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Note

Concerning 2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-diamino-6,6′-dideoxy-α,α-trehalose

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We sought improved methods for preparing 2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-diamino-6,6′-dideoxy-α,α-trehalose as an intermediate for the synthesis of certain "pseudo cord factors". We describe facile syntheses of the corresponding 6,6′-dichloro and -diazido hexaacetate. However catalytic reduction of the hexa-*O*-acetyl diazide does not lead to the diamine hexa-acetate as earlier described by others. Instead 6,6′-diacetamido-2,3,2′,3′-tetra-*O*-acetyl-6,6′-dideoxy-α,α-trehalose is obtained, presumably *via* O→N acetyl migration. This product has the physical characteristics earlier described for the diamine. Small amounts of presumed amine recovered from reduction of the diazide in acid are rapidly and spontaneously converted into ninhydrin-unreactive material showing amide absorption in the i.r. spectrum. It seems likely, therefore, that the original (diamine) structural assignment was incorrect.

In a recent report from this laboratory, the synthesis of "pseudo cord factors" from (α-D-glucopyranosyluronic acid)(α-D-glucopyranosiduronic acid) was described[1]. The "pseudo cord factors" were synthesized by attaching lipid substituents to the carboxyl groups of the disaccharide either by ester or amide linkages. An alternative class of diamide pseudo cord factors might also be prepared from lipid carboxylic acids and a suitably protected 6,6′-diamino derivative of trehalose (as originally suggested to us by Dr. B. C. Das). In the present study, we sought to prepare and to employ for this purpose 2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-diamino-6,6′-dideoxy-α,α-trehalose, a presumed known substance whose preparation from the corresponding 6,6′-ditosyl derivative was earlier described by Ježo[2] who employed azide displacement followed by catalytic hydrogenation. The ditosyl hexaacetate may be obtained by a rather tedious four-step synthesis from trehalose[3]. Alternatively, the ditosyl trehalose may be prepared from the disaccharide by selective tosylation[4], but the yield is poor (39% of a crude product).

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In an effort to facilitate access to the desired hexa-*O*-acetyl diamine as an intermediate for pseudo cord factor synthesis, we have successfully achieved a more-direct synthesis of 2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-dichloro-6,6′-dideoxy trehalose (**1**) from the carbohydrate. This product is readily converted into the diazide. However, in our hands, reduction of the diazide did not lead to the free diamine—as interpreted by Ježo—but instead, by O→N acetyl migration, afforded 6,6′-diacetamido-2,3,2′,3′-tetra-*O*-acetyl-6,6′-dideoxy-α,α-trehalose.

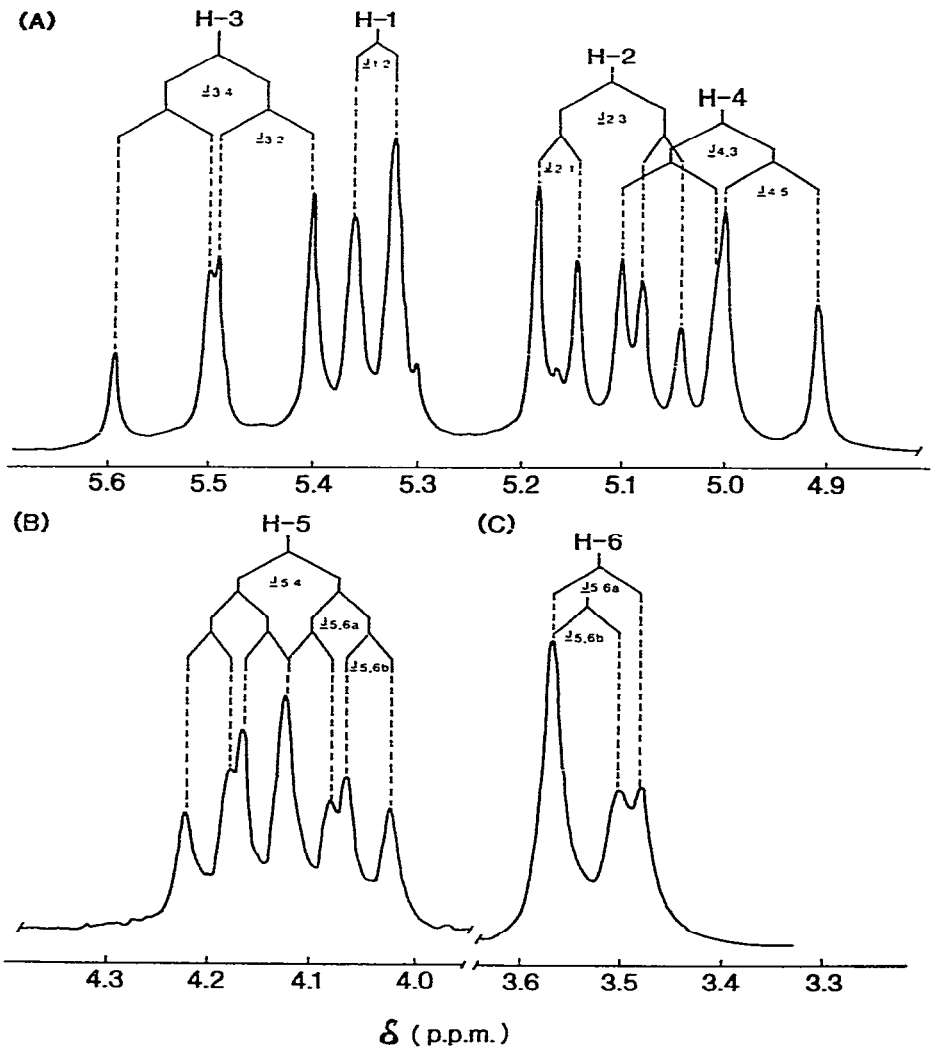
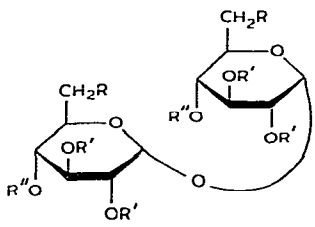


Fig. 1. Partial 100-MHz ^{1}H-n.m.r. spectrum of 2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-dichloro-6,6′-dideoxy-α,α-trehalose (**1**).

DISCUSSION

Treatment of α,α-trehalose with methanesulfonyl chloride in *N,N*-dimethylformamide as described for the synthesis of 6-chloro-6-deoxy-D-glucose[5], followed by acetylation with acetic anhydride and pyridine, afforded the crystalline 6,6′-dichloro hexaacetate **1** in very good yield (88%). Synthesis of **1** from α,α-trehalose has been reported[6]; however, the present method is more convenient and affords a higher yield. [We were unaware of the prior report of synthesis[6] (1973) until it was kindly called to our attention by one of the Referees. This compound has not, to our knowledge, been indexed in *Chemical Abstracts*]. The 100-MHz n.m.r. spectrum of compound **1** (not heretofore reported) was well resolved (Fig. 1), and the data obtained were in agreement with the assigned structure. Deacetylation of **1** with ammonium hydroxide in methanol gave 6,6′-dichloro-6,6′-dideoxytrehalose (**2**). Treatment of compounds **1** and **2** with sodium azide in *N,N*-dimethylformamide gave the known[4] 6,6′-diazido derivatives **3** and **4**, respectively. With the acetylated chloride **1**, the azide displacement was accompanied by partial deacetylation, so that it was necessary to re-acetylate the product. Compound **4** was also prepared from **3** by acetylation. It is worth mentioning that synthesis of a 6-azido sugar from the corresponding chloride has been described[7].



α,α-trehalose	R	=	OH, R′	= H, R″ = H
1	R	=	Cl, R′	= Ac, R″ = Ac
2	R	=	Cl, R′	= H, R″ = H
3	R	=	N_3, R′	= H, R″ = H
4	R	=	N_3, R′	= Ac, R″ = Ac
5	R	=	NH_2, R′	= Ac, R″ = Ac
6	R	=	NHAc, R′	= Ac, R″ = H
7	R	=	NHAc, R′	= Ac, R″ = Ac
8	R	=	NHAc, R′	= H, R″ = H

Catalytic hydrogenation of **4** gave a crystalline product having the same physical properties as those reported[2] for 2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-diamino-6,6′-dideoxy-α,α-trehalose (**5**), but the product was instead shown to be 6,6′-diacetamido-2,3,2′,3′-tetra-*O*-acetyl-6,6′-dideoxy-α,α-trehalose (**6**). The identity of **6** became clear only after numerous, unsuccessful attempts at acylation with lipid carboxylic acids, when the presumed "diamine" was always recovered unchanged. Characterization of this "known" compound by i.r. spectrometry, and its failure to react with ninhydrin, provided the first necessary data to support our interpretation (confirmed by the ^{1}H-n.m.r. spectrum). We have presumably been successful in achieving the desired reduction of the diazide to amine, but *to only a limited extent*, by conducting the hydrogenation in acidic medium. However, even under these conditions, the product is principally the diacetamide, as is immediately obvious from the i.r. spectrum. Ninhydrin-reactive amine that is at first present in the product mixture is rapidly converted into ninhydrin-unreactive material. Acetylation of **6**

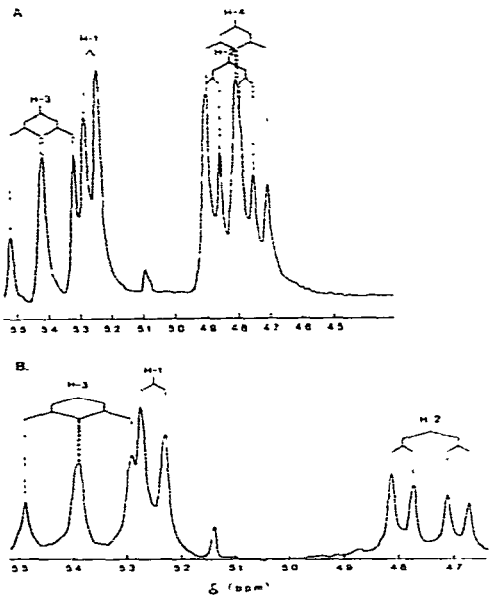


Fig. 2. Partial ^{1}H-n.m.r. spectrum of 6,6′-diacetamido-2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-dideoxy-α,α-trehalose (A) and of 6,6′-diacetamido-2,3,2′,3′-tetra-*O*-acetyl-6,6′-dideoxy-α,α-trehalose (B). In the latter, the H-4 signal does not appear (compare A and Fig. 1). No longer de-shielded by an *O*-acetyl group, it is shifted to higher field.

gave the crystalline peracetate (**7**). *O*-Deacetylation of **6** afforded 6,6′-diacetamido-6,6′-dideoxy-α,α-trehalose (**8**), identical with the diacetamido derivative obtained in the same way from **7**, indicating that, during the hydrogenation of the diazide **4**, an O→N acetyl migration had taken place. The structure of **6** was further confirmed by ^{1}H-n.m.r. spectroscopy. Comparison of the n.m.r. spectrum of **6** (Fig. 2), with that of the peracetate **7** (and also with spectra of other hexa-*O*-acetyl derivatives of trehalose[4-8]) shows that, in the case of the tetra-acetate compound **6**, the H-4 signal is shifted to higher field, probably owing to the fact that H-4 is no longer de-shielded by an adjacent *O*-acetyl group.

The hydrogenation of **4** was studied under various conditions in an attempt to obtain the desired hexa-*O*-acetyl-6,6′-diamino-6,6′-dideoxytrehalose. In all of these experiments, only **6** was obtained as a recoverable product, supporting the interpretation that the acetyl migration occurs very readily. It seems likely, therefore, that the product of Ježo was probably identical with ours, and that the hexa-*O*-acetyl diamine (**5**) is not a known compound.

We have developed alternative routes to the synthesis of suitably protected 6,6′-diamino derivatives of trehalose, which are described in the accompanying paper[9].

EXPERIMENTAL

Melting points were measured in capillary tubes in a modified Thiele–Hershberg

apparatus and are not corrected. Optical rotations were determined with a Jasco DIP-4 polarimeter, for whose use we thank Dr. John Stewart. N.m.r. spectra were recorded with a Jeol FX 100 spectrometer with tetramethylsilane as internal standard and $CDCl_3$ as solvent. Thin-layer chromatograms were run on Eastman Kodak silica gel plates. Chromatography columns were packed with silical gel (E. Merck, No. 7734). Microanalyses were performed by Galbraith Laboratories, Knoxville, Tennessee.

2,3,4,2',3',4'-Hexa-O-acetyl-6,6'-dichloro-6,6'-dideoxy-α,α-trehalose (**1**). — α,α-Trehalose dihydrate (1.0 g) was (evidently adequately) dehydrated by dissolving it in *N,N*-dimethylformamide (25 mL) and concentrating the resulting solution to ~15 mL. The concentrated solution was cooled to room temperature and methanesulfonyl chloride (2 mL) was added. The mixture was stirred for 21 h at 75°. 1-Propanol (2 mL) was added and the mixture was stirred at the same temperature for an additional 3 h. Water was added and the mixture was evaporated to give a colored liquid that was treated directly with acetic anhydride (5 mL) and pyridine (20 mL). The mixture was kept at room temperature overnight. Ice–water was added and the crystalline material was filtered off and washed several times with water. It was dried and recrystallized from acetone–hexane to give 1.47 g (88%), of **1**, m.p. 158–161°. $[\alpha]_D^{23}$ +140° (*c* 0.82, chloroform) [lit.[6] m.p. 165–166°; α_D +134°]. N.m.r. (see Fig. 1): δ 5.50 (dd, 2 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9 Hz, H-3 and H-3'), 5.34 (d, 2 H, $J_{1,2}$ 4 Hz, H-1 and H-1'), 5.11 (dd, 2 H, $J_{2,1}$ 4, $J_{2,3}$ 10 Hz, H-2 and H-2'), 5.0 (dd, 2 H, $J_{3,4}$ 9.0, $J_{4,5}$ 10.0 Hz, H-4 and H-4'), 4.12 (ddd, 2 H, $J_{4,5}$ 10.0, $J_{5,6a}$ 5.7 $J_{5,6b}$ 4.3 Hz, H-5 and H-5'), 3.50 (two superposed doublets, 4 H, $J_{5,6b}$ 5.7, $J_{5,6a}$ 4.3 Hz, 6-CH_2 and 6'-CH_2), and 2.11, 2.07 and 2.03 (18 H, 3s 6 OAc).

Anal. Calc. for $C_{24}H_{32}Cl_2O_{15}$: C, 45.64; H, 5.07; Cl, 11.25. Found: C, 45.53; H, 5.19; Cl, 11.39.

6,6'-Dichloro-6,6'-dideoxy-α,α-trehalose (**2**). — The dichloro-hexaacetate **1** (165 mg) was suspended in methanol (5 mL) and ammonium hydroxide solution (0.5 mL) was added. The mixture was stirred for 4 h at room temperature and evaporated. The residue was dissolved in methanol and applied to an ion-exchange column [AG50 WX-8 (H^+), 1.2 × 15 cm]. Elution with methanol followed by evaporation of the solvent gave an amorphous residue (85 mg, 85%) which was homogeneous in t.l.c. Crystallization from ethyl alcohol gave an analytical sample: m.p. 178–180° (dec.), $[\alpha]_D^{24}$ +167° (*c* 0.67, methanol).

Anal. Calc. for $C_{12}H_{20}Cl_2O_9$: C, 37.99; H, 5.27; Cl, 18.73. Found: C, 37.74; H, 5.41, Cl, 18.49.

6,6'-Diazido-6,6'-dideoxy-α,α-trehalose (**3**). — A mixture of the dichloro derivative **2** (67 mg) and sodium azide (69 mg) in *N,N*-dimethylformamide (3 mL) was heated for 18 h at 130–135°. The solid material was filtered off and washed with *N,N*-dimethylformamide, and the filtrate was evaporated to give a solid residue. This residue was de-ionized by dissolving it in methanol and passing the solution through an ion-exchange column (AG50 WX-8 (H^+), 1.2 × 15 cm). Evaporation of the effluent yielded an amorphous residue (60 mg, 87%) found homogeneous in t.l.c. A crystalline sample could be obtained by crystallization from ethyl alcohol–ether;

m.p. 208–211°, $[\alpha]_D^{23}$ +162° (*c* 0.64, 3:1 methanol–water) (lit.[4] m.p. 209–211°, $[\alpha]_D$ +158°).

2,3,4,2',3',4'-Hexa-O-acetyl-6,6'-diazido-6,6'-dideoxy-α,α-trehalose (**4**). — (*A*) From compound **1**. A mixture of the dichloro hexaacetate **1** (510 mg) and sodium azide (600 mg) in *N,N*-dimethylformamide (10 mL) was heated for 19 h at 135°. The insoluble material was filtered off and washed with *N,N*-dimethylformamide and the filtrate was evaporated. The product obtained was reacetylated conventionally with acetic anhydride and pyridine. The mixture was kept overnight at room temperature and ice–water was then added. The crystalline product was filtered off and washed with water. It was recrystallized from methanol to give fine needles; yield 376 mg (72%), m.p. 121–125°, $[\alpha]_D^{24}$ +138° (*c* 0.94, chloroform) (lit.[2] m.p. 119–119.5°, $[\alpha]_D$ +137°).

(*B*) From compound **3**. The 6,6'-diazido derivative **3** (20 mg) was treated with acetic anhydride and pyridine overnight. Ice–water was added and the crystalline material was filtered off and washed with water. It was recrystallized from methanol to give a pure sample (26 mg, 79%), having the same physical properties as those of compound **4** obtained by route *A*.

6,6'-Diacetamido-2,3,2',3'-tetra-O-acetyl-6,6'-dideoxy-α,α-trehalose (**6**). — The diazido derivative **4** (50 mg) was suspended in ethyl alcohol (40 mL) and hydrogenated in the presence of palladium-on-charcoal catalyst (10%, 50 mg) at 34 lb.in.$^{-2}$ for 2 h. The catalyst was filtered off and washed with ethyl alcohol, and the filtrate was evaporated. The product, which was practically homogeneous in t.l.c. was crystallized from ethyl alcohol–ether; yield 30 mg (65%), m.p. 197–200°, $[\alpha]_D^{24}$ +130° (*c* 0.84, 3:1 methanol–water) (lit.[2], reported for 2,3,4,2',3',4'-hexa-*O*-acetyl-6,6'-diamino-6,6'-dideoxy-α,α-trehalose m.p. 187–190°, $[\alpha]_D$ +128.4°).

N.m.r. (see Fig. 2): δ 6.43 (m, 2 H, NH), 5.42 (dd, 2 H, $J_{2,3}$ and $J_{3,4}$ 10 Hz, H-3 and H-3'), 5.27 (d, 2 H, $J_{1,2}$ 4.4 Hz, H-1 and H-1'), 4.76 (dd, 2 H, $J_{1,2}$, 4.4, $J_{2,3}$ 10 Hz, H-2 and H-2'), 3.79–2.75 (complex m, 8 H, H-4, H-4', H-5, H-5', 6-CH_2 and 6'-CH_2), and 2.10, 2.06 and 1.93 (s, 18 H, 6 acetate groups).

Anal. Calc. for $C_{24}H_{36}N_2O_{15} \cdot H_2O$: C, 47.21; H, 6.23; N, 4.59. Found: C, 47.31; H, 6.49; N, 4.54.

6,6'-Diacetamido-2,3,4,2',3',4'-hexa-O-acetyl-6,6'-dideoxy-α,α-trehalose (**7**). — The 6,6'-diacetamido derivative (**6**; 75 mg) was acetylated conventionally with acetic anhydride and pyridine. Evaporation of the mixture gave a homogeneous product that crystallized from ethyl acetate–hexane; yield 70 mg (81%), m.p. 92–98°, $[\alpha]_D^{24}$ +155° (*c* 1.0, chloroform); n.m.r. (see Fig. 2): δ 5.75 (m, 2 H, NH), 5.49 (dd, 2 H, $J_{2,3}$ 10, $J_{3,4}$ 9.5 Hz, H-3 and H-3'), 5.33 (d, 2 H, $J_{1,2}$ 3.9 Hz, H-1 and H-1'), 4.90 (dd, 2 H, $J_{1,2}$ 3.9, $J_{2,3}$ 10 Hz, H-2 and H-2'), 4.88 (t, $J_{3,4}$ and $J_{4,5}$ 10 Hz, H-4 and H-4'), 3.91–3.10 (6 H, complex m, H-5 and H-5', 6-CH_2 and 6'-CH_2), and 2.07, 2.02 and 1.99 (24 H, s, 8 acetate groups).

Anal. Calc. for $C_{28}H_{40}N_2O_{17}$: C, 49.70; H, 5.91; N, 4.14. Found: C, 49.51; H, 6.02; N, 4.09.

6,6'-Diacetamido-6,6'-dideoxy-α,α-trehalose (**8**). — To a solution of the hexa-

acetate (7, 82 mg) in methanol (2 mL) was added M sodium methoxide (in methanol, a few drops), and the solution was kept for 3 h at room temperature. The mixture was then made neutral with acetic acid and applied to an ion-exchange column [AG50 WX-8 (H^+), 1.5 × 20 cm]. The product was eluted with 1 : 1 aqueous methanol (40 mL) and evaporation of the effluent gave an amorphous residue (55 mg, 94%) that was homogeneous in t.l.c. Trituration with acetone gave an analytically pure, solid sample, $[\alpha]_D^{24}$ +110° (*c* 0.74, methanol).

Anal. Calc. for $C_{16}H_{28}N_2O_{11}$: C, 45.28; H, 6.65; N, 6.60. Found: C, 45.27; H, 6.64; N, 6.48.

ACKNOWLEDGMENTS

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REFERENCES

1 M. B. Goren and K.-S. Jiang, *Chem. Phys. Lipids*, 25 (1979) 209–224.
2 I. Ježo, *Chem. Zvesti*, 25 (1971) 364–368.
3 H. Bredereck, *Ber.*, 63 (1930) 959–965.
4 G. Birch and A. C. Richardson, *Carbohydr. Res.*, 8 (1968) 411–415.
5 M. E. Evans, L. Long, Jr., and F. W. Parrish, *J. Org. Chem.*, 33 (1968) 1074–1076; M. E. Evans and F. W. Parrish, *Methods Carbohydr. Chem.*, 6 (1972) 193–196.
6 S. Hanessian and P. Lavallée, *Carbohydr. Res.*, 28 (1973) 303–311.
7 D. Horton, A. E. Luetzow, and J. C. Wease, *Carbohydr. Res.*, 8 (1968) 366–367.
8 A. Liav and M. B. Goren, *Carbohydr. Res.*, 84 (1980) 171–174.
9 A. Liav and M. B. Goren, *Carbohydr. Res.*, 87 (1980) 153–155.