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Total Synthesis of Trehalase Inhibitor, Trehazolin

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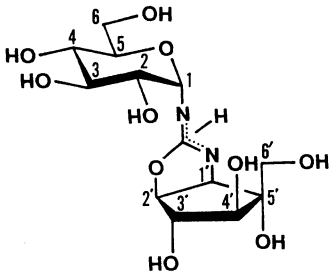
The total synthesis of trehalase inhibitor, trehazolin has been accomplished by coupling the optically active aminocyclopentanepentaol with α-D-glucopyranosylisothiocyanate derivative, followed by subsequent oxazoline-ring formation and removal of the protecting groups, thereby confirming its absolute configuration.

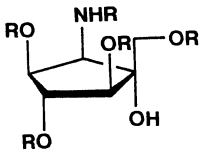
In 1991 trehazolin **1**, a potent inhibitor against trehalase, was isolated by Ando *et al.*[1] from the culture broth of *Micromonospora* strain SANK 62390, and it was shown most likely to be identical to trehalostatin previously isolated by Murao *et al.*[2-4] from *Amycolatopsis trehalostatica*. The two structures, being only epimeric at C-4', have been proposed by two groups, and the correct one **1** has finally been established by an unambiguous synthesis[5] of the branched aminocyclopentanepentaol moiety **3** as the penta-*N,O*-acetyl derivative **2** and comparison of its physical and spectroscopic data with those of the equivalent derivative obtained from **1**.

In this communication, we wish to report the first complete synthesis of **1** and its diastereoisomer **10** by coupling of DL-(1,3/2,4,5)-5-amino-1-*C*-(hydroxymethyl)cyclopentane-1,2,3,4-tetraol (**3**), obtained by treatment of **2**[5] with 2 M hydrochloric acid, and 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosylisothiocyanate (**4**),[6] followed by formation of the oxazoline ring with mercuric oxide and removal of the protecting groups.

Thus, reaction of the racemic amine **3** with the isothiocyanate **4** in a mixture of *N,N*-dimethylformamide (DMF) and methanol afforded a 93% yield of a diastereoisomeric mixture of the thiourea derivatives[7] **5a** and **5b**: IR (neat) 1540 cm^{-1} (NH). Without separation, the mixture was successively treated with mercuric oxide in diethyl ether, resulting in a simultaneous ring-closure through attack of the neighbouring *cis*-hydroxy group to give rise to an inseparable mixture (96%) of the α-glucosylamino-oxazolines[7] **6a** and **6b**: IR (neat) 1670 cm^{-1} (C=N), which was treated with acetic anhydride in pyridine to convert to the tetra-*N,O*-acetyl derivatives **7a,b**. Similar

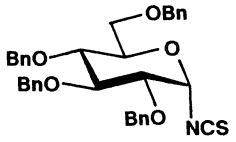


Trehazolin 1

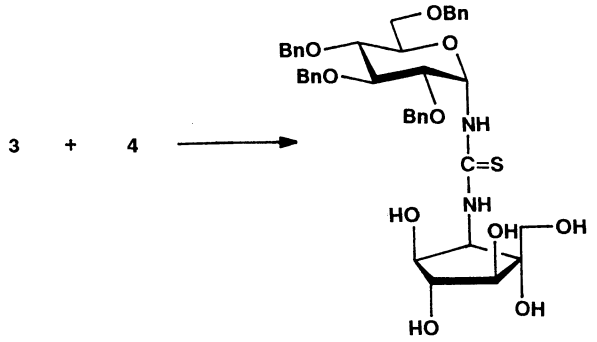


2: R = Ac

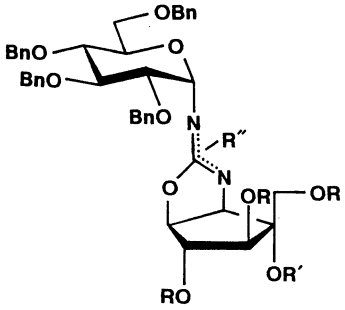
3: R = H



4



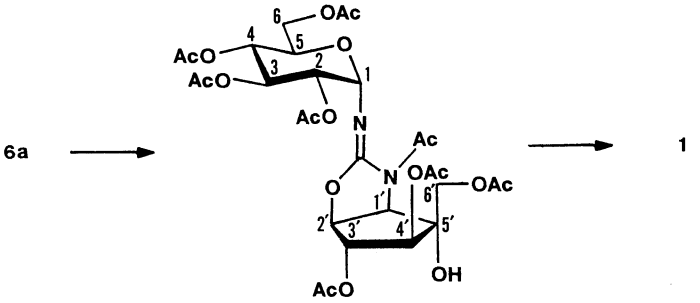
5a,b



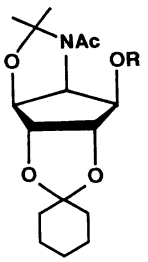
6a,b: R = R' = R" = H

7a,b: R = R" = Ac, R' = H

8a,b: R = R' = R" = Ac

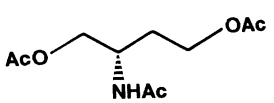


9a



11 : R = H

12 : R =



13

7b → 6b → 9b → 10

Trehazolin diastereoisomer

acetylation in the presence of 4-dimethylaminopyridine (DMAP) gave penta-*N*,*O*-acetyl derivatives **8a**,**b**. The diastereoisomeric mixture was readily separable by a silica gel chromatography to afford **7a** (47%), $[\alpha]_D^{24}$ +100° (c 2.5, $CHCl_3$), and **7b** (47%), $[\alpha]_D^{24}$ +29° (c 2.5, $CHCl_3$). Compounds **8a** and **8b** were also separated and obtained in 40 and 42% yields, respectively. Removal of the acyl groups of **7a** or **8a** was readily effected by treatment with methanolic sodium methoxide to give the tetraol **6a**[7] (100%).

O-Debenzylation of **6a** was then carried out in liquid ammonia with sodium at -78° to give the crude inhibitor **1** that was isolated as the octa-*N*,*O*-acetyl derivative **9a** (77%), $[\alpha]_D^{25}$ +104° (c 1.7, $CHCl_3$), the ^{1}H-NMR spectrum of which was identical with that reported for the equivalent derivative[8] derived[1] from natural **1**. *N*,*O*-Deacetylation of **9a** with methanolic sodium methoxide in methanol proceeded cleanly at room temperature to afford, after elution from a column of Dowex 50W-X2 (H^+) resin with aqueous 4% ammonia, the free base **1**, $[\alpha]_D^{23}$ +105° (c 0.36, H_2O), in 71% yield, the ^{1}H NMR spectrum of which was superimposable on that of an authentic sample,[1] $[\alpha]_D^{25}$ +99.5° (c 0.41, H_2O), of trehazolin. Likewise, the diastereoisomer **10**, $[\alpha]_D^{25}$ +63° (c 0.40, H_2O), of **1** was prepared from **6b** through the octa-*N*,*O*-acetyl derivative **9b**,[7] $[\alpha]_D^{25}$ +30° (c 1.6, $CHCl_3$), obtained from the tetraol **6b**.

Biological assay[9] of the synthetic **1** and **10** showed inhibitory activity IC_{50} 11.6 and 35.9 ng/ml, respectively, against porcine trehalase (*cf.* an authentic sample of **1**: IC_{50} 9.39 ng/ml). Very interestingly, the diastereoisomer **10** was shown to possess about 30% of activity.

Concerning the position of the C=N bond in **1**, there has so far been no firm spectroscopic evidence to differenciate between the two tautomers. In fact, the tautomers of **6a** or **6b** seem to be rapidly interchangeable at room temperature, considering from a pH-dependent property of its ^{1}H NMR spectrum. Therefore, it remains unknown which structure plays a role as the inhibitor.

Attempts to establish the absolute configuration of **1** have been carried out by optical resolution of the alcohol **11**[5] as the (*S*)-(+)-*O*-acetylmandelate **12** and conversion into (*S*)-2-acetamido-1,4-butanediol diacetate (**13**), $[\alpha]_D^{29}$ -42.7° (c 0.9, $CHCl_3$), by the sequence of reaction: deoxygenation via the methylthiothiocarbonate, periodate oxidation after de-*O*-ketallization followed by reduction with sodium borohydride and acetylation. This compound was shown to be identical to an authentic sample, $[\alpha]_D^{32}$ -42.3° (c 1, $CHCl_3$), derived by acetylation of the amino alcohol[10] obtained from L-aspartic acid by three-step reaction. Compound **1** could similarly be synthesized by use of the optically active **3** derived from **11**, establishing the absolute configuration as **1** depicted.

In summary, the present communication described the first total synthesis of trehazolin **1** and determination of its absolute configuration.

The authors express sincere thanks to Dr. Shuji Takahashi (Sankyo Co. Ltd., Tokyo) for comparison of the ^{1}H NMR spectra of **1** and **9a** with those of authentic samples[1] and for biological assay.

References

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7) All new compounds were characterized by 270 MHz ^{1}H NMR and IR, spectrometric and elemental analyses. Selected ^{1}H NMR (270 MHz) data for **5a,b** (in $CDCl_3$) δ 7.80-7.72 (2 H, 2 d, 2 NH), 6.74-6.65 (2 H, 2 br s, 2 NH). For **6a,b** ($CDCl_3$) δ 5.40 (1 H, d, $J_{1,2}$ 4 Hz, 1-H), 5.28 (1 H, d, $J_{1,2}$ 5.1 Hz, 1-H). For **10** (in D_2O) δ 5.25 (1 H, d, $J_{1,2}$ 5.9 Hz, 1-H), 5.22 (1 H, dd, $J_{1',2'}$ 8.4, $J_{2',3'}$ 1.1 Hz, 2'-H), 4.43 (1 H, d, 1'-H), 4.25 (1 H, dd, $J_{3',4'}$ 3.7 Hz, 3'-H), 3.90 (1 H, d, 4'-H), 3.72-3.48 (5 H, m, 2, 6, and 6'-H), 3.52 (1 H, dd, J 8.4 and 9.9 Hz, 3-H or 4-H), 3.42-3.36 (1 H, m, 5-H), 3.30 (1 H, dd, J 8.8 and 9.9 Hz, 4-H or 3-H).
8) Two isomeric octa-*N,O*-acetyl derivatives were initially prepared by Murao *et al.*[4] by treatment of trehalostatin with acetic anhydride in pyridine. On the other hand, one octa-*N,O*-acetyl derivative was reported to be obtained from trehazolin **1** by Ando *et al.*,[1] and it was identified with **9a** obtained here. Compound **9a** is most likely to be identical to one of the two described by Murao *et al.*, judging from the ^{1}H NMR spectroscopic data.[4]
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10) P. Karrer, P. Portmann, and M. Suter, *Helv. Chim. Acta.*, **31**, 1617 (1948).

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