1826 BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 51 (6), 1826—1829(1978) [Vol. 51, No. 6

Synthesis of Stachyose Tetradecaacetate and An Isomer

Tetsuo SUAMI,* Toshiki OTAKE, Shigeru NISHIYAMA, Ryoichi ADACHI, and Katsumi NAKAMURA
Department of Applied Chemistry, Faculty of Engineering, Keio University, Hiyoshi, Yokohama 233

(Received November 9, 1977)

Condensation of 1′,2,2″,3,3′,3″,4,4′,4″,6′-deca-*O*-acetylraffinose (**1**) with 2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl chloride in the presence of tetraethylammonium chloride, followed by deacetylation, debenzylation and subsequent acetylation afforded stachyose tetradecaacetate. An isomer in which a terminal D-galctopyranosyl group is attached to the 6″-*O* of raffinose in β-anomeric linkage, was prepared by condensing **1** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide in the presence of mercury(II) cyanide. ^{13}C NMR spectra of stachyose tetradecaacetate and the isomer were discussed.

Stachyose was first discovered from a root of *Stachys tuberifera* (*Chorogi* in Japanese) by von Planta and Schulze in 1890.[1] Later this tetrasaccharide was found in other plants such as *Labiate*, *Pinuceae*, and *Leguminosae*.[2] The structure of stachyose was established by Onuki,[3] Laidlaw and Wylam,[4] and Hérissey and Courtois[5] as *O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-glucopyranosyl β-D-fructofuranoside.

In continuation of a previous study on sucrochemistry,[6] attempts were made to synthesize stachyose tetradecaacetate by condensing 1′,2,2″,3,3′,3″,4,4′,4″,6′-deca-*O*-acetylraffinose[6] (**1**) with 2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl chloride[7] (**2**). We now wish to report a successful synthesis of stachyose tetradecaacetate (**5**) by the condensation in the presence of tetraethyl ammonium chloride,[8–12] followed by deacetylation, debenzylation and subsequent acetylation.

Results and Discussion

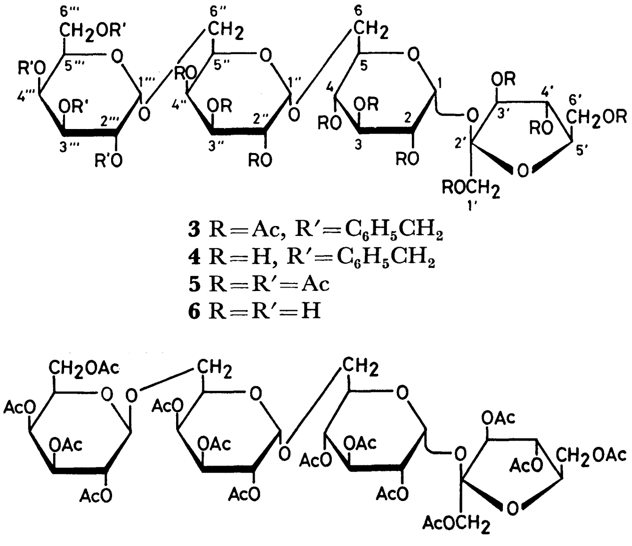
When **1** was condensed with **2** in dichloromethane in the presence of tetraethylammonium chloride, *N*,*N*-diisopropylethylamine and molecular sieves at ambient temperature for a prolonged period, *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-α-D-galactopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl) 1,3,4,6-tetra-*O*-acetyl-β-D-fructofuranoside (**3**) was obtained as crystals. Deacetylation of **3** in methanolic sodium methoxide gave 2‴,3‴,4‴,6‴-tetra-*O*-benzylstachyose (**4**). Debenzylation of **4** in aqueous methanol in the presence of palladium catalyst under hydrogen atmosphere, followed by acetylation gave **5**. Compound **5** was identical with authentic stachyose tetradecaacetate.[3] Deacetylation of **5** gave stachyose (**6**) which was identical with an authentic sample.[3] The present synthesis substantiates the effectiveness and the high stereoselectivity of the Lemieux's condensation system.[8–11]

In contrast, when **1** was condensed with **2** in the presence of mercury(II) cyanide in benzene, the product was contaminated with an isomer (isostachyose derivative)[22]: *O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-α-D-galactopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl) 1,3,4,6-tetra-*O*-acetyl-β-D-fructofuranoside. In this case, isolation of stachyose tetradecaacetate in a pure state was unsuccessful, owing to a similar chromatographic behavior of a contaminant in each step of the reactions. The contamination with isostachyose tetradecaacetate[22] (**7**) was demonstrated by measurement of ^{13}C NMR spectra of **5** and **7**. An authentic sample of **7** was prepared by condensing **1** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide[15] in the presence of mercury(II) cyanide in nitromethane.

^{13}C NMR spectra of sucrose octaacetate,[16] raffinose undecaacetate,[13] isoraffinose undecaacetate,[14] **5** and **7** have been determined. Assignments of the signals, obtained in accordance with the data for sucrose,[17–19] raffinose[19] and stachyose,[19] are given in Table 1.

The signals of the anomeric carbon atoms are readily recognized in the lower field (90.0—101.0 ppm). The chemical shift values for the anomeric carbon atoms of the α-D-glucopyranosyl moieties are almost the same in these cases (90.0—90.3 ppm). The anomeric carbon atoms of the α-D-galactopyranosyl groups (C-1″ and 1‴) reveal their signals at 95.9—96.2 ppm.

The signal of the anomeric carbon atom of the terminal β-D-galactopyranosyl group (C-1‴) in **7** (101.0 ppm) shifts downfield markedly from that of the corresponding α-D-galactopyranosyl group in **5** (96.2 ppm). This is in line with the fact that the C-1 of methyl α-D-glucopyranoside gives its signal in a 4.1 ppm higher field than that of the β-D-glucopyranoside.[20] This is also the



3 R=Ac, R′=$C_6H_5CH_2$
4 R=H, R′=$C_6H_5CH_2$
5 R=R′=Ac
6 R=R′=H

7

Scheme 1.

---

* To whom correspondence should be addressed.

TABLE 1. VALUES OF ^{13}C NMR CHEMICAL SHIFTS[a) OF SUCROSE OCTAACETATE, RAFFINOSE UNDECAACETATE, ISORAFFINOSE UNDECAACETATE, STACHYOSE TETRADECAACETATE (**5a**), AND ISOSTACHYOSE TETRADECAACETATE (**7**)

	Sucrose octaacetate[16)	Raffinose undecaacetate[13)	Isoraffinose undecaacetate[14)	Stachyose tetradecaacetate **5**	Isostachyose tetradecaacetate **7**
C-2′	104.1	104.9	104.4	104.8	104.6
C-1‴				96.2	101.0
C-1″		95.9	101.1	96.1	96.2
C-1	90.0	90.2	90.3	90.3	90.2
C-3′ (or 4′)	79.2	79.9	79.4	80.1	79.5
C-4′ (or 3′)	75.9	76.4	76.1	76.6	76.1
C-5′	75.2	75.9	75.4	76.1	75.4
Methine and methylene carbons of D-glucosyl and D-galactosyl moieties	70.4	70.5	71.2	70.4	70.9
	69.6	69.5	70.9	69.7 (2)	70.7
	68.6	69.4	70.5	68.7 (2)	70.5
	68.4	68.9	69.9	68.4	69.8
		68.3 (2)	69.2	68.3	69.6
		67.5	68.8	67.74	68.6 (4)
		66.4	68.6	67.70	67.7 (2)
		66.0	67.2	67.5	67.1 (2)
			67.0	67.1	65.8
				66.7	
				66.2	
				65.7	
C-1′, 6′, 6 (or 6″ or 6‴)	63.6	63.6	63.6	63.7	63.5
	62.9	61.9	62.9	62.1	62.5
	61.9	61.8	61.3	62.0	61.2

a) Chemical shifts are expressed in parts per million (ppm) downfield from the ^{13}C NMR resonance of tetramethylsilane.

case of the anomeric carbon atoms of the terminal D-galactopyranosyl groups in raffinose and isoraffinose undecaacetates (Table 1).

The presence of **7** as an impurity in stachyose tetradecaacetate was demonstrated by measuring its ^{13}C NMR spectrum and determining the optical rotation. The crude product of **5** showed a trace of the signal at 101.0 ppm and a slightly lower value of specific rotation as compared with that of an authentic sample.

Experimental

General Methods. Stachyose tetrahydrate (Aldrich Chemical Company, Inc., U. S. A.), mp 100—104 °C, $[\alpha]_D^{24}$ +133 °C (*c* 4, water), was used as an authentic sample. Melting points were determined in capillary tubes and are corrected. Solutions were concentrated under reduced pressure below 40 °C. Optical rotations were measured with a Japan Spectroscopic DIS-SL polarimeter. ^{1}H NMR spectra were recorded with a Varian XL-100 spectrometer at 100 MHz, peak positions being given in δ values. ^{13}C NMR spectra were determined with a Varian CFT-20 spectrometer at 20 MHz, the resonance signals being expressed in ppm downfield from the signal of tetramethylsilane. In both spectra, deuteriochloroform was used as a solvent and tetramethylsilane as an internal standard. IR spectra were recorded with a Hitachi 225 spectrophotometer in KBr disks. TLC was performed on Wakogel B-10 (Wako Pure Chemical Co. Ltd.) plates, silica gel (Wakogel C-300) being used for column chromatography. Acetylation was carried out with acetic anhydride in pyridine as usual.

1′,2,2″,3,3′,3″,4,4′,4″,6′-Deca-O-acetylraffinose (1). The compound was prepared by the method of Suami *et al.*[6) The product used in the present synthesis contained a trace amount of 1′,2,2″,3,3′,3″,4,4′,6′,6″-deca-*O*-acetylraffinose[6) which was detectable on TLC.

O-(2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-α-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-α-D-glucopyranosyl) 1,3,4,6-tetra-O-acetyl-β-D-fructofuranoside (3). (a) Tetraethylammonium chloride (4.04 g), *N,N*-diisopropylethylamine (3.25 g) and molecular sieves 3A (5.2 g) were added to a solution of **1** (1.43 g) and 2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl chloride[7) (**2**, 12.1 g) in dichloromethane (40 ml). After 21 days at ambient temperature, the mixture was filtered and the filtrate was washed with cold water. The aqueous layer was extracted with chloroform, the chloroform layer being combined with the dichloromethane solution. After being dried over Na_2SO_4, the combined solution was concentrated. The residue was acetylated with acetic anhydride and pyridine in order to make the subsequent chromatographic separation easy. The acetylation product was purified on a silica gel column using 1:5 (v/v) 2-butanone–toluene. Fractions homogeneous on TLC (R_f 0.30) in the same solvent were combined and concentrated. The residue was recrystallized from 2-propanol to give 711 mg (32%) of **3**, mp 70—72 °C, $[\alpha]_D^{20}$ +91.9 ° (*c* 0.86, chloroform).

Found: C, 59.95; H, 5.98%. Calcd for $C_{72}H_{86}O_{31}$: C, 59.74; H, 5.99%.

Fractions homogeneous on TLC (R_f 0.17) in the same solvent were combined and concentrated. The residue was recrystallized from 3:2:5 (v/v) ethanol–methanol–water to give 349 mg (23%) of raffinose undecaacetate, mp 97—100 °C (Lit,[13) mp 99—101 °C).

(b) Mercury(II) cyanide (1.5 g) and drierite (3.0 g) were added to a solution of **1** (1.60 g) and **2** (3.51 g) in dry benzene (30 ml). The mixture was heated under reflux for 20 h with

mechanical agitation. The mixture was filtered and the filtrate was washed with sodium hydrogencarbonate solution and cold water. After being dried over Na_2SO_4, the solution was concentrated. The residue was purified on a silica gel column analogously as described in (a). Fractions practically homogeneous on TLC (R_f 0.30) in the same solvent as in (a) were combined and concentrated to dryness.
The residue was recrystallized from 2-propanol to give 1.12 g (45%) of crude **3**, mp 66—68 °C, $[\alpha]^{23}_{\mathrm{D}}$ +88.2 ° (*c* 1.02, chloroform).

Found: C, 59.99; H, 5.87%. Calcd for $C_{72}H_{86}O_{31}$: C, 59.74; H, 5.99%.

Fractions homogeneous on TLC (R_f 0.54) in 20:1 (v/v) chloroform-ethanol were combined and concentrated to give 205 mg (13%) of 1′,2,2″,3,3′,3″,4,4′,6′,6″-deca-*O*-acetylraffinose.[14]

Besides the products described above, 650 mg of a mixture of **3** and an isomer (isostachyose derivative)[22]: *O*-(2,3,4,6-tetra-*O*-benzyl-*β*-D-galactopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-α-D-galactopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl) 1,3,4,6-tetra-*O*-acetyl-*β*-D-fructofuranoside, was recovered from the column. The mixture showed two spots on TLC (R_f 0.30 and 0.29) in the same solvent system as described in (a) in an approximate ratio of 1:1. An attempt to separate each component by repeated column chromatography was unsuccessful.

2‴,3‴,4‴,6‴-Tetra-O-benzylstachyose (4). (a) Compound **3** (669 mg) was deacetylated in 0.1 M methanolic sodium methoxide (10 ml) at ambient temperature overnight. After being neutralized with Amberlite IRA-120(H+) resin, the solution was concentrated. The residue was purified on a silica gel column using 6:18:6:1 (v/v) acetone–hloroc-form–methanol–water. Fractions homogeneous on TLC (R_f 0.58) in 4:9:4:1 (v/v) acetone–chloroform–methanol–water were combined and concentrated to give 471 mg (100%) of **4** as a glass, $[\alpha]^{20}_{\mathrm{D}}$+81.7° (c 0.6, chloroform).

(b) Crude **3** (896 mg) was deaectylated analogously as described above to giv 596 mg (94%) of crude **4**, $[\alpha]^{20}_{\mathrm{D}}$+96.2° (*c*, 1.04, chloroform).

1′,2,2″,2‴,3,3′,3″,3‴,4,4′,4″,4‴,6′,6‴-Tetradeca-O-acetylstahyose (**5**). (a) Compound **4** (423 mg) was hydrogenated in 90% aqueous methanol (15 ml) in the presence of freshly prepared palladium black in hydrogen atmosphere (343 kPa) at 35 °C for a week in a Parr apparatus. During the course of hydrogenation, the pH of the solution was adjusted to 5 by adding a few drops of glacial acetic acid. The catalyst was filtered off and the filtrate was concentrated to dryness. The residue was acetylated, the product being purified on a silica-gel column using 1:7 (v/v) acetone–benzene. Fractions homogeneous on TLC (R_f 0.62) in 1:1 (v/v) 2-butanone–toluene were combined and concentrated. The residue was recrystallized from 2-propanol to give 248 mg (48%) of **5**, mp 93—95 °C, $[\alpha]^{21}_{\mathrm{D}}$+123.0° (*c* 0.88, ethanol). The compound is identical with an authentic sample in ^{1}H NMR and IR spectra, showing no depression in mixed melting point determination with an authentic sample. Lit,[3] mp 95—96 °C, $[\alpha]^{22.5}_{\mathrm{D}}$+120.2° (*c* 1.02, ethanol). ^{1}H NMR: δ 1.95 (s, 3, OAc), 2.02 (s, 3, OAc), 2.07 (s, 12, 4×OAc), 2.11 (s, 18, 6×OAc), 2.15 (s, 3, OAc), 2.19 (s, 3, OAc), 4.84 (dd, 1, $J_{1,2}$=4.2 Hz, $J_{2,3}$=10.0 Hz, H-2), 5.65 (d,1, H-1).

Found: C, 49.72; H, 5.62%. Calcd for $C_{52}H_{70}O_{35}$: C, 49.76; H, 5.62%.

(b) Crude **4** (600 mg) was worked up analogously as described above to give 236 mg (32%) of crude **5**, mp 93—95 °C, $[\alpha]^{22}_{\mathrm{D}}$+117.9 ° (*c* 3.30, ethanol).

Found: C, 49.53; H, 5.47%. Calcd for $C_{52}H_{70}O_{35}$: C, 49.76; H, 5.62%.

Stachyose (6). Compound **5** (56 mg) was deacetylated in 0.1 M methanolic sodium methoxide (2 ml) at ambient temperature overnight. The solution was diluted with cold water and neutralized with Amberlite IR-120(H+) resin. The solution was concentrated to dryness, and the residue was recrystallized from aqueous ethanol to give 14.2 mg (48%) of **6**. After 24 h in a vacuum desiccator with calcium chloride, the product melted at 122—123 °C, $[\alpha]^{20}_{\mathrm{D}}$+140.2° (*c* 0.84, water). After being dried over phosphorus pentaoxide *in vacuo* at 45 °C for 72 h, the product gave correct analysis as a monohydrate, $[\alpha]^{20}_{\mathrm{D}}$+147.3° (*c* 0.8, wster), its IR spectrum being superimposable on that of an authentic sample. Lit, $[\alpha]_{\mathrm{D}}$+148.1° (*c* 9, water) (anhyd ous),[1] $[\alpha]^{20}_{\mathrm{D}}$ +146° (*c* 2.05, water) (anhydrous),[3] $[\alpha]^{23}_{\mathrm{D}}$+133° (*c* 4.5, water) (tetrahydrate).[21]

Found: C, 41.99; H, 6.70%. Calcd for $C_{24}H_{42}O_{21}$. H_2O: C, 42.11; H, 6.48%.

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-α-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-α-D-glucoyranosyl) 1,3,4,6-tetra-O-acetyl-β-D-fructofuranoside (7). Mercury(II) cyanide (2.0 g) and drierite (2.0 g) were added to a solution of **1** (0.81 g) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide[15] (1.03 g) in dry nitromethane (20 ml). After being left to stand for 4.5 h in the dark, the mixturr was filtered and the filtrate was concentrated. The residue was purified on a silica gel column using 2:5 (v/v) 2-butanone–toluene. Fractions homogeneous on TLC (R_f 0.37) in 3:5 (v/v) 2-butanone–toluene were combined and coccentrated. The residue was recrystallized from 2-propanol to give 588 mg (54%) of **7**, mp 89—91 °C, $[\alpha]^{21}_{\mathrm{D}}$+69.4° (*c* 1.34, chloroform). ^{1}H NMR: δ 1.95 (s, 3, OAC), 1.97 (s, 3, OAc), 2.03 (s, 3, (OAc, 2.05 (s, 3, OAc), 2.06 (s, 3, OAc), 2.10 (s, 9, 3× OAc), 2.11 (s, 3, OAc), 2.12 (s, 3, OAc), 2.13 (s, 3, OAc), 2.15 (s, 3, OAc), 2.17 (s, 3, OAc), 2.20 (s, 3, OAc), 4.49 (d, 1, $J_{1''',2'''}$= 8.0 Hz, H-1‴), 4.85 (dd, 1, $J_{1,2}$=4.0 Hz, $J_{2,3}$=10.5 Hz, H-2), 5.73 (d, 1, H-1).

Found: C, 49.60; H. 5.58%. Calcd for $C_{52}H_{70}O_{35}$: C, 49.76; H, 5.62%.

We wish to express our thanks to Mr. Toshihide Ikeda for his technical assistance and to Mr. Saburo Nakada for the elemental analyses.

References

1) A. von Planta and E. Schulze, *Ber.*, **23**, 1692 (1890).

2) D. French, *Advances Carbohyd. Chem.*, **9**, 149 (1954).

3) M. Onuki, *J. Agr. Chem. Soc. Jpn.*, **8**, 445 (1932).

4) R. A. Laidlaw and C. B. Wylam, *J. Chem. Soc.*, **1953**, 567.

5) H. Hérissey, A. Wickstrom, and J. Courtois, *Bull· Soc. Chim. Biol.*, **33**, 642 (1951).

6) T. Suami, T. Otake, T. Ikeda, and R. Adachi, *Bull. Chem. Soc. Jpn.*, **50**, 1612 (1977).

7) P. W. Austin, F. E. Hardy, J. G. Buchanan, and J. Baddily, *J. Chem. Soc.*, **1964**, 2128; P. W. Austin, F. E. Hardy, J. G. Buchanan, and J. Baddily, *J. Chem. Soc.*, **1965**, 1419.

8) R. U. Lemieux, K. B. Hendriks, R. V. Stick, and K. James, *J. Am. Chem. Soc.*, **97**, 4056 (1975).

9) R. U. Lemieux and H. Driguez, *J. Am. Chem. Soc.*, **97**, 4063 (1975).

10) R. U. Lemieux and H. Driguez, *J. Am. Chem. Soc.*, **97**, 4069 (1975).

11) R. U. Lemieux, D. R. Bundle, and D. A. Barker, *J. Am.*

Chem. Soc., **97**, 4076 (1975).

12) D. D. Cox, E. K. Metzner, and E. J. Reist, Abstract of the 172nd ACS National Meeting San Fraccisco, Calif., U. S. A., Aug. 30, 1976.

13) C. Scheiber and H. Mittelmeier, *Ber.*, **23**, 1438 (1980); C. Tanret, *Bull. Soc. Chim. Fr.*, **13**, 261 (1895); R. L. Whistler and J. N. BeMiller, *Methods Carbohyd. Chem.*, **1**, 366 (1962).

14) T. Suami, T. Otake, T. Nishimura, and T. Ikeda, *Carbohydr. Res.*, **26**, 234 (1973); An isomeric raffinose where the terminal D-galactopyranosyl group attaches to the 6-*O* of sucrose in β anomeric linkage is tentatively referred to isoraffinose for the sake of convenience

15) H. Ohle, W. Marecek, and W. Bourjan, *Chem. Ber.*, **62**, 833 (1929).

16) C. S. Hudson and J. M. Johnson, *J. Am. Chem. Soc.*, **37**, 2748 (1915); T. Suami, T. Otake, S. Ogawa, T. Shoji, and N. Kato, *Bull. Chem. Soc. Jpn.*, **43**, 1219 (1970).

17) D. Doddrell and A. Allerhand, *J. Am. Chem. Soc.*, **93**, 2779 (1971).

18) R. Khan, *Adv. Carbohyd. Chem. Biochem.*, **33**, 278 (1976).

19) A. Allerhand and D. Doddrel, *J. Am. Chem. Soc.*, **93**, 2777 (1971).

20) D. E. Dorman and J. D. Roberts, *J. Am. Chem. Soc.*, **92**, 1355 (1970).

21) C. Tanret, *C. R. Acad. Sci.*, **134**, 1586 (1902); *Bull. Soc. Chim. Fr.*, **27**, 947 (1902); *Bull. Soc. Chim. Fr.*, **29**, 888 (1903).

22) An isomeric stachyose in which the terminal D-galactopyranosyl group attaches to the 6″-*O* of raffinose in β anomeric linkage is tentatively referred to isostachyose.