[Chem. Pharm. Bull.]
25(8)2134-2137 (1977)

UDC 547.458.04 : 547.652.1'546.33.04

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

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(Received January 5, 1977)

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 B⁴⁾ 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 A⁸⁾ (**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

3

4a : R = H

4b : R = Cbz

5a : R₁ = Ms, R₂ = COC₆H₅5b : R₁ = R₂ = H

6

Cbe = COOC₂H₅, Cbz = COOCH₂C₆H₅, Ms = SO₂CH₃

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
<i>Staphylococcus aureus</i> 209P	0.1	0.1	0.1
<i>Staphylococcus aureus</i> 109	25	—	3.1
<i>Escherichia coli</i> NIHJ	0.8	0.4	3.1
<i>Escherichia coli</i> 665 ^{a)}	200	3.1	12.5
<i>Klebsiella</i> 806	0.8	0.8	3.1
<i>Proteus vulgaris</i> 025	0.8	0.8	12.5
<i>Proteus mirabilis</i> 1306	50	50	100
<i>Pseudomonas aeruginosa</i> Ser	6.2	3.1	12.5
<i>Pseudomonas aeruginosa</i> 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 A⁵⁾ as shown in the Table.

In the case of a kanamycin derivative, naphthalene-sodium reaction of 2"-O-benzoyl-penta-N-ethoxycarbonyl-4",6"-O-cyclohexylidene-3',4'-di-O-mesykanamycin 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.

Experimental⁹⁾

Methyl 2,3,4,6-Tetradecoxy-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 $\nu_{\text{max}}^{\text{Nujol}}$ cm⁻¹: 3350, 1700, 1535, 1170. Anal. Calcd. for C₁₅H₂₈N₂O₁₂S₂: 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 N₂ 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 CHCl₃ several times and the combined extracts were 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 CHCl₃, evaporation of the solvent and recrystallization of the residue from EtOH gave 2a, mp 141–142°, prisms. Yield, 40 mg (65%). NMR (CDCl₃) δ : 5.72 (2H, s, H-3, 4), 3.28 (3H, s, -OCH₃). Anal. Calcd. for C₁₃H₂₂N₂O₆: 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-tetradecoxy-3-eno- α -D-erythro-hexopyranoside (2b)—Mesylation of methyl 2,6-dideoxy-2,6-dibenzyloxycarbonylamino- α -D-glucopyranoside¹⁾ gave a 3,4-dimesylate 1b, mp 174–176°, prisms (from EtOH). IR $\nu_{\text{max}}^{\text{Nujol}}$ cm⁻¹: 3350, 1700, 1540, 1180. Anal. Calcd. for C₂₅H₃₂N₂O₁₂S₂: 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. Na₂CO₃. Then, after 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 $\nu_{\text{max}}^{\text{Nujol}}$ cm⁻¹: 3220, 1690, 1540. NMR (CDCl₃) δ : 5.64 (2H, s, H-3, 4). Anal. Calcd. for C₂₃H₂₆N₂O₆·1/4H₂O: 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-benzoyloxycarbonyl-3',4'-di-O-mesybutirosin A⁸⁾ (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 (NH₄⁺). 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 CO₂ and lyophilization gave 60 mg of 4a as an amorphous powder. Anal. Calcd. for C₂₁H₃₉N₅O₁₀·2H₂CO₃: 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. Na₂CO₃ 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 B⁹⁾ in pyridine in the usual manner gave a 3',4'-dimesylate 5a, mp 162–164°, powder (from hexane-AcOEt). NMR (CDCl₃) δ : 3.10 (3H, s, CH₃SO₂-), 3.24 (3H, s, CH₃SO₂-). Anal. Calcd. for C₄₈H₇₃N₅O₂₅S₂·H₂O: 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 $\text{C}_{39}\text{H}_{63}\text{N}_5\text{O}_{18}$: C, 52.57; H, 7.13; N, 7.86. Found: C, 52.28; H, 7.32; N, 7.75.

Benzoylation 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⁾

[Chem. Pharm. Bull.
25(8) 2137—2139 (1977)]

UDC 615.652.011.5.074 : 546.11 02.3.08

Radioimmunoassay of Pregnanediol

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(Received January 5, 1977)

Rabbit anti-pregnanediol antiserum was obtained using 3 α ,20 α -dihydroxy-5 β -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; 3 α ,20 α -dihydroxy-5 β -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, 3 α ,20 α -dihydroxy-5 β -pregnan-7-one O-carboxymethyloxime, was synthesized in this laboratory by the method reported previously.³⁾ 5 β -Pregnane-3 α ,20 α -diol-1,2-³H (45

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