SYNTHESIS OF TREHAZOLIN β-ANOMER

YOSHIYUKI KOBAYASHI and MASAO SHIOZAKI*

Exploratory Chemistry Research Laboratories, Sankyo Co., Ltd.,
1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140, Japan

(Received for publication October 12, 1993)

Synthesis of trehazolin β-anomer (**3**) from a D-glucose derived azido alcohol (**4**), was accomplished. 2-Chloro-1-methylpyridinium iodide was used in place of 2-chloro-3-ethylbenzoxazolium tetrafluoroborate as a means of preventing concomitant anomerization. Evaluation of compound (**3**) reveals that the stereochemistry of the anomeric position is significant for generation of inhibitory activities towards trehalases.

Trehazolin (**1**), reported by ANDO and co-workers in 1991, is a unique natural pseudodisaccharide isolated from culture broths of *Micromonospora* SANK 62390 and *Amicolatopsis* SANK 60791. It exhibits strong specific activities towards various trehalases[1]. The absolute configuration of trehazolin aminocyclitol moiety (**2**) is [1*R*-(1α, 2β, 3α, 4β, 5β)] as determined by previous synthetic studies of trehazolin[2~5].

Trehazolin β-anomer (**3**) was synthesized in order to investigate the structure-activity relationship of trehazolin derivatives (Fig. 1).

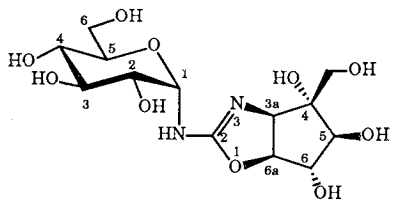
Herein, we report the synthesis of trehazolin β-anomer utilizing the azido alcohol intermediate (**4**)[2], and its activity towards trehalases obtained from two different natural sources.

Synthesis of Trehazolin β-Anomer

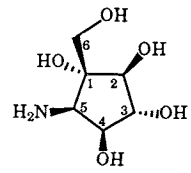
After azido reduction of compound **4**, treatment of corresponding aminotriol hydrochloride with 2,3,4,6-tetra-*O*-benzyl-1-deoxy-β-D-glucopyranosyl isothiocyanate[6] and triethylamine afforded the thiourea **5**.

Cyclization of compound **5** with 2-chloro-1-methylpyridinium iodide[7,8] and triethylamine yielded

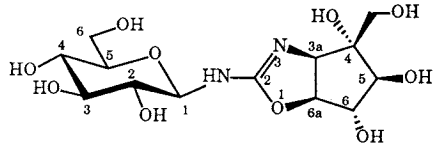
Fig. 1. The structures of trehazolin (**1**), trehazolin aminocyclitol moiety (**2**) and trehazolin β-anomer (**3**).



Trehazolin (**1**)

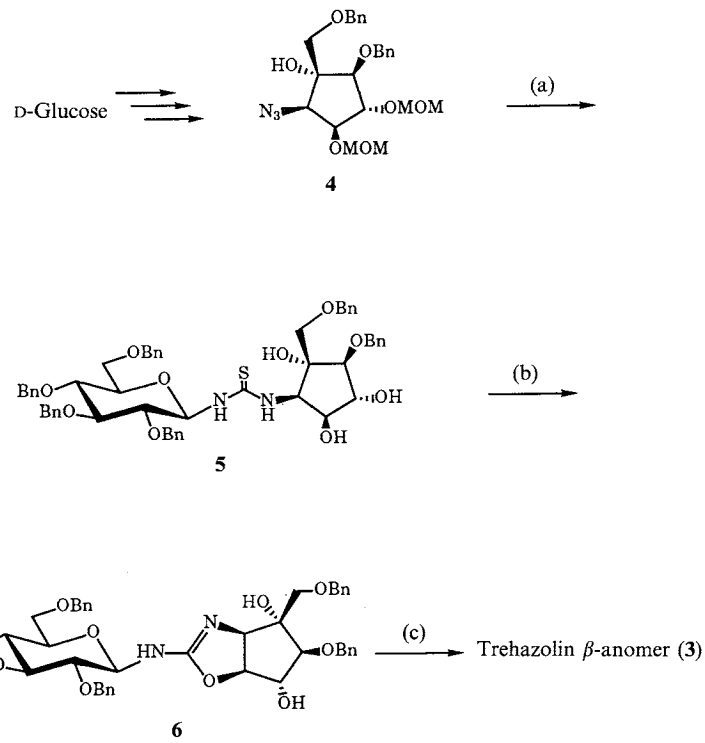


Trehazolin aminocyclitol moiety (**2**)



Trehazolin β-anomer (**3**)

Scheme 1.



(a) 4.0 equiv of $LiAlH_4$, Et_2O, 0°C, 4 hours, 5% HCl-MeOH, 60°C, 4 hours, and then 1.2 equiv of 2,3,4,6-tetra-*O*-benzyl-1-deoxy-β-D-glucopyranosyl isothiocyanate, 1.6 equiv of Et_3N, THF, room temperature, 13 hours, 79%. (b) 〈Method A〉 1.7 equiv of 2-chloro-3-ethylbenzoxazolium tetrafluoroborate, MeCN, 0°C, 1 hour, then quenched with 3.3 equiv of Et_3N, 0°C, 1 hour, 43% isolated. 〈Method B〉 2.0 equiv of 2-chloro-1-methylpyridinium iodide, MeCN, 0°C, 1 hour, then quenched with 4.0 equiv of Et_3N, 0°C, 1 hour, 70%. (c) H_2, $Pd(OH)_2$-C, MeOH, 60°C, 30 minutes, 51%.

compound **6** in 70%. Using 2-chloro-3-ethylbenzoxazolium tetrafluoroborate[9] in lieu of 2-chloro-1-methylpyridinium iodide, to promote cyclization caused anomerization and contaminated compound **6** with the α-anomer. This is presumably due to the tetrafluoroborate species acting as a Lewis acid. Cyclization conditions using 2-chloro-1-methylpyridinium iodide may be applicable for the synthesis of acid sensitive aminooxazolines.

Table 1. Inhibitory activities of trehazolin β-anomer (**3**) and trehazolin (**1**) towards silkworm and porcine trehalases (IC_{50}).

Enzyme	Trehazolin β-anomer (**3**)	Trehazolin (**1**)
Silkworm trehalase	0.19 μl/ml	0.011 μg/ml
Porcine trehalase	0.013 μg/ml	0.006 μg/ml

Finally, hydrogenolysis of compound **6** using palladium hydroxide on carbon as a catalyst to cleave six benzyl groups, afforded compound **3** (Scheme 1).

This compound was identical to an unknown product which was slowly generated during exposure of trehazolin (**1**) to aqueous NH_3.

Inhibition of Trehalases

Activities of trehazolin β-anomer (**3**) towards the two trehalases were found to be much weaker than that of trehazolin (**1**). (Table 1)

This reveals that the inhibitory activities are influenced by the stereochemistry of the anomeric position at D-glucose moiety of trehazolin.

Experimental

General

270 MHz ^{1}H NMR spectra were recorded on a JEOL JNM-EX-270 or JNM-GX-270 spectrometer using tetramethylsilane as an internal reference. Infrared spectra were recorded using a JASCO FT/IR-8900 or A-102 spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Low and high resolution mass spectra were obtained from a JEOL JMS-AX-505H spectrometer. FAB and FAB high resolution mass spectra were recorded from a JEOL HX-100 or SX-102A spectrometer. Analytical chromatography was performed on Merck Art 5715 silica gel 60-F_{254} plates. Flash chromatography was performed on Merck Art 9385 silica gel 60 (230~400 mesh). Tetrahydrofuran (THF) was distilled from $LiAlH_4$ and used immediately thereafter. Diethylether (Et_2O) was dried by passing through ICN Alumina *N*-Super I. Acetonitrile (MeCN) was dried by storing over 3A-molecular sieves. All other commercially obtained reagents were used directly as received.

N-(2,3,4,6-Tetra-*O*-benzyl-*β*-D-glucopyranosyl)-*N'*-[[1*R*-(1*α*,2*β*,3*α*,4*β*,5*β*)]-2-benzyloxy-1-benzyloxymethyl-1,3,4-cyclopentanetriol-5-yl] thiourea (**5**)

A solution of **4** (53 mg, 0.11 mmol) in Et_2O (3.0 ml) was added dropwise to a suspension of $LiAlH_4$ (19 mg, 0.44 mmol) in Et_2O (2.6 ml) with stirring at 0°C under nitrogen atmosphere. After completion of the reaction (4 hours), this reaction mixture was diluted with Et_2O, and saturated aqueous Na_2SO_4 was added to the mixture at 0°C. After being stirred at room temperature for 1 hour, the mixture was extracted twice with Et_2O. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a crude product, which was chromatographed on a silica gel column. Elution with CH_2Cl_2-MeOH (15:1) gave 46 mg (91%) of the corresponding amine as a colorless oil: Rf=0.36 (CH_2Cl_2-MeOH, 15:1). To a solution of this amine (46 mg) in MeOH (0.7 ml) was added 10% hydrogen chloride in methanol (0.7 ml) with stirring at 0°C, and after 5 minutes, this mixture was warmed to 60°C and stirred for 4 hours. After completion of the reaction, this reaction mixture was concentrated *in vacuo* to give a residue, which was dried under reduced pressure for 3 hours. To a solution of this residue in THF (2.3 ml) was added a solution of 2,3,4,6-tetra-*O*-benzyl-1-deoxy-*β*-D-glucopyranosyl isothiocyanate (72 mg, 0.12 mmol) in THF (2.3 ml) and Et_3N (0.0215 ml, 0.16 mmol) with stirring at 0°C. After being stirred for 5 minutes at 0°C, this mixture was warmed to room temperature and stirred for 13 hours. After completion of the reaction, this reaction mixture was diluted with EtOAc and washed with brine. The aqueous layer was extracted twice with EtOAc. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a crude product, which was chromatographed on a silica gel column. Elution with benzene-EtOAc (5:2) gave 77 mg (79% from **4**) of **5** as a colorless syrup: $[\alpha]_D^{24}$ +12.2° (*c* 0.83, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3650, 3400, 3300, 3000, 2930, 2850, 1600, 1530, 1495, 1450, 1400, 1355, 1140, 1065, 1020, 940, 910, 820, 690, 660, 590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.50~7.10 (30H, m), 6.17 (1H, br s), 5.32 (1H, br s), 5.00~4.32 (13H, m), 4.06 (1H, dd, J=8.0, 3.5 Hz), 4.00~3.45 (10H, m), 3.25 (1H, br s), 2.35 (2H, br s); FAB-MS, positive *m/z* 941.4 $(M+H)^+$; negative *m/z* 939.5 $(M-H)^-$; Rf=0.33 (benzene-EtOAc, 5:2); FAB HR-MS: Calcd. for $C_{55}H_{61}N_2O_{10}S$: 941.4047; Found *m/z* 941.4070 $(M+H)^+$.

1-[[3a*R*-(3a*α*,4*α*,5*β*,6*α*,6a*α*)]-5-Benzyloxy-4-benzyloxymethyl-3a,5,6,6a-tetrahydro-4*H*-cyclopentoxazole-4,6-diol-2-yl]amino-1-deoxy-2,3,4,6-tetra-*O*-benzyl-*β*-D-glucopyranoside (**6**)

〈Method A〉 To a solution of 2-chloro-3-ethylbenzoxazolium tetrafluoroborate (49 mg, 0.18 mmol) in MeCN (6.0 ml) was added a solution of **5** (105 mg, 0.11 mmol) in MeCN (3.0 ml) with stirring at 0°C under nitrogen. After being stirred for 1 hour, to this mixture was added Et_3N (0.05 ml, 0.36 mmol) maintaining 0°C. After completion of the reaction (1 hour), this reaction mixture was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$. The aqueous layer was extracted twice with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a crude product, which was chromatographed on a silica gel column. Elution with benzene-MeCN (2:1) gave 43 mg (43%) of **6** as a colorless foamy glass.

〈Method B〉 Using the above procedure but by addition of 2-chloro-1-methylpyridinium iodide in lieu of 2-chloro-3-ethylbenzoxazolium tetrafluoroborate, **5** was converted to **6** in 70% without anomerization: $[\alpha]_D^{24}$ +4.6° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3675, 3500, 3425, 3050, 3000, 2900, 2850, 1678, 1600, 1520, 1495, 1450, 1410, 1360, 1300, 1290, 1200, 1155, 1090, 1065, 1025, 910, 695, 660, 600, 450 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.45~7.05 (30H, m), 4.95 (1H, d, J=7.9 Hz), 4.88 (1H, d, J=10.3 Hz), 4.83 (1H, d, J=10.3 Hz), 4.80 (1H, d, J=11.5 Hz), 4.76 (1H, d, J=9.2 Hz), 4.71 (2H, s), 4.58 (1H, d, J=11.5 Hz), 4.57 (1H, d, J=11.5 Hz), 4.54 (2H, s), 4.53 (1H, d, J=7.9 Hz), 4.47 (1H, d, J=11.5 Hz), 4.45 (1H, d, J=11.5 Hz), 4.39 (1H, d, J=11.5 Hz), 4.23 (1H, s), 3.97 (1H, d, J=9.9 Hz), 3.80~3.57 (6H, m), 3.48 (1H, dd, J=8.4, 2.0 Hz), 3.31 (1H, t, J=8.4 Hz), 3.03 (3H, br s); FAB-MS, positive *m/z* 907 $(M+H)^+$, negative *m/z* 905.7 $(M-H)^-$; Rf=0.33 (benzene-MeCN, 2:1); FAB HR-MS: Calcd for $C_{55}H_{59}N_2O_{10}$: 907.4170; Found *m/z* 907.4190 $(M+H)^+$.

1-Deoxy-1-[[3a*R*-(3aα,4α,5β,6α,6aα)]-4-hydroxymethyl-3a,5,6,6a-tetrahydro-4*H*-cyclopentoxazole-4,5,6-triol-2-yl]amino-β-D-glucopyranoside (Trehazolin β-Anomer) (**3**)

To a solution of **6** (41 mg, 0.05 mmol) in MeOH (8.2 ml) was added $Pd(OH)_2$ on carbon (1.25 g) at room temperature and the mixture was hydrogenolyzed at 60°C for 30 minutes. After completion of the reaction, this reaction mixture was filtered, and concentrated *in vacuo* to give a crude product, which was chromatographed on Amberlite CG-50 (NH_4^+ type/H^+ type, 3:2, 5 ml). Elution with 0.5 M aqueous NH_3 gave 7.8 mg (51%) of **3** as a white powder: $[\alpha]_D^{25}$ +5.3° (*c* 0.78, H_2O); IR (KBr) ν_{max} 3360, 1667, 1065 cm^{-1}; ^1H NMR (D_2O/external TMS) δ 4.73 (1H, dd, J=8.8, 2.9 Hz), 4.15 (1H, d, J=8.8 Hz), 4.00 (1H, dd, J=5.0, 2.9 Hz), 3.75 (1H, d, J=5.9 Hz), 3.68 (1H, dd, J=12.7, 2.0 Hz), 3.62 (1H, d, J=12.2 Hz), 3.55 (1H, dd, J=12.7, 4.4 Hz), 3.51 (1H, d, J=12.2 Hz), 3.35 (1H, t, J=8.8 Hz), 3.31 (1H, ddd, J=8.8, 4.4 and 2.0 Hz), 3.24 (1H, t, J=8.8 Hz), 3.18 (1H, t, J=8.8 Hz); FAB-MS, positive *m/z* 367 $(M+H)^+$, negative *m/z* 365 $(M-H)^-$; Rf=0.38 (MeCN-H_2O-AcOH, 13:5:2); FAB HR-MS: Calcd for $C_{13}H_{23}N_2O_{10}$: 367.1353; Found *m/z* 367.1353 $(M+H)^+$.

Acknowledgments

We are indebted to Mr. O. ANDO of the Biomedical Research Laboratories at Sankyo Co., Ltd. for taking measurements of the inhibitory activities of trehazolin β-anomer.

References

1) ANDO, O.; H. SATAKE, K. ITOI, A. SATO, M. NAKAJIMA, S. TAKAHASHI, H. HARUYAMA, Y. OHKUMA, T. KINOSHITA & R. ENOKITA: Trehazolin, a new trehalase inhibitor. J. Antibiotics 44: 1165~1168, 1991
2) KOBAYASHI, Y.; H. MIYAZAKI & M. SHIOZAKI: Syntheses and absolute configurations of trehazolin and its aglycon. J. Am. Chem. Soc. 114: 10065~10066, 1992
3) KOBAYASHI, Y.; H. MIYAZAKI & M. SHIOZAKI: Synthesis and absolute configuration of trehazolin aminocyclitol moiety. Tetrahedron Lett. 34: 1505~1506, 1993
4) KOBAYASHI, Y.; H. MIYAZAKI & M. SHIOZAKI: Syntheses of trehazolin, trehalamine and its aminocyclitol moiety: Determination of absolute configuration of trehazolin. J. Org. Chem., in press
5) OGAWA, S. & C. UCHIDA: Synthesis of aminocyclitol moieties of trehalase inhibitors, trehalostatin and trehazolin. Confirmation of the correct structure of the inhibitor. J. Chem. Soc. Perkin Trans. I 1992: 1939, 1992
6) CAMARASA, M. J.; P. F-RESA, M. T. GARCIALOPEZ, F. G DE RAS HERAS, P. P. M-CASTRILLON & A. S. FELIX: A new procedure for the synthesis of glycosyl isothiocyanates. Synthesis 1984: 509~510, 1984
7) SHIBANUMA, T.; M. SHIONO & T. MUKAIYAMA: A convenient method for the preparation of carbodiimides using 2-chloropyridinium salt. Chem. Lett. 575~576, 1977
8) MUKAIYAMA, T.: New synthetic reactions based on the onium salts of aza arenes. Angew. Chem. Int. Ed. Engl. 18: 707~721, 1979
9) TAKEDA, T. & T. MUKAIYAMA: Asymmetric total synthesis of indolmycin Chem. Lett. 1980: 163~166, 1980