Chem. Pharm. Bull.
30(4)1169—1174(1982)

Chemical and Biochemical Studies on Carbohydrate Esters. XIII.[1] Synthesis of 6-*O*-, 6,6′-Di-*O*-, and 4,6,4′,6′-Tetra-*O*-stearoyl-α,α-trehaloses

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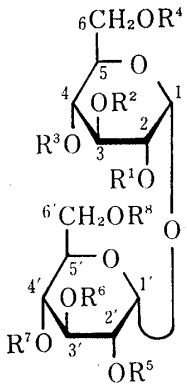
(Received, August 24, 1981)

2,3,2′,3′-Tetra-*O*-benzyl-α,α-trehalose was synthesized conveniently in improved yield according to the known pathway but under modified reaction conditions: namely, α,α-trehalose was treated with α,α-dimethoxytoluene to give its 4,6: 4′,6′-di-*O*-benzylidene derivative, which was benzylated with benzyl chloride in dimethylsulfoxide in the presence of sodium hydride, and subsequently debenzylidenated by hydrolysis with 80% acetic acid. Selective acylation of the key intermediate by reaction with 1.4 molar equivalents of stearoyl chloride, followed by catalytic hydrogenolysis over palladium black, afforded 6-*O*-stearoyl-α,α-trehalose, mp 116—122°C, $[\alpha]_D^{18}$ +108.2° (c=1.0, chloroform), and 6,6′-di-*O*-stearoyl-α,α-trehalose, mp 157—160°C, $[\alpha]_D^{18}$ +80.8° (c=1.0, chloroform), in an approximate molar ratio of 1: 3.8. Similarly, 4,6,4′,6′-tetra-*O*-stearoyl-α,α-trehalose, mp 95—97°C and 108—110°C (double melting point), $[\alpha]_D^{17}$ +54.5° (c=1.0, chloroform), was also obtained by the use of 4 molar equivalents of acid chloride. Based on comparison of the carbon-13 nuclear magnetic resonance (^{13}C NMR) spectral data and thin-layer and gas-liquid chromatographic behavior, the major components contained in the mono- and diester preparations which had been produced in our previous work by transesterification of α,α-trehalose with methyl stearate and shown to possess interesting biological activities were identified as the 6- and 6,6′-stearates, respectively.

Keywords——synthesis; 6-*O*-stearoyl-α,α-trehalose; 6,6′-di-*O*-stearoyl-α,α-trehalose; 4,6,4′,6′-tetra-*O*-stearoyl-α,α-trehalose; 4,6: 4′,6′-di-*O*-benzylidene-α,α-trehalose; 2,3,2′,3′-tetra-*O*-benzyl-α,α-trehalose; ^{13}C NMR

In previous studies of this series, we have found that an isomeric monoester mixture (TS-C(mono)) obtained by the transesterification of α,α-trehalose (**1**) with methyl stearate exerts an antitumor effect against Ehrlich ascites carcinoma,[2] and that an analogous diester preparation (TS-C(di)) has the ability to enhance the phagocytic activity of the reticuloendothelial system in mice.[1] Although the major components contained in TS-C(mono) and TS-C(di) were tentatively assigned as 6-*O*- (**2**) and 6,6′-di-*O*-stearoyl-α,α-trehalose (**3**), respectively, conclusive evidence for this identification is still required. At this time, it is also uncertain whether or not the major constituents are really responsible for the biological activities of these preparations. In order to elucidate these points, our present paper deals with the regioselective synthesis of **2** and **3**, as well as of 4,6,4′,6′-tetra-*O*-stearoyl-α,α-trehalose (**4**).

As a key intermediate, we employed a known compound, 2,3,2′,3′-tetra-*O*-benzyl-α,α-trehalose (**5**), which was first synthesized by Hough *et al.*[3] They prepared **5** by converting **1** into its 4,6: 4′,6′-di-*O*-benzylidene derivative (**6**) (benzaldehyde and zinc chloride), followed by benzylation (benzyl chloride and sodium hydoxide) and subsequent debenzylidenation (acid-catalyzed ethanolysis). We accomplished the synthesis of **5** according to the same pathway but with modified conditions in each reaction. For benzylidenation of **1**, we applied the method of Evans, who has recently succeeded in preparing methyl 4,6-*O*-benzylidene-α-D-glucopyranoside by treatment of methyl α-D-glucopyranoside with α,α-dimethoxytoluene (benzaldehyde dimethyl acetal).[4] By application of the procedure, the desired diacetal **6** was obtained in good yield (86%) after only 2 h; the yields reported by Hough *et al.* were 66 and 78% after 72 and 120 h, respectively. In the present study, benzylation of **6** was



Compd.	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8
1	H	H	H	H	H	H	H	H
2	H	H	H	COR′[a]	H	H	H	H
3	H	H	H	COR′	H	H	H	COR′
4	H	H	COR′	COR′	H	H	COR′	COR′
5	CH_2Ph	CH_2Ph	H	H	CH_2Ph	CH_2Ph	H	H
6	H	H	>CHPh		H	H	>CHPh	
7	CH_2Ph	CH_2Ph	>CHPh		CH_2Ph	CH_2Ph	>CHPh	
8	CH_2Ph	CH_2Ph	H	COR′	CH_2Ph	CH_2Ph	H	H
9	CH_2Ph	CH_2Ph	H	COR′	CH_2Ph	CH_2Ph	H	COR′
10	CH_2Ph	CH_2Ph	COR′	COR′	CH_2Ph	CH_2Ph	COR′	COR′

a) $R' = -(CH_2)_{16}CH_3$

Chart 1

conducted according to the method recommended by Iwashige and Saeki:[5] namely, **6** was treated with benzyl chloride in dimethylsulfoxide (DMSO) in the presence of sodium hydride at room temperature to afford 2,3,2′,3′-tetra-*O*-benzyl-4,6: 4′,6′-di-*O*-benzylidene-α,α-trehalose (**7**) in 79% yield. Subsequent removal of the benzylidene groups of **7** was achieved by hydrolysis with 80% acetic acid to give **5** in 92% yield. The modified procedures adopted here in each step seem preferable for the synthesis of **5**.

There are several pieces of evidence to indicate that the order of reactivity of the hydroxyl groups in **1** toward acylation is as follows: HO-6, -6′>HO-2, -2′>HO-3, -3>HO-4, -4′.[6] It was therefore, anticipated that preferential production of 6-*O*-and 6,6-di-*O*-stearoyl derivatives (**8** and **9**) could be attained by partial acylation of **5**. After several preliminary experiments, we decided to perform the selective esterification of **5** by treating it with 1.4 molar equivalents of stearoyl chloride in pyridine–methylene chloride at room temperature overnight. On thin-layer chromatography (TLC), the syrupy product obtained showed a small spot and a much larger spot, which were later assigned as **8** and **9**, respectively; other theoretically possible esters were virtually absent. Without further purification, the mixture of **8** and **9** was subjected to catalytic hydrogenolysis over palladium black, and the resulting product was recrystallized to afford the 6,6′-di-*O*-stearate **3**, $C_{48}H_{90}O_{13}$, mp 157—160°C, $[\alpha]_D^{18}$ +80.8° (c=1.0, chloroform). The mother liquor was concentrated and fractionated on a silica gel column to furnish first an additional amount of **3** and then the 6-*O*-stearate **2**, $C_{30}H_{56}O_{12}$, mp 116—122°C, $[\alpha]_D^{18}$ +108.2° (c=1.0, chloroform). The yields of **2** and **3** from 2g of **5** were 0.33 g (19%) and 1.81 g (73%), respectively.

Unequivocal proof of the acyl locations in **2** and **3** was obtained by measuring the carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra in the Fourier-transform mode with complete proton decoupling (Table I). Because of the symmetry of the molecule, the disaccharide **1** itself gave only six signals, which appeared in the region between 60 and 100 ppm. Those signals were assigned as shown in Table I, based on a comparison of the chemical shifts with the data reported for methyl α-D-glucopyranoside:[7] the signal due to the carbons of exocyclic hydroxymethyl groups (C-6 and C-6′) appears at fairly high field (62.8 ppm) and can be differentiated readily from those (>70 ppm) of the remaining carbons. As expected, the spectrum of **3** also showed only six signals in the above region, indicating that, upon acylation, the signal due to C-6 and C-6′ and the signal due to the adjacent carbons, C-5 and C-5′, were considerably shifted downfield (+1.5 ppm) and upfield (−2.7 ppm), respectively, whereas other signals exhibited little change. Thus, **3** was confirmed to be the diester possessing the stearoyl functions at 6- and 6′-positions. On the other hand, the spectrum of **2** gave twelve signals in the same region: six of them proved to correspond to the signals shown by **1** and the rest to those detected with **3**. Thus, **2** was concluded to be the monostearate carrying its acyl moiety at the C-6 position.

Table I. ^{13}C NMR Spectral Data for **2**, **3**, **4** and Related Specimens (Sugar Moiety Only)[a]

Sample	Chemical shifts[b,c]											
	C-1	C-1′	C-2	C-2′	C-3	C-3′	C-4	C-4′	C-5	C-5′	C-6	C-6′
α,α-Trehalose	95.4		73.5		74.9		72.3		74.3		62.8	
6-Monostearate, **2**	95.7	95.6	73.5	73.4	74.9	74.8	72.0	72.3	71.5	74.4	64.3	62.8
6,6′-Distearate, **3**	95.8		73.3		74.9		72.0		71.6		64.3	
4,6,4′,6′-Tetra-stearate **4**	95.9		73.3		72.4		72.2[d]		69.3		63.1	
TS-C(mono)[e]	95.6		73.4		74.9	74.8	72.0	72.3	71.5	74.4	64.3	62.8
	(93.1, 96.2)		(73.2)								(62.4)	
TS-C(di)	95.7		73.3		74.8		71.9		71.5		64.3	
	(95.3, 92.3)		(72.9)		(74.4)		(72.2)				(64.6, 62.7, 62.4)	

a) 25.0 MHz, in pyridine-d_5 at 24.5°C.
b) In ppm from TMS.
c) Figures in parentheses indicate the chemical shifts of the minor signals.
d) This assignment was confirmed by ^1H selective hetero-spin decoupling.
e) The signals due to C-1 (C-2) and C-1′ (C-2′) overlap each other.

In our previous study, compositional analysis of TS-C(mono) was carried out by TLC, as well as by gas liquid chromatography (GLC), showing that the preparation was composed of a major monoester (*ca.* 80%) and certain minor isomers.[2] The TLC and GLC behavior of the presently synthesized 6-monostearate **2** was identical with that of the major component contained in TS-C (mono). On TLC, TS-C(di) gave a chromatogram consisting of a large spot and a small spot, and the *Rf*-value of the former proved to agree well with that of **3**: GLC was practically unsuitable for analysis of the diesters owing to their inadequate volatility. Furthermore, a gross similarity could be observed when the ^{13}C-NMR spectra of TS-C(mono) and TS-C(di) were compared with those of **2** and **3**, respectively, though some additional small signals were detectable in the spectra of both TS-C(mono) and TS-C(di). On the basis of these findings, the principal constituents contained in TS-C(mono) and TS-C(di) have now been demonstrated to be **2** and **3**, respectively. At this time, we are unable to assign the minor esters existing in these preparations to any specific isomers. However, in view of the aforementioned order of reactivity of the hydroxyl groups in **1**, the chief minor isomer in TS-C(mono) may be assumed to be the 2-stearate, while that in TS-C(di) may be the 2,2′-, 2,6-, or 2,6′-distearate.

So far, extensive studies on the syntheses of cord-factor (α,α-trehalose-6,6′-dimycolate) and related simpler analogs have been carried out by various workers interested in their diverse biological activities.[8] However, as pointed out by Liav and Goren in 1980, most of the methods reported have certain deficiencies (poor yields, production of multiesters, formation of undesired by-products, *etc.*).[8a] Our present procedure represents a new approach to the convenient synthesis of this class of compounds.

To date, only two examples of stearoyl derivatives of **1**, that is, the octa- and 2,3,4,2′,3′,4′-hexa-*O*-stearates, appear in the literature.[9] During the course of the present work, 4,6,4′,6′-tetra-*O*-stearate **4**, $C_{84}H_{158}O_{15}$, mp 95—97°C and 108—110°C double melting point), $[\alpha]_D^{17}$ +54.5° (c=1.0, chloroform), was newly obtained in addition to **2** and **3**. The synthesis of **4** was readily accomplished *via* 2,3,2′,3′-tetra-*O*-benzyl-4,6,4′,6′-tetra-*O*-stearoyl-α,α-trehalose (**10**), mp 56—57°C, which was prepared by treatment of **5** with 4 molar equivalents of stearoyl chloride. As expected, the ^{13}C NMR spectrum of **4** showed only six signals in the 60—100 ppm region (Table I). Considerable acylation shift could be observed in the signal due to C-3 and C-3′ (−2.5 ppm), as well as in the signal due to C-5 and C-5′ (−5.0 ppm), but not in those of the remaining carbons. The spectral features of **4** are comparable with the data reported for methyl 4,6-di-*O*-myristoyl-α-D-glucopyranoside:[10] at this time, the reason for the small

change in the chemical shifts of the carbons at which the acyl functions were introduced is still obscure.

The biological activities of the chemically pure samples obtained in this work, as well as of related analogs, are now being tested and the results will be reported in a forthcoming paper.

Experimental

Unless otherwise stated, solutions were concentrated in a rotary evaporator below 40°C under a vacuum. Melting points were determined with a Yanagimoto MP-S2 micro melting point apparatus, and are uncorrected. Optical rotation was measured with a JASCO DIP-SL automatic polarimeter. Infrared (IR) spectra were recorded with a JASCO IR-G spectrometer. ^1H and ^{13}C NMR spectra were taken at 100 and 25 MHz, respectively, with a JEOL FX-100 FT NMR spectrometer, using tetramethylsilane (TMS) as an internal reference, and chemical shifts are expressed in terms of ppm; some ^1H NMR spectra were measured at 60 MHz with a JNM-PMX-60 spectrometer. TLC was performed on precoated silica gel plates (Kieselgel 60 GF_{254}; 0.25 mm thick), and detection was effected by spraying 10% H_2SO_4 followed by heating. GLC was carried out with a Shimadzu gas chromatograph GC4CM-PF coupled with a hydrogen flame ionization detector, using a glass column. Samples to be analysed by GLC were trimethylsilylated according to the method of Sweeley.[11]

4,6: 4′,6′-Di-*O*-benzylidene-α,α-trehalose (6)——This compound was prepared by application of the procedure of Evans.[4] The commercial α,α-trehalose dihydrate (Nakarai Chemicals Ltd.) was heated at 70°C *in vacuo* over P_2O_5 for 5 h to remove moisture. For preparation of α,α-dimethoxytoluene, a solution of benzaldehyde (21 g) and trimethylorthoformate (24 g) in MeOH (100 ml) containing Amberlite IR-120 (H^+) ion exchange resin (1 g, 20—50 mesh, washed with MeOH) was boiled for 3 h under reflux. After removal of the resin by filtration, the filtrate was concentrated below 35°C, and the residual product was distilled at 104—108°C/40 Torr to give α,α-dimethoxytoluene in *ca.* 85% yield. A mixture of the dry α,α-trehalose dihydrate (**1**) (9.7 g, 26 mmol), freshly distilled α,α-dimethoxytoluene (15.2 g, 100 mmol), dry dimethylformamide (DMFA) (50 ml), and *p*-toluenesulfonic acid monohydrate (0.07 g) was placed in a 200 ml round-bottomed flask; this was attached to a rotary evaporator and rotated in a water-bath at 65°C under reduced pressure (40 mmHg), so that DMFA refluxed in the vapor duct. After 2 h, the DMFA was distilled off by decreasing the pressure to 20 mmHg and raising the temperature of the water-bath to 100°C. The resulting residue was applied to a short column (4×6 cm) of Florisil (Floridin Co.), and developed successively with benzene, $CHCl_3$, and acetone. Recrystallization of the acetone eluate from aq. MeOH gave the diacetal **6** as a monohydrate (11.8 g, 86%), mp 192—194°C, which was used in the subsequent experiment without being dried. A portion of this product was dehydrated by evaporation from pyridine according to the procedure described by Birch[12] for the dehydration of α,α-trehalose dihydrate. The crystalline residue was washed with ether and dried to afford **6** as an anhydrate, mp 195—196°C, $[\alpha]_D^{20}$ +82.6° (c=0.5, MeOH) [Lit. i) Hanessian *et al.*:[13a] hydrate (H_2O, 2.4%), mp 195°C; anhydrate, mp 195°C, $[\alpha]_D^{25}$ +80.3° (c=0.424, MeOH). ii) Hough *et al.*:[13b] hemihydrate, mp 198—199°C, $[\alpha]_D^{20}$ +92.4° (c=1.0, acetone)]. ^1H NMR (in $CDCl_3$, at 60 MHz) δ: 5.35 (2H, d, J=3.5 Hz, $C_{1.1}'$-H), 5.53 (2H, s, PhC<u>H</u>(×2), 7.2—7.5 (10H, aromatic protons). *Anal.* Calcd for $C_{26}H_{30}O_{11}$: C, 60.22; H, 5.83. Found: C, 60.18; H, 5.97.

2,3,2′,3′-Tetra-*O*-benzyl-4,6: 4′,6′-di-*O*-benzylidene-α,α-trehalose (7)——The benzylation reaction was carried out according to the procedure described by Iwashige.[5] The commercial NaH (50%, coated with mineral oil) was washed thoroughly by stirring it with *n*-hexane and decanting the wash. The NaH was added to dimethyl sulfoxide (DMSO) (15 ml) with stirring under an argon atmosphere and a solution of **6** (4.0 g, 7.5 mmol as monohydrate) in DMSO (15 ml) was added dropwise. The whole was further stirred at room temperature for 1 h to complete the salt formation. Then, a solution of benzyl chloride (6.4 g, 50 mmol) in DMSO (10 ml) was added dropwise and stirring was continued for a further 2 h in an argon atmosphere. The reaction mixture was poured into ice-water and extracted with CH_2Cl_2. The extract was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography on silica gel (4×6 cm), developing with CH_2Cl_2, to furnish **7** as a viscous syrup; yield, 5.0 g (79%). The IR spectrum (in nujol) did not show OH-absorption bands. ^1H NMR (in $CDCl_3$, at 60 MHz) δ: 5.51 (2H, s, PhC<u>H</u>(×2), 7.1—7.5 (30H, aromatic protons).

2,3,2′,3′-Tetra-*O*-benzyl-α,α-trehalose (5)——Compound **7** (4.95 g) was heated with 80% aq. AcOH (60 ml) under reflux for 30 min. The reaction mixture was evaporated to dryness under reduced pressure, and the residue was recrystallized from a mixed solvent of ether and *n*-hexane to afford **5** as colorless needles; yield, 3.7 g (92%). mp 186—189°C, $[\alpha]_D^{20}$ +124° (c=1.0, $CHCl_3$). [Lit.[3] mp 186—188°C, $[\alpha]_D$ +120° (c=(c=1.0, $CHCl_3$)]. ^1H NMR (in $CDCl_3$, at 100 MHz) δ: 3.40—4.10 (12H, $C_{2,3,4,5,6,2',3',4',5',6'}$-H), 4.70, 4.83, 4.98 (8H, $PhCH_2$-×4), 5.17 (2H, d, J=3.4 Hz, $C_{1.1}'$-H), 7.30—7.37 (20H, aromatic protons). *Anal.* Calcd for $C_{40}H_{46}O_{11}$: C, 68.36; H, 6.60. Found: C, 68.11; H, 6.58.

2,3,2′,3′-Tetra-*O*-benzyl-6,6′-di-*O*-stearoyl-α,α-trehalose (9) and 2,3,2′,3′-Tetra-*O*-benzyl-6-*O*-stearoyl-α,α-trehalose (8)——Compound **5** (2 g, 2.85 mmol) was dissolved in a mixed solvent of pyridine and CH_2Cl_2

(20 ml each). To the solution, 1.4 molar equivalents of stearoyl chloride (Eastman Kodak Co.) (1.2 g) was added at 0°C under stirring, and then the mixture was stirred at room temperature overnight. On TLC with $CHCl_3$–MeOH (60: 1 v/v), the final reaction mixture showed a large spot A_1 (*Rf* 0.83) and a small spot A_2 (*Rf* 0.60) in addition to a faint spot due to unchanged **5** (*Rf* 0.26). The mixture was poured into ice-water and extracted with $CHCl_3$. The extract was evaporated to a small volume and applied to a column of Florisil (2×5 cm). Elution was effected first with *n*-hexane and then with $CHCl_3$. On the basis of TLC monitoring, the fractions corresponding to the spots A_1 and A_2 were collected and combined. Removal of the solvents afforded a mixture of **9** and **8** as a colorless semi-solid; yield, 3.1 g.

6,6′-Di-*O*-stearoyl-α,α-trehalose (3) and 6-*O*-Stearoyl-α,α-trehalose (2)——The mixture of **9** and **8** (3.1 g) was hydrogenolyzed in MeOH–CH_2Cl_2 (40 ml each) for 4 h, using Pd-black as a catalyst. The catalyst was collected by filtration, and washed with hot MeOH. The combined filtrate and washings were evaporated to dryness. The residue was recrystallized from MeOH to afford **3** as colorless needles (1.51 g). mp 157—160°C, $[\alpha]_D^{18}$ +80.8° (*c*=1.0, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1735 (ester). ^1H NMR (in pyridine-d_5, at 100 MHz) δ: 0.88 (6H, t, *J*=6.0 Hz, $-CH_3\times2$), 1.10—1.84 (60H, $-OCOCH_2(CH_2)_{15}CH_3\times2$), 2.32 (4H, t, *J*=7.5 Hz, $-OCO-CH_2-\times2$), 4.00—5.16 (12H, m, $C_{2,3,4,5,6,2',3',4',5',6'}$–H), 5.84 (2H, d, *J*=3.6 Hz, $C_{1,1'}$–H). ^{13}C NMR (in pyridine-d_5, at 25 MHz): see Table I. *Anal.* Calcd for $C_{48}H_{90}O_{13}$: C, 65.87; H, 10.37. Found: C, 65.53; H, 10.24. After recrystallization, the mother liquor was concentrated *in vacuo* and the residue was chromatographed on a silica gel column (2×15 cm). Elution with $CHCl_3$–MeOH (15: 1) gave an additional amount of **3** (0.30 g; total yield of **3**, 1.81 g). Further elution with $CHCl_3$–MeOH (10: 1) furnished **2** as a colorless amorphous powder (0.33 g). mp 116—122°C, $[\alpha]_D^{18}$ +108.2° (*c*=1.0, $CHCl_3$) IR ν_{max}^{KBr} cm^{-1}: 1735 (ester). ^1H NMR (in pyridine-d_5, at 100 MHz) δ: 0.87 (3H, t, *J*=6.0 Hz, $-CH_3$), 1.10—1.90 (30H, $-OCOCH_2-(CH_2)_{15}CH_3$), 2.33 (2H, t, *J*=7.5 Hz, $-OCOCH_2-$), 4.00—4.50 (2H, C_6'–H), 4.5—6.2 (10H, $C_{2,3,4,5,6,2',3',4',5'}$–H), 5.88 (2H, d, *J*=3.7 Hz, $C_{1,1'}$–H). ^{13}C NMR (in pyridine-d_5, at 100 MHz): see Table I. *Anal.* Calcd for $C_{30}H_{56}O_{12}$: C, 59.19; H, 9.27. Found: C, 58.97; H, 9.11.

Identification of Major Components in TS-C(di) and TS-C(mono) with 3 and 2, respectively——The procedures employed for the production of TS-C(di) and TS–C(mono) were essentially similar to those described in our previous paper.[2] As stated in ref. 2, transesterification of α,α-trehalose with methyl stearate yielded a crude product designated as "Preparation A," which contained mono-, di-, and polyesters. On TLC with $CHCl_3$–MeOH–AcOH–H_2O (79: 11: 8: 2), the crude product gave a large spot (*Rf* 0.07—0.14) and several minor spots (*Rf* 0.37, 0.56, 0.58, and 0.62). On silica gel column chromatography, a monoester mixture, TS–C(mono) (designated as "Preparation C" in ref. 2), corresponding to the large spot and a diester mixture, TS–C(di), corresponding to the chief minor spot with *Rf* 0.37, were isolated. Under these TLC conditions, **3** and **2** showed the same mobilities as TS–C(di) and TS–C(mono), respectively. When TLC was conducted by triple developments with $CHCl_3$–MeOH (82: 18) and with $CHCl_3$–MeOH (5: 1), partial resolution of isomeric components in TS–C(mono) and of those in TS–C(di), respectively, could be achieved. *Rf*-values: TS–C-(mono), 0.49—0.63(main) and 0.66(minor); TS–C(di), 0.62(main) and 0.57(minor). The *Rf*-values of the main spots detected with TS–C(di) and TS–C(mono) were identical with those of **3** and **2**, respectively. In ref. 2, the GLC behavior of the trimethylsilylated "Preparation C" was examined under the following conditions: column packing, 1.5% OV-1 on Shimalite W; column size, 2 m×4 mm i.d.; column temperature, 295°C (isothermal); carrier gas, N_2 (flow-rate, 50—55 ml/min). The resulting chromatogram consisted of a large peak and some much smaller peaks: the retention times (t_R) relative to the octa-trimethylsilyl ether of α,α-trehalose (=1.00 (1.65 min)) and the peak area ratios (%) were as follows: 10.02(11.0%), 11.42(9.2%),13.12 (2.3%), and 14.84(77.6%). In these GLC conditions, TS–C(mono) gave a chromatogram similar to that reported for "Preparation C," and the t_R-value of the major peak was confirmed to be identical with that of **2**. As discussed in the text, supporting evidence for the identity of the major components contained in TS-C(di) and TS-C(mono) with **3** and **2**, respectively, was also provided by comparison of the ^{13}C-NMR spectral data (Table I) for these preparations with those for **3** and **2**.

4,6,4′,6′-Tetra-*O*-stearoyl-α,α-trehalose (4)——To a solution of **5** (3.69 g, 5 mmol) in dry pyridine (50 ml), 4.0 molar equivalents of stearoyl chloride (5.86 g) dissolved in CH_2Cl_2 (50 ml) was added dropwise at 0°C, and the mixture was stirred for 48 h at room temp. The mixture was then poured into ice-water and extracted with $CHCl_3$. The extract was washed successively with H_2O, 2% HCl, and H_2O, dried over Na_2SO_4, and concentrated under reduced pressure to give a slightly brownish residue. On TLC (*n*-hexane–ether (1: 2 v/v)), the crude product showed a major spot at *Rf* 0.81 along with minor spots at *Rf* 0.46, 0.27, and 0.15 which were presumably ascribable to the 4,6,6′- and 4,6,4′-triesters and 6,6′-diester, respectively. The mixture was passed through a silica gel column (Wako gel C-200) with *n*-hexane–ether (2: 1 v/v) to furnish Frs. 1 (1.86 g), 2(5.76 g), 3(0.64 g), and 4(0.47 g). TLC: Fr. 1, *Rf* 0.81; Fr. 2, *Rf* 0.81 and 0.46; Fr. 3, *Rf* 0.46 and 0.27; Fr. 4, *Rf* 0.27 and 0.15. Fr. 2 (5.76 g) was rechromatographed over Florisil with *n*-hexane to afford Fr. 2a (2.99 g) which consisted almost solely of the ester corresponding to the major spot (*Rf* 0.81). Fr. 1 and Fr. 2a were combined and crystallized from petr. ether to give **10** as a colorless crystalline powder; total yield, 6.5 g (72%). mp 56—57°C. IR ν_{max}^{KBr} cm^{-1}: 1735 (ester); OH-absorption bands were not detected. Compound **10** (2.0 g) was dissolved in $CHCl_3$ (50 ml) and hydrogenolyzed over Pd-black (0.5 g) for 3 h at room temp. under atmospheric pressure. Removal of the solvent and the catalyst left an amorphous powder (1.60 g), which was crystallized from CH_2Cl_2 to give **4** as colorless leaflets; yield, 1.23 g (77%). mp 95—97°C

and 108—110°C (double mp). $[\alpha]_D^{17}$ +54.5° (c=1.0, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1732, 1717 (ester). ^1H NMR (in pyridine-d_5, at 100 MHz): δ 0.88 (12H, t-like, $-C\underline{H}_3\times3$), 1.0—1.90 (120H, $CH_3(C\underline{H}_2)_{15}CH_2-\times4$), 1.16—1.58 (8H, $CH_3(CH_2)_{15}C\underline{H}_2CO\times4$), 4.10—5.20 (10H), 5.66 (2H, t, J=9.5 Hz, $C_{4,4}'$-H), 5.78 (2H, d, J=3.7 Hz, $C_{1,1}'$-H). ^{13}C NMR (in pyridine-d_5, at 25 MHz): see Table I. *Anal.* Calcd for $C_{84}H_{158}O_{15}$: C, 71.65; H, 11.31. Found: C, 71.42; H, 11.56.

Acknowledgement The authors are indebted to Mr. Y. Itatani of this Faculty for elemental analyses and spectral measurements.

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