a tendency to form the tailing spots by application of such solvent systems. At present, we recommend a mixed solvent of ether–hexane (2: 1) for the separation of this type of compounds. By employment of the solvent, well round shaped spots were found to be attainable. The resulting *Rf* values are listed in Table I. On the other hand, satisfactory separation of these compounds was achieved by GLC, using SE-30, OV-1, or OV-17 as a stationary phase. The relative retention times (rt_R) obtained by either isothermal or linear temperature programmed (LTPG) operation are presented in Table III. As can be seen in Fig. 4, a total synthetic mixture of compounds I—XI was simultaneously separated by LTPG operation. When adopted the isothermal operations, linear relationship could be observed between the log rt_R values and the number of carbon atoms contained in the acyl functions (Fig. 5). The relation may be utilized for identification of the unknown analogs. The calibration curves for compounds I, V, VIII, and X were prepared under the conditions mentioned in Table IV. As illustrated in Fig. 6, they showed good linearity without exception. The recorder response factors thus determined are shown

Table III. Relative Retention Times of 1-O-Acyl-β-D-glucopyranose Tetraacetates

Compd. No.	Relative retention times								
	SE-30[a]			OV-17[b]			OV-1[c]		
	Isothermal		LTPG[d] 230°→260°	Isothermal		LTPG[d] 240°→290°	Isothermal		LTPG[d] 240°→290°
	230°	250°		240°	290°		240°	290°	
I	1.22		0.12	1.20		0.10	1.24		0.13
II	1.52		0.15	1.47		0.13	1.47		0.15
III	1.96		0.19	1.90		0.16	1.82		0.19
IV	2.52		0.24	2.43		0.19	2.29		0.23
V	4.26		0.37	4.17	0.27	0.31	3.76		0.36
VI	5.35	0.34	0.46	5.50	0.33	0.38	4.82	0.50	0.44
VII	7.26	0.42	0.57	7.20	0.40	0.47	6.24	0.57	0.55
VIII	12.22	0.68	0.84	12.67	0.58	0.68	10.47	0.83	0.82
IX		1.13	1.19		0.88	0.93		1.21	1.19
X		1.89	(1.59)[e]		1.35	1.22		1.79	1.62
XI		3.22	(2.16)[e]		2.02	1.52		2.64	2.12
Internal standard									
G[f]	1.00 (2.3 min)			1.00 (3.0 min)			1.00 (1.7 min)		
S[g]		1.00 (16.1 min)	1.00 (20.4 min)		1.00 (8.6 min)	1.00 (30.3 min)		1.00 (4.2 min)	1.00 (16.5 min)

a) 1.5% SE-30 on Chromosorb W (AW-DMCS) (60—80 mesh); 2.5 m×4 mm I.D.; N_2, 50 ml/min
b) 1.5% OV-17 on Shimalite W (80—100 mesh); 2 m×4 mm I.D.; N_2, 50 ml/min
c) 1.5% OV-1 on Shimalite W (80—100 mesh); 2 m×4 mm I.D.; N_2, 50 ml/min
d) LTPG (linear temperature programmed) operation at 1°/min; Representative gas chromatogram is shown in Fig. 4.
e) data obtained by isothermal hold at 260° after LTPG operation
f) G: β-D-glucopyranose pentaacetate
g) S: sucrose octaacetate

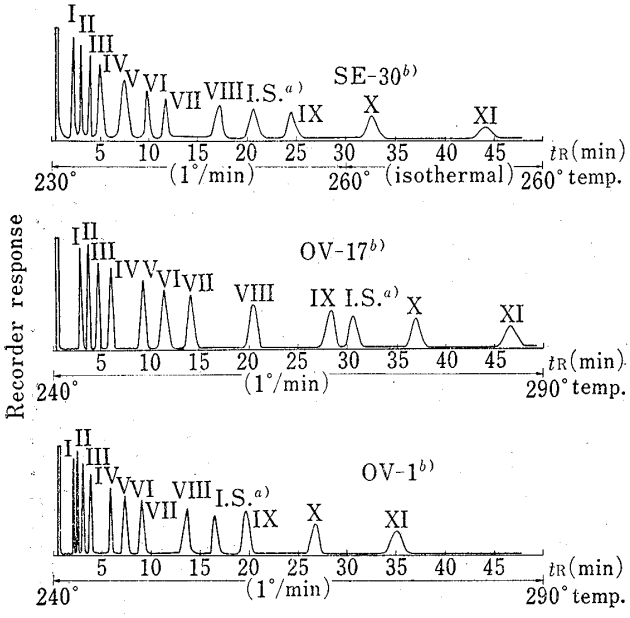

Fig. 4. Representative Gas Chromatograms of a Synthetic Mixture of Compounds I-XI[c]

a) I.S. (internal standard), sucrose octaacetate
b) Details of the operating conditions and the relative retention times are shown in Table III.
c) composition (mg): I, 3.9; II, 4.3; III; 3.9; IV, 4.1; V, 4.0; VI, 3.7; VII, 3.7; VIII, 4.3; IX, 5.1; X, 4.9; XI, 5.6; I.S., 4.3

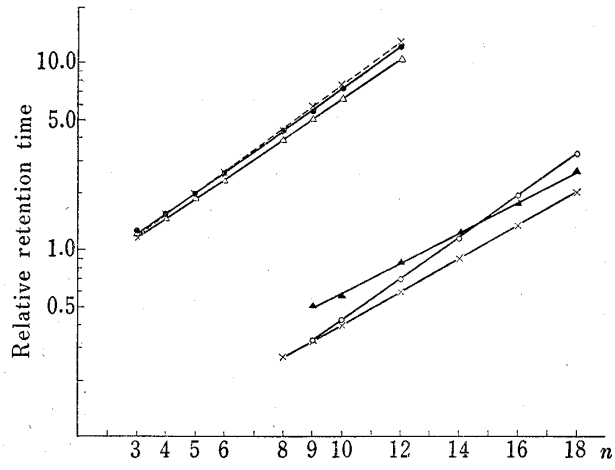

Fig. 5. Relationship between the Relative Retention Times and the Number of Carbon Atoms in Acyl Groups

conditions[a]	I.S.[b]
---×---: OV-17, at 240°	G
—●—: SE-30, at 230°	G
—△—: OV-1, at 240°	G
—○—: SE-30, at 250°	S
—▲—: OV-1, at 290°	S
—×—: OV-17, at 290°	S

a) details, see Table III.
b) G, β-D-glucopyranose pentaacetate; S, sucrose octaacetate

Table IV. Recorder Response Factors for Compounds I, V, VIII, and X

Compound No.	rt_R[a]	Internal standard[b] (t_R (min))	GLC Conditions: Column[c]	GLC Conditions: Temp.[d] (°C)	Calibration curve No.[e]	Recorder response factor
I	1.36	G (5.3)	OV-17	230	1	1.02
V	1.20	P (5.1)	OV-17	260	2	0.88
VIII	2.93	P (3.8)	OV-17	270	3	0.70
X	1.40	S (7.5)	OV-17	295	4	0.50
I	1.22	G (2.3)	SE-30	230	5	0.98
V	1.27	P (2.6)	SE-30	260	6	0.95
VIII	3.23	P (2.6)	SE-30	260	7	0.47

a) retention times relative to the internal standard (1.00)
b) G, β-D-glucopyranose pentaacetate; P, phenyl β-D-glucopyranoside tetraacetate; S, sucrose octaacetate
c) details, see foot-notes in Table III.
d) isothermal operation
e) See Fig. 6.

in Table IV. From these results, it has been indicated that GLC provides a useful tool for both the qualitative and quantitative analyses of 1-O-acylglycose tetraacetates.

The biological activities of the present products are now being tested, and the results will be reported soon elsewhere.

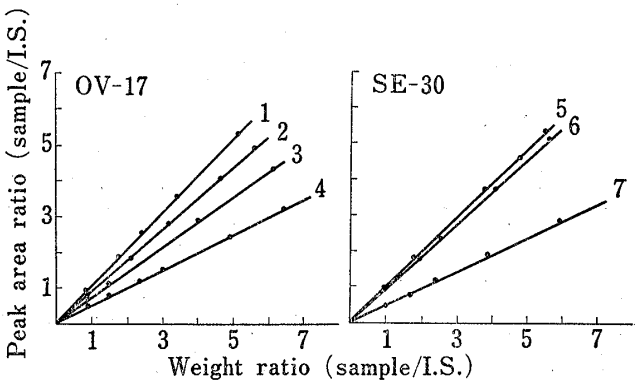

Fig. 6. Calibration Curves
The experimental conditions employed are shown in Table IV.

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