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Deoxysugar Synthesis. III.[1] Removal of Vicinal Mesyloxy Groups with Naphthalene-Sodium

Teruo Hayashi, Noriko Takeda, Hiromichi Saeki, and Eiji Ohki

Central Research Laboratories, Sankyo Co., Ltd.[2]

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Naphthalene–sodium reaction of 3,4-dimesylates of N-protected 2,6-diamino-2,6-dideoxy-α-D-glycosides (**1a**, **1b**) resulted in a good yield of 3-eno compounds (**2a**, **2b**). Application of this anion–radical reaction to butirosin 3′,4′-dimesylate (3) gave 3′,4′-dideoxy-3′-eno butirosin A (**4a**) which shows a broad inhibitory activity against bacteria which are both sensitive and resistant to butirosin. Kanamycin 3′,4′-dimesylate (**5a**) also analogously gave a 3′-eno derivative (**6**).

Keywords——deoxygenation; anion-radical; naphthalene-sodium; aminoglycoside antibiotics; kanamycin; butirosin

One of the important chemical modifications in the field of aminoglycoside antibiotics includes a removal of the 3′ and 4′[3] hydroxy groups of these antibiotics to convert them into the corresponding deoxy derivatives which successfully prevent certain types of enzymatic inactivations caused by resistant bacteria, as exemplified by 3′,4′-dideoxykanamycin B[4] 3′,4′-dideoxybutirosin A,[5] and others. The deoxygenation process heretofore in use involved treatment of 3′,4′-dimesyl derivatives with zinc and sodium iodide. This process was developed by Tipson and Cohen.[6] In 1972, Carnahan, *et al.*[7] demonstrated that treatment of dimesylates of vicinal diols with anthracene or naphthalene-sodium results in a high yield conversion into the corresponding alkenes. We have applied this deoxygenation procedure to 3,4-dimesylates of 2,6-diamino-2,6-dideoxy-D-glucose or to the corresponding moieties of some aminoglycoside antibiotics in place of the Tipson–Cohen reaction.

Following the procedure of Carnahan, *et al.*,[7] treatment of methyl 2,6-dideoxy-2,6-diethoxycarbonylamino-3,4-di-O-mesyl-α-D-glucopyranoside (**1a**) with naphthalene-sodium afforded a 3-eno compound (**2a**) in good yield. This anion-radical reaction was also carried out using naphthalene or anthracene-lithium or biphenyl-sodium; and almost the same results were obtained. In case of the benzyloxycarbonyl analog of **1a** (**1b**), the deoxygenation reaction also proceeded, but was accompanied by removal of the protecting groups. On successive treatment of the reaction product with benzyloxycarbonyl chloride, a 3-eno analog **2b** was obtained and characterized.

Naphthalene–sodium reaction of tetra-N-benzyloxycarbonyl-3′,4′-di-O-mesylbutirosin A[8] (3) was also carried out analogously but resulted in a lower yield of 3′,4′-dideoxy-3′-enobutirosin (**4a**) whose N-benzyloxycarbonyl derivative **4b** was identified with the sample obtained by the Tipson–Cohen reaction of **3**. The 3′-enobutirosin (**4a**) thus obtained has no 3′ and

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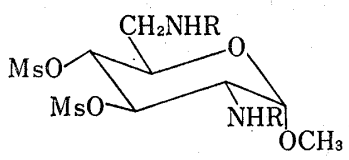
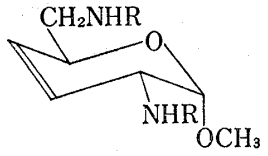
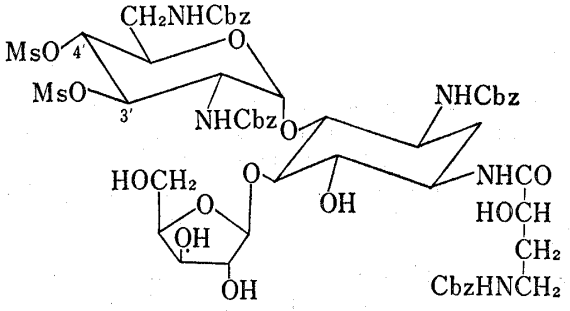


1a : R=Cbe
1b : R=Cbz



2a : R=Cbe
2b : R=Cbz



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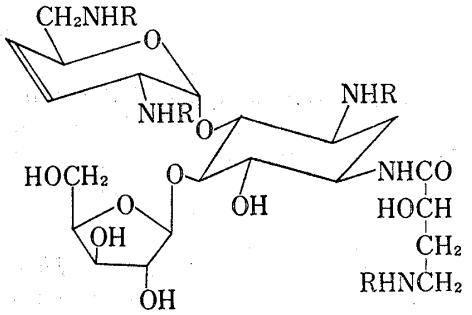
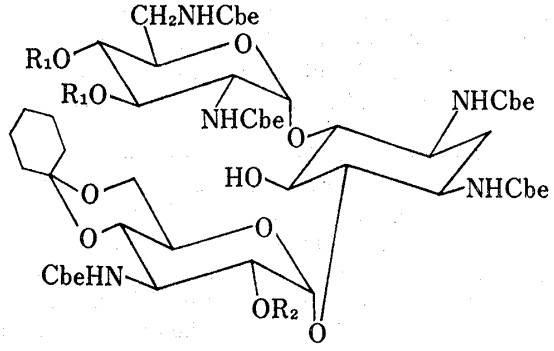
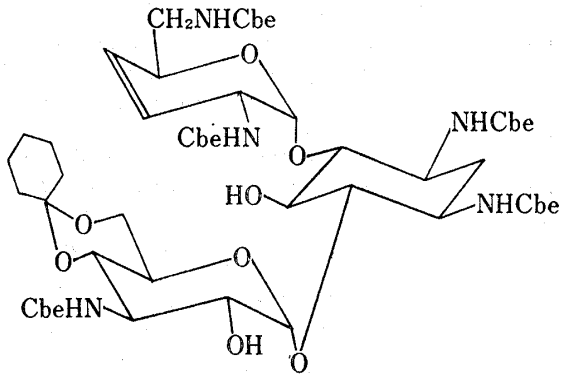


4a : R=H
4b : R=Cbz



5a : R_1=Ms, R_2=COC_6H_5
5b : R_1=R_2=H



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Cbe=$COOC_2H_5$, Cbz=$COOCH_2C_6H_5$, Ms=SO_2CH_3

Chart 1

TABLE I. Minimum Inhibitory Concentration of Butirosin, 3′,4′-Dideoxybutirosin A, and 3′,4′-Dideoxy-3′-eno butirosin A (in mcg/ml, heat infusion agar)

Test organisms	Butirosin	3′,4′-Dideoxy-butirosin A	3′,4′-Dideoxy-3′-eno butirosin A
Staphylococcus aureus 209P	0.1	0.1	0.1
Staphylococcus aureus 109	25	—	3.1
Escherichia coli NIHJ	0.8	0.4	3.1
Escherichia coli 665[a)]	200	3.1	12.5
Klebsiella 806	0.8	0.8	3.1
Proteus vulgaris 025	0.8	0.8	12.5
Proteus mirabilis 1306	50	50	100
Pseudomonas aeruginosa Ser	6.2	3.1	12.5
Pseudomonas aeruginosa 1055[a)]	400	6.2	50

a) Resistant strains proved to inactivate butirosin through phosphorylation at the 3′-hydroxy group. See S. Sugawara, S. Inaba, M. Madate, and H. Saeki, *Sankyo Kenkyusho Nempo*, **25**, 56 (1973).

4′-hydroxy functions and also exhibited broad activities against both sensitive and resistant bacteria; however, its activity is considerably less than that of 3′,4′-dideoxybutirosin A[5] as shown in the Table.

In the case of a kanamycin derivetive, naphthalene-sodium reaction of 2″-O-benzoyl-penta-N-ethoxycarbonyl-4″,6″-O-cyclohexylidene-3′,4′-di-O-mesylkanamycin B (**5a**) also afforded a 3′-eno compound (**6**) with removal of the 2′-benzoyl group. This yield of **6** was fairly good in comparison with that afforded by the Tipson–Cohen reaction of **5a**. The by-product was a parent deprotected compound (**5b**) which may have arisen from a radical fission of the O–S bonds in the mesyloxy groups during this reaction.

Experimental[9]

Methyl 2,3,4,6-Tetradeoxy-2,6-diethoxycarbonylamino-3-eno-α-D-*erythro*-hexopyranoside (2a)——Methyl 2,6-dideoxy-2,6-diethoxycarbonylamino-α-D-glucopyranoside, mp 158—163°, needles (from EtOH), was prepared according to the method used in synthesis of the benzyloxycarbonyl analog.[1] Mesylation in pyridine in the usual manner gave a 3,4-dimesylate **1a**, mp 187—188°, needles (from EtOH). IR ν_{max}^{Nujol} cm^{-1}: 3350, 1700, 1535, 1170. *Anal.* Calcd. for $C_{15}H_{28}N_2O_{12}S_2$: C, 36.58; H, 5.73; N, 5.69; S, 13.02. Found: C, 36.84; H, 6.37; N, 5.74; S, 13.05.

According to Carnahan, *et al.*,[7] the reagent was prepared by stirring a mixture of 94 mg of sodium, 520 mg of naphthalene, and 8 ml of tetrahydrofuran (THF) at room temperature for 2 .5 hr under N_2 atmosphere. The resulting solution (4 ml) was added dropwise to a solution of 100 mg of **1a** in 3 ml of THF and the green color of the solution was maintained throughout addition. The mixture was further stirred for 15 min, then, after addition of 0.3 ml of MeOH and successively of a small amount of water, the mixture was evaporated *in vacuo* to dryness. The residue was extracted with $CHCl_3$ several times and the combined extracts was dried and evaporated, giving a mixture of **2a** and naphthalene which was charged on a column of silica gel with benzene. Washing with benzene, elution with $CHCl_3$, evaporation of the solvent and recrystallization of the residue from EtOH gave **2a**, mp 141—142°, prisms. Yield, 40 mg (65%). NMR ($CDCl_3$) δ: 5.72 (2H, s, H-3, 4), 3.28 (3H, s, $-OCH_3$). *Anal.* Calcd. for $C_{13}H_{22}N_2O_6$: C, 51.64; H, 7.34; N, 9.27. Found: C, 51.71; H, 7.36; N, 9.11.

Methyl 2,6-Dibenzyloxycarbonylamino-2,3,4,6-tetradeoxy-3-eno-α-D-*erythro*-hexopyranoside(2b)——Mesylation of methyl 2,6-dideoxy-2,6-dibenzyloxycarbonylamino-α-D-glucopyranoside[1] gave a 3,4-dimesylate **1b**, mp 174—176°, prisms (from EtOH). IR ν_{max}^{Nujol}cm^{-1}: 3350, 1700, 1540, 1180. *Anal.* Calcd. for $C_{25}H_{32}N_2O_{12}S_2$: C, 48.23; H, 5.11; N, 4.01; S, 10.40. Found: C, 48.23; H, 5.11; N, 4.01; S, 10.30.

To a solution of 200 mg of **1b** and 200 mg of naphthalene in 5 ml of THF was added portionwise 50 mg of sodium at the rate of maintaining the green color of the solution at room temperature. Then the mixture was neutralized with AcOH and evaporated *in vacuo* to dryness. The residue was shaken with a mixture of ether and water and the aqueous layer was collected, acidified and successively was made basic with dil. Na_2CO_3. Then, afetr 0.1 ml of carbobenzyloxy chloride was added, the mixture was stirred for 1 hr and extracted with AcOEt. The extract was washed with water, dried, and evaporated. Trituration of the residue with hexane gave a powder whose recrystallization from EtOH gave **2b** as needles, mp 150—153°. The sample was identified with the sample obtained by the Tipson-Cohen reaction of **1b**. IR ν_{max}^{Nujol} cm^{-1}: 3220, 1690, 1540. NMR ($CDCl_3$) δ: 5.64 (2H, s, H-3, 4). *Anal.* Calcd. for $C_{23}H_{26}N_2O_6 \cdot 1/4H_2O$: C, 64.10; H, 6.20; N, 6.50. Found: C, 64.22; H, 6.04; N, 6.15.

3′,4′-Dideoxy-3′-enobutirosin A (4a)——To a solution of naphthalene-sodium prepared from 1.28 g of naphthalene and 0.3 g of sodium in 20 ml of THF was added dropwise a solution of 0.62 g of tetra-*N*-benzyloxycarbonyl-3′,4′-di-O-mesylbutirosin A[8] (3) in 5 ml of THF as described above. After further stirring for 20 min, 5 ml of MeOH was added with cooling and the mixture was neutralized with 3 N methanolic HCl and evaporated *in vacuo* to dryness. The residue was dissolved in 10 ml of water, acidified with 2 N HCl, filtered, and washed with ether. The aqueous layer was neutralized with Amberlite IR-45 (OH^-) and charged on 15 ml of Amberlite CG–50 (NH_4^+). After washing with water, the column was eluted with 0.3 N ammonia. Thus, collection of the fractions containing **4a**, evaporation below 50°, saturation with CO_2 and lyophylization gave 60 mg of **4a** as an amorphous powder. *Anal.* Calcd. for $C_{21}H_{39}N_5O_{10} \cdot 2H_2CO_3$: C, 40.52; H, 6.95; N, 10.28. Found: C, 40.73; H, 7.21; N, 10.09.

Benzyloxycarbonylation of **4a** thus obtained in aq. Na_2CO_3 gave **4b** which was identified with the authentic sample by means of nuclear magnetic resonance (NMR) spectrometry and thin-layer chromatography.

Penta-N-ethoxycarbonyl-4″,6″-O-cyclohexylidene-3′,4′-dideoxy-3′-enokanamycin B (6)——Mesylation of 2″-O-benzoyl-penta-N-ethoxycarbonyl-4″,6″-O-cyclohexylidenekanamycin B[8] in pyridine in the usual manner gave a 3′,4′-dimesylate **5a**, mp 162—164°, powder (from hexane–AcOEt). NMR ($CDCl_3$) δ: 3.10 (3H, s, CH_3SO_2-), 3.24 (3H, s, CH_3SO_2-). *Anal.* Calcd. for $C_{48}H_{73}N_5O_{25}S_2 \cdot H_2O$: C, 47.95; H, 6.29; N, 5.83; S, 5.33. Found C, 47.56; H, 6.01; N, 5.61; S, 5.10.

9) Melting points are not corrected.

Naphthalene–sodium reagent prepared from 345 mg of sodium and 2.04 g of naphthalene in 30 ml of THF was added dropwise to a solution of 1 g of **5a** in 30 ml of THF as described above. The mixture was stirred for 30 min and was quenched by addition of MeOH. Work-up as described above gave 825 mg of a syrup which was charged on 15 g of silica gel and eluted with $CHCl_3$–MeOH (25: 1, v/v). The fast running fractions were collected and recrystallized from aq. MeOH to give 295 mg of **6**, mp 213—215°, powder. NMR (pyridine-d_5) δ: 5.85 (2H, br. s, H-3′,4′). *Anal.* Calcd. for $C_{39}H_{63}N_5O_{18}$: C, 52.57; H, 7.13; N, 7.86. Found: C, 52.28; H, 7.32; N, 7.75.

Benzoylaion of **6** in pyridine gave a 2″-benzoate, mp 202—203°, powder which was identified with the sample prepared from **5a** *via* the known Tipson–Cohen method.

After the collection of fractions including **6**, the column was eluted with $CHCl_3$–MeOH (10: 1, v/v); thus, 170 mg of **5b** was obtained and identified with the authentic sample reported earlier.[8]

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Radioimmunoassay of Pregnanediol

Itsuo Yoshizawa, Ryoko Ohuchi, and Akiko Nakagawa

Hokkaido College of Pharmacy[1]

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Rabbit anti-pregnanediol antiserum was obtained using 3α,20α-dihydroxy-5β-pregnan-7-one O-carboxymethyloxime coupled to bovine serum albumin as the antigen. A radioimmunoassay procedure for pregnanediol utilizing the highly specific antiserum was developed and permitted the measurement of this material in body fluids.

Keywords——radioimmunoassay; pregnanediol; 3α,20α-dihydroxy-5β-pregnan-7-one O-carboxymethyloxime; anti-pregnanediol antiserum; progesterone metabolism

Introduction

Progesterone is an important steroid not only as a sexual hormone but also as a key intermediate to adrenocortical and/or other hormones in the steroid biosynthesis. Although the main pathways of the metabolism of progesterone have been elucidated, many aspects of its detailed metabolism remain obscure.

Pregnanediol is a major reductive metabolite of progesterone and excretion of its glucuronide in urine has been extensively used as a parameter of circulating progesterone. The measurement of pregnanediol in urine has a definite clinical value.[2]

In the course of our research on the alternation of various steroid during the menstrual cycle, it became necessary to determine the plasma pregnanediol, especially by a simple method. We have, therefore, undertaken to develop the method of radioimmunoassay of pregnanediol and this paper describes the details of the methodology.

Experimental

Materials——The haptenyl compound, 3α,20α-dihydroxy-5β-pregnan-7-one O-carboxymethyloxime, was synthesized in this laboratory by the method reported previously.[3] 5β-Pregnane-3α,20α-diol-1,2-^{3}H (45

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