Synthesis of Corticosteroid Derivatives Containing the 20β-Ol-21-al Side Chain

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Received November 25, 1975

A general synthesis of steroids containing the 20β-ol-21-al side chain is described. The ketol side chain was oxidized to 20-oxo-21-al, and treated with hydroxylamine to yield the 20-oxo-21-oxime in good yield. Reduction with sodium borohydride followed by hydrolysis afforded the final product. The procedure is illustrated by the synthesis of 20β-hydroxy-3-oxopregn-4-en-21-al, 17,20β-dihydroxy-3-oxopregn-4-en-21-al, 11β,20β-dihydroxy-3-oxopregn-4-en-21-al, and 11β,17,20β-trihydroxy-3-oxopregn-4-en-21-al.

Kendall et al.[1] proposed in 1934 that the physiologically essential steroid hormone secreted by the mammalian adrenal cortex contained a hydroxy aldehyde side chain. Subsequent studies by Reichstein and his co-workers[2] demonstrated that Kendall had erred in his interpretation of the data, and that the side chain was a ketol. Corticosteroids with the 17-hydroxy aldehyde configuration were as a consequence no longer seriously considered in a physiological context. Recently, as a result of in vivo and in vitro studies of corticosteroid metabolism in humans[3-5] and hamsters,[6] we revived the possibility that 20-hydroxy 21-aldehydes are important metabolic intermediates. In this context we therefore investigated the chemical synthesis of this class of compound.

The synthesis of corticosteroids with the hydroxy aldehyde side chain has been attempted by others.[7-13] In general, the isolation of pure products was troublesome, and the yields were low. Approaches used included selective oxidation of 20,21,22-triols with periodate,[7,8] reduction of 20-oxo-21-al dimethyl acetal and deacetalation,[9,10] and reduction of 20-oxo-21-aldehydes with 20β-hydroxy steroid dehydrogenase.[11]

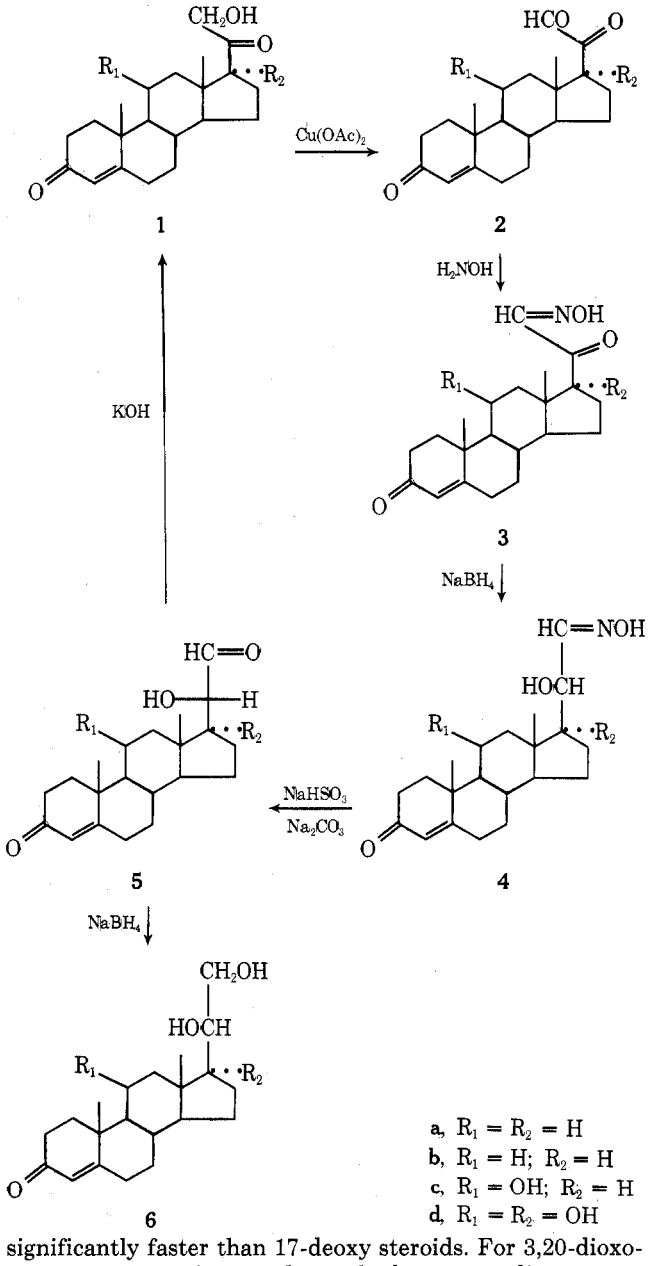
The procedure we use is illustrated in Scheme I. The formation of aldoxime (3) from keto aldehyde (2) and hydroxylamine hydrochloride was carried out at room temperature using stoichiometric amounts of reactants at neutral pH. The proportion of ethanol to water in the solvent mixture was adjusted to permit the aldehyde to stay in solution, and the product to precipitate out. When ethanol was used in excess, products and reactants stayed in solution, resulting in a decrease in the purity and yield of the product.

Initially, lithium aluminum hydride was employed to reduce the 20-carbonyl group. Attempts to reoxidize the resulting 3-hydroxy group selectively with manganese dioxide were unsuccessful, because the =NOH group at position 21 facilitated the reoxidation of 20-ol as well. Both 3-oxo-4-ene and 20-oxo-21-oxime are electronically equivalent since both are α,β-unsaturated ketones.

With sodium borohydride, reduction (3 → 4) was influenced by temperature and solvent. At ice bath temperatures reduction of the 20-oxo group occurred preferentially. As the temperature rose, selectivity of reduction was directed increasingly to the 3-oxo group. The nature of the solvent had a strong directing effect on the selectivity of the reduction. The 3-oxo group was favored over the 20-oxo group in methanol, ethanol, 2-propanol, tetrahydrofuran, methanol–water mixtures, or tetrahydrofuran–water mixtures. In dimethylformamide–methanol (2/1 v/v), reduction of the 20-oxo group was significantly favored over the 3-oxo group. In this reagent the reaction proceeded more slowly than in other solvents; more sodium borohydride was needed to effect complete reduction. It is probable that the dielectric constant of the medium plays an important role in determining selectivity and reduction rate.

It was found that 17α-hydroxylated steroids were reduced significantly faster than 17-deoxy steroids. For 3,20-dioxopregn-4-en-21-oxime and 11β-hydroxy-3,20-dioxopregn-4-en-21-oxime, a large excess of sodium borohydride was necessary to effect reduction, which required 2–3 h to complete. With 17-hydroxy-3,20-dioxopregn-4-en-21-oxime, the reaction was completed within 1 h and required much less reducing agent. The latter two steroids were reduced with a greater selectivity favoring the 20 position.

Scheme I



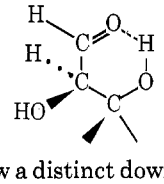
a, $R_1 = R_2 = H$
b, $R_1 = H$; $R_2 = H$
c, $R_1 = OH$; $R_2 = H$
d, $R_1 = R_2 = OH$

Faster reduction at the 3 position in methanol seems to be due to easier accessibility of the borohydride reagent to this exposed position. We propose that the 3 position is shielded by dimethylformamide, and is thus made less accessible to the reagent, but that bulky groups in the region of the D ring permit the selective approach of the borohydride over dimethylformamide to the reactive 20 position. The presence of a 17-hydroxyl group polarizes the 20-oxo group, and increases the reduction rate. The reaction is stereospecific, yielding only 20β-hydroxy enantiomers.

When excess sodium borohydride was decomposed with acetic acid, further reduction took place rapidly which led to undesired products and decreased yield of the hydroxy oxime. Consequently, the product was extracted directly at the completion of the reaction without attempting to destroy residual sodium borohydride.

Hydrolysis of the oximes (**4** → **5**) was performed by a modification of the method described by Pines et al.[14] We found that it was not possible to cleave the oxime by refluxing the steroid in ethanol–water (1:1) with excess sodium bisulfite as recommended by these authors, since the resulting hydroxy aldehydes were unstable under these conditions. At 45–55 °C, hydrolysis by excess sodium bisulfite proceeds smoothly with little decomposition. It was not possible to decompose the bisulfite adduct with dilute acid, since hydroxy aldehydes are unstable under these conditions. Sodium carbonate readily generated the desired steroidal 20-ol-21-al. In order to minimize decomposition at this stage, all manipulations were performed in an ice bath. If the hydroxy aldehydes remained in aqueous solution or suspension, they rapidly polymerized to intractable materials that were insoluble in ethyl acetate. Therefore, as the free aldehyde was released during sodium carbonate hydrolysis, it was extracted directly into ethyl acetate.

The steroid hydroxy aldehydes are stable as pure, dry products, especially at low temperature. Alkali catalyzes their isomerization to the corresponding ketols. The isomerization of 20β-hydroxy-3-oxopregn-4-en-21-al and 11β,20β-dihydroxy-3-oxopregn-4-en-21-al in base was clean and quantitative. Isomerization of 17,20β-dihydroxy-3-oxopregn-4-en-21-al was slower and less efficient, possibly because the aldehyde is stabilized to some degree by hydrogen bonding with the 17-hydroxy group.



The NMR data show a distinct downfield shift for the 20α proton of the 17-hydroxylated steroids from 4.1 to 4.4 ppm. This deshielding effect is consistent with the more rapid reduction of 17-hydroxy steroids by sodium borohydride. The extreme downfield singlet at 9.7 ppm corresponds to the 21-aldehyde proton. There was no evidence of splitting, and it is tentatively suggested that the 21-H is oriented at right angles to the adjacent proton at position 20. Further explorations of these stereochemical relationships require that the 20α epimer be prepared.

Experimental Section

Corticosteroids were bought from Steraloids, Pawling, N.Y. The 3-methyl-2-benzothiazolone hydrazone hydrochloride (MBTH) was purchased from Aldrich Chemical Co. Hydroxylamine hydrochloride and other reagents were used as purchased without further purification. Prepacked chromatographic columns, size A and B, containing silica gel 60 were purchased from Em Laboratories, Elmsford, N.Y.

Melting Points. Melting points were determined with a Hoover capillary apparatus and are uncorrected. The true melting points are within a degree of those reported.

Absorption Spectra. The ultraviolet spectra were determined in absolute methanol in a Cary Model 15 recording spectrophotometer. Infrared spectra were obtained in KBr pellets with a Perkin-Elmer Model 221 spectrophotometer.

Nuclear Magnetic Resonance. NMR spectra were determined in $CDCl_3$ solution on Varian A-60A or HA-100 spectrophotometers. Chemical shift data are reported in parts per million downfield from internal tetramethylsilane (δ scale).

Mass Spectrometry. A Du Pont Model 21-492B mass spectrometer with 21-094 computerized data system was used. The mass spectra were recorded at an electron ionizing energy of 70 eV. Source temperature was 210 °C and the range of probe temperature was from room temperature to about 300 °C. Spectra above 100 °C are reported.

MBTH Reaction. About 0.5-mg sample was weighed out and dissolved in 2 ml of ethanol. To 100 or 200 μl of the sample solution was added 0.5 ml of 0.1% MBTH followed by 1.2 ml of 0.1 M glycine buffer (pH 4.0). After total volume was adjusted to 2.5 ml by adding water, the mixture was heated in a boiling water bath for 7 min. When the solution had cooled, 2.5 ml of ethanol was added and absorbancy was determined spectrophotometrically between 200 and 450 nm.

3,20-Dioxopregn-4-en-21-al (2a) from 21-Hydroxypregn-4-ene-3,20-dione (1a). The method of Monder and Furfine[15] was followed with minor modification. To a solution of 3 g (9.10 mmol) of **1a** in 250 ml of methanol was added a solution of 0.75 g (4.1 mmol) of cupric acetate in 375 ml of methanol. After standing for 10 min, air was bubbled through the mixture for 30 min. To the clear blue solution, 150 ml of 0.1 M aqueous sodium carbonate containing 1.5 g of EDTA (pH 9.0) was added; most of the methanol was removed under vacuum at 25 °C. Steroid was extracted with 200 ml of ethyl acetate after adding 1200 ml of water. About 100 g of sodium sulfate was dissolved into the water phase which was then extracted with three 150-ml portions of ethyl acetate. The combined solvent extract was washed with 2% sodium bicarbonate in saturated sodium chloride, then with saturated sodium chloride and dried over anhydrous sodium sulfate. Ethyl acetate was evaporated to dryness and the residual product was dissolved in 50 ml of acetone. Crystallization was initiated by carefully adding 300 ml of phosphate buffer (0.001 M, pH 7.5) with a dropper under the surface. The turbid solution was left overnight at 5 °C. Crystals were collected by filtration and washed with water. A second crop was obtained by partial evaporation of solvent. The combined yield was 2.7 g (90%). Chromatographic mobility on thin layer plates and ir spectrum matched exactly those of an authentic sample: mp 90–92 °C dec; λ_{max} (alcohol) 241 nm (ϵ 15 400); MBTH derivative, λ_{max} 374 nm; ir 5.85, 6.02, 8.13, 9.30 μ.

Anal. Calcd for $C_{21}H_{28}O_3 \cdot H_2O$ (346.45): C, 72.80; H, 8.73. Found: C, 72.71; H, 8.66.

17-Hydroxy-3,20-dioxopregn-4-en-21-al (2b) from 17,21-Dihydroxypregn-4-ene-3,20-dione (1b). Conversion of **1b** to **2b** was performed as described for the synthesis of **1a**, except that the cupric acetate catalyzed oxidation proceeded for 2 h. Yield was 2.6 g (87%): mp 96–100 °C dec; λ_{max} (alcohol) 241 nm (ϵ 15 400); MBTH derivative, λ_{max} 387 nm; ir 5.80, 6.06 μ.

Anal. Calcd for $C_{21}H_{28}O_4 \cdot H_2O$: C, 69.58; H, 8.34. Found: C, 69.39; H, 8.25.

11β-Hydroxy-3,20-dioxopregn-4-en-21-al (2c) from 11β,21-Dihydroxypregn-4-ene-3,20-dione (1c). Conversion of **1c** to **2c** was performed as described for the synthesis of **2a** except that the cupric acetate catalyzed oxidation proceeded for 60 min. Yield was 2.6 g (87%): mp 113–116 °C dec; λ_{max} (alcohol) 241 nm (ϵ 15 300); MBTH derivative λ_{max} 373 nm; ir 5.84, 6.05, 9.20, 9.60 μ.

Anal. Calcd for $C_{21}H_{28}O_4 \cdot H_2O$: C, 69.58; H, 8.34. Found: C, 69.91; H, 8.70.

11β,17-Dihydroxy-3,20-dioxopregn-4-en-21-al (2d) from 11β,21-Trihydroxypregn-4-ene-3,20-dione (1d). Conversion of **1d** to **2d** was performed as described for the synthesis of **2a** except that the cupric acetate catalyzed oxidation proceeded for 90 min. Yield was 2.4 g (80%): mp 160–162 °C dec; λ_{max} (alcohol) 241 nm (ϵ 15 700); MBTH derivative λ_{max} 386 nm; ir 5.81, 6.06, 9.60 μ.

Anal. Calcd for $C_{21}H_{28}O_5 \cdot H_2O$: C, 66.65; H, 7.99. Found: C, 66.79; H, 8.01.

3,20 Dioxopregn-4-en-21-oxime (3a from 2a). To 500 mg (1.5 mmol) of **2a** in 16 ml of 75% aqueous ethanol, 120 mg (1.7 mmol) of hydroxylamine hydrochloride was added and 104 mg (0.8 mmol) of sodium carbonate monohydrate in 1 ml of water was added slowly with stirring. Stirring was continued for 60 min at room temperature. Water (50 ml) was added. The suspension was cooled in an ice bath for 10 min, filtered, and washed with cold water. The collected product was dried in vacuo over P_2O_5. Yield was 510 mg (98%): mp 208–210 °C dec; ir 2.96–3.20 (multiple bands), 3.34, 3.45, 6.0–6.20, 6.8–7.0 (multiple bands), 7.23, 7.33, 7.57, 7.80, 7.88, 8.02, 8.12, 8.26, 8.42, 8.58,

9.00, 9.92 μ.

Anal. Calcd for $C_{21}H_{29}O_3N$: C, 73.49; H, 8.52; N, 4.08. Found: C, 73.36; H, 8.86; N, 4.58.

3,20-Dioxo-17-hydroxypregn-4-en-21-oxime (3b from 2b). To 500 mg (1.5 mmol) of **2b** in 11 ml of 77% aqueous ethanol was added 104 mg (1.5 mmol) of hydroxylamine hydrochloride and 93 mg (0.75 mmol) of sodium carbonate monohydrate in 1 ml of water. The mixture was stirred for 45 min and worked up as for **3a.** Yield was 505 mg (96%): mp 195–197 °C dec; ir 2.92, 3.40, 3.48, 5.9–6.2 (multiple bands), 6.8–7.05 (multiple bands), 7.22, 7.37, 7.52, 7.89, 8.11, 8.22, 8.40 8.90, 9.20, 9.33 μ.

Anal. Calcd for $C_{21}H_{29}O_4N$: C, 70.19; H, 8.13; N, 3.90. Found: C, 69.74; H, 8.28; N, 3.79.

3,20-Dioxo-11β-hydroxypregn-4-en-21-oxime (3c from 2c). To 345 mg (1.0 mmol) of **2c** in 11 ml of 73% aqueous ethanol was added 0.069 g (1.0 mmol) of hydroxylamine hydrochloride and 0.062 g (0.5 mmol) of sodium carbonate monohydrate in 1 ml of water and treated as for **3a.** Yield was 340 mg (94%): mp 184–188 °C dec; ir 2.95–3.20 (multiple bands), 3.48 5.95–6.20 (multiple bands), 6.96, 7.03, 7.26, 7.45, 7.92, 8.16, 8.49, 8.74, 9.02, 9.25, 9.40 μ.

Anal. Calcd for $C_{21}H_{29}O_4N$: C, 70.19; H, 8.13; N, 3.90. Found: C, 70.31; H, 8.22; N, 3.90.

3,20-Dioxo-11β,17-dihydroxypregn-4-en-21-oxime (3d from 2d). To 360 mg (1.0 mmol) of **2d** in 17 ml of 70% aqueous ethanol was added 0.069 g (1.0 mmol) of hydroxylamine hydrochloride and 0.062 g (0.5 mmol) of sodium carbonate monohydrate in 1 ml of water. The mixture was stirred for 1 h and concentrated to 5 ml under vacuum. Water (15 ml) was added and the precipitate was collected by filtration. Yield was 350 mg (93%): mp 202–203 °C; ir 2.90–3.10 (multiple bands), 3.42, 5.87–6.25 (multiple bands), 6.93, 6.98, 7.42, 7.45, 7.87, 8.12, 8.22, 8.42, 8.64, 8.76, 8.99, 9.27, 9.42, 9.65, 9.78 μ.

Anal. Calcd for $C_{21}H_{29}O_5N$: C, 67.18; H, 7.79; N, 3.37. Found: C, 66.76; H, 7.89; N, 3.26.

20-Hydroxy-3-oxopregn-4-en-21-oxime (4a from 3a). To 500 mg (1.5 mmol) of **3a** in 15 ml of dimethylformamide–methanol (2/1 v/v) in an ice bath was added slowly with stirring 125 mg (3.3 mmol) of sodium borohydride in 7 ml of the same solvent. The mixture was stirred in an ice bath for 3 h. Methanol was removed under vacuum. Ethyl acetate was added followed by 85 ml of water and 5 ml of saturated aqueous sodium chloride with vigorous stirring. The ethyl acetate extract was washed twice with water, twice with saturated sodium chloride, and dried with anhydrous sodium sulfate. The ethyl acetate was removed under vacuum. Product was purified on a silica gel column (E. Merck, size B) with chloroform–methanol (97/3 v/v) as eluent. Yield of purified product was 240 mg (48%): mp 203–205 °C; ir 2.92, 3.43, 3.47, 6.05–6.17 (multiple bands), 6.90, 6.98, 7.27, 7.37, 7.50, 7.85, 8.05, 8.12, 8.37, 9.0, 9.40, 9.72, 9.87 μ.

Anal. Calcd for $C_{21}H_{31}O_3N$: C, 73.01; H, 9.05; N, 4.05. Found: C, 72.02; H, 8.97; N, 4.03; *m/e* 345.230616 (calcd, 345.230404).

11β,20-Dihydroxy-3-oxopregn-4-en-21-oxime (4c from 3c). To 500 mg (1.4 mmol) of **3c** in 16 ml of dimethylformamide (2/1 v/v) was added 140 mg (3.7 mmol) of sodium borohydride in 6 ml of the same solvent. The mixture was stirred for 3 h and worked up as described for **4a.** The product was purified on a silica gel column with chloroform–methanol (96/4 v/v) as developing solvent. The purified yield was 230 mg (46%): mp 65–67 °C dec; ir 2.39, 3.41, 3.47, 6.00–6.25 (multiple bands), 6.85–7.07 (multiple bands), 7.45, 7.57, 7.85, 8.05, 8.13, 8.42, 8.66, 9.40, 9.73 μ.

Anal. Calcd for $C_{21}H_{31}O_4N$: C, 69.8; H, 8.64; N, 3.88. Found: C, 66.3; H, 8.63; N, 3.51; *m/e* 361.226758 (calcd, 361.225319).

17,20-Dihydroxy-3-oxopregn-4-en-21-oxime (4b from 3b). 3b (500 mg, 1.4 mmol) in 12 ml of dimethylformamide–methanol (2/1 v/v) was treated with 25 mg of sodium borohydride in 2 ml of the same solvent and stirred for 60 min in an ice bath. The product was purified on a silica gel column with chloroform–methanol (95/5 v/v) as eluent. The purified product was 310 mg (62%): mp 204 °C dec; ir 2.94, 3.39, 3.48, 5.95–6.20 (multiple bands), 6.76–7.05 (multiple bands), 7.26, 7.37, 7.51, 7.89, 8.06, 8.49, 8.94, 9.60, 9.77, 10.05 μ.

Anal. Calcd for $C_{21}H_{31}O_4N$: C, 69.8, H, 8.64; N, 3.88. Found: C, 68.0; H, 8.68; N, 3.47; *m/e* 361.226226 (calcd, 361.225319).

11β,17,20-Trihydroxy-3-oxopregn-4-en-21-oxime (4d from 3d). 4d was prepared by following the same method as for **4b.** Yield was 290 mg (58%): mp 85–87 °C; ir 2.92, 3.41, 3.47, 6.0–6.19 (multiple bands), 6.89, 6.98, 7.24, 7.45, 7.87, 8.13, 8.45, 8.87, 9.48, 9.67, 9.75 μ.

Anal. Calcd for $C_{21}H_{31}O_5N$: C, 66.82; H, 8.28; N, 3.71. Found: C, 67.47; H, 8.52; N, 3.31; *m/e* 377.220502 (calcd, 377.220502).

Purification of products **4a–d** was difficult, since some decomposition to unidentified products occurred, even though the various reduced oximes were well separated. Degradation products were removed by extraction with ethyl acetate after conversion of the oxime to the bisulfite adduct which was then hydrolyzed to the 20-hydroxy 21-aldehyde.

20-Hydroxy-3-oxopregn-4-en-21-al (5a). To 70 mg (0.2 mmol) of **4a** in 30 ml of 50% aqueous ethanol, 700 mg (6.7 mmol) of powdered sodium bisulfite was added. The mixture was stirred for 3 h in a water bath at 50 °C. Ethanol was evaporated thoroughly, then 10 ml of water was added to the residue. The solution was washed with ethyl acetate four times. If a white precipitate insoluble in ethyl acetate developed, more water was added. The washed water layer was cooled to 3 °C. Ethyl acetate (15 ml) and 6 ml of saturated sodium carbonate solution was added with vigorous mixing, which was continued for 10 min. The organic layer was separated and extracted with ethyl acetate once more. The combined extract was washed twice with water and twice with saturated sodium chloride solution, then dried with anhydrous sodium sulfate. Ethyl acetate was removed under vacuum at below 0 °C. The residue was isolated from dichloromethane–hexane. The residual white powder was dried under high vacuum. Yield was 34 mg (51%): mp 140 °C dec; ir 2.92, 3.41, 3.48. 5.80, 6.01, 6.20, 6.90, 6.98, 7.27, 7.30, 7.52, 7.87, 8.07, 8.14, 8.43, 8.95, 9.34, 9.66, 9.86 μ.

Anal. Calcd for $C_{21}H_{30}O_3 \cdot 0.5H_2O$: C, 74.4; H, 9.20. Found: C, 75.0; H, 8.95.

λ_{max} (methanol) 241 nm (ε 16 400); MBTH derivative λ_{max} 312 nm (ε 26 900); NMR 18-CH_3, 0.88; 19-CH_3, 1.19; 4-H, 5.69; 20-CHOH, 4.06 (multiplet; doublet in D_2O, J = 10 Hz); 21-CHO, 9.65 ppm; mass spectrum *m/e* 330 (M^+, $C_{21}H_{30}O_3$), 11.4%; 301 [M^+ – (HC=O)], 7.3%; 300 [M^+ – (CHO) – (H)], 3.5%; 299 [M^+ – (CHO) – (2 H)], 100%; 271 [M^+ – (CHO – CHOH)], 47.4%.

17,20-Dihydroxy-3-oxopregn-4-en-21-al (5b). 5b was prepared from **4b** as described for **5a.** Yield 31 mg (46%): mp 98–101 °C; ir 2.89, 3.38, 3.46, 5.75, 6.05, 6.18, 6.90, 6.97, 7.07, 7.25, 7.37, 7.84, 8.05, 8.12, 8.44 8.90, 9.40, 9.60, 9.82, 10.42, 10.59 μ.

Anal. Calcd for $C_{21}H_{30}O_4$: 72.80; H, 8.70. Found: C, 72.82; H, 8.84.

λ_{max} (methanol) 241 nm (ε 15 900); MBTH derivative λ_{max} 312 nm (ε 25 100); NMR 18-CH_3, 0.95; 19-CH_3, 1.20; 4-H, 5.72; 20-H, 4.36 (20-HCO–); 21-H, 9.72 ppm (21-HC=O); mass spectrum *m/e* 346 (M^+, $C_{21}H_{30}O_4$), 16.0%; 317 [M^+ – (HC=O)], 15.2%; 316 [M^+ – (CHO) – (H)], 29.2%; 288 [M^+ – (CHOCHOH) + (H)], 22.2%; 287 [M^+ – (CHOCHOH)], 100%.

11β,20-Dihydroxy-3-oxopregn-4-en-21-al (5c). The same method was followed as for **5a.** Yield 33 mg (49%): mp 138 °C dec; ir 2.92, 3.41, 3.47, 5.81, 6.07, 6.19, 6.90, 7.26, 7.41, 7.86, 8.12, 8.43, 8.68, 11.50 μ.

Anal. Calcd for $C_{21}H_{30}O_4 \cdot 0.25H_2O$: C, 71.90; H, 8.80. Found: C, 71.50; H, 9.03.

λ_{max} (methanol) 241 nm (ε 15 100); MBTH derivative λ_{max} 312 nm (ε 17 500); NMR 18-CH_3, 1.12; 19-CH_3, 1.46; 4-H, 5.68; 20-H, 4.10 (multiplet); 21-H, 9.71 (21-HC=O); 11-H, 4.32; mass spectrum *m/e* 346 (M^+, $C_{21}H_{30}O_4$), 8.6%; 317 [M^+ – (HC=O)], 18.0%; 316 [M^+ –(CHO) – (H)], 40.0%; 315 [M^+ – (CHO) – (2 H)], 100%; 270 [M^+ – (CHOCHO) – (H_2O)], 76.4%.

11β,7,20-Trihydroxy-3-oxopregn-4-en-21-al (5d). 5d was synthesized as described for **5a.** Yield 27 mg (40%): mp 107 °C; ir 2.92, 3.41, 3.46 5.82, 6.05–6.15 (multiple bands), 6.77–7.01 (multiple bands), 7.24, 7.45, 7.85, 8.10, 8.42, 8.62, 8.85, 9.25–9.95 μ (multiple bands).

Anal. Calcd for $C_{21}H_{30}O_5 \cdot 0.25H_2O$: C, 68.73; H, 8.38. Found: C, 68.62; H, 8.52.

λ_{max} (methanol) 241 nm (ε 14 900); MBTH derivative λ_{max} 312 nm (ε 25 600); NMR 18-CH_3, 1.18; 19-CH_3, 1.45; 4-H, 5.69; 11-H, 4.39; 20-H, 4.39; 21-H, 9.73 (21-HC=O); mass spectrum *m/e* 362 (M^+, $C_{21}H_{30}O_5$), 28.6%; 333 [M^+ – (HC=O)], 14.7%; 344 [M^+ – (H_2O)], 20.8%; 332 [M^+ – (CHO) – (H)], 11.1%; 314 [M^+ – (CHO) – (H) – (H_2O)], 16.7%; 302 [M^+ – (CHOCHOH) – (H_2O)], 83.5%; 163 [M^+ – (199)], 100%.

Isomerization of 5a–d to Corticosteroids. To 3 mg (0.01 mmol) of **5a–d** was added 0.15 mmol of potassium hydroxide in 0.3 ml of methanol. The solution was stirred for 25 min at room temperature and 0.17 mmol of glacial acetic acid was added. Methanol was evaporated, then steroid was extracted with ethyl acetate after 0.3 ml of water was added. The extract was washed with water, then dried with anhydrous sodium sulfate and solvent was evaporated. Identification of the resulting ketols was made in each case on the basis of infrared spectra and chromatographic mobilities.

Determination of Stereochemistry at C-20. 5a–d (5 mg) were reduced to 20,21-glycol by adding 0.2 mg of sodium borohydride in 0.3 ml of methanol in an ice bath. After 20 min stirring, methanol was evaporated and ethyl acetate and water were added. Ethyl acetate extract was chromatographed with authentic standard on a silica gel coated TLC plate which had been dipped into 0.1 M sodium borate solution (pH 9.0) and dried at 45 °C overnight. R_f values with chlo-

roform–methanol (90:10) as developing solvent were (mobilities of authentic standards in parentheses) **6a**, 0.37, (20α = 0.26; 20β = 0.37); **6b**, 0.47 (20α = 0.37; 20β = 0.47); (chloroform–methanol, 85:15) **6c**, 0.32 (20α = 0.24; 20β = 0.32); **6d**, 0.25 (20α = 0.18, 20β = 0.25).

Acknowledgments. This investigation was supported by U.S. Public Health Service Grants CA 14914, AM 09006, and RR 5589. S. w. O. is a trainee of the National Cancer Institute (Grant CA 5215).

Registry No.—**1a**, 64-85-7; **1b**, 152-58-9; **1c**, 50-22-6; **1d**, 50-23-7; **2a**, 853-27-0; **2b**, 20287-95-0; **2c**, 20287-97-2; **2d**, 14760-49-7; **3a**, 59005-48-0; **3b**, 59005-49-1; **3c**, 59005-50-4; **3d**, 59005-51-5; **4a**, 59005-52-6; **4b**, 59005-53-7; **4c**, 59005-54-8; **4d**, 59005-55-9; **5a**, 59005-56-0; **5b**, 59005-57-1; **5c**, 59005-58-2; **5d**, 59005-59-3.

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Synthesis of Substituted Glycopeptides Containing a 2-Acetamido-2-deoxy-β-D-glucopyranosyl Residue and the Amino Acid Sequence 18–22 of Bovine Pancreatic Deoxyribonuclease A[1]

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Received December 22, 1975

Condensation of 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-4-oyl]-2-deoxy-β-D-glucopyranosylamine (1) with protected L-alanine, L-alanyl-L-threonine, L-alanyl-L-threonyl-L-leucine, and L-alanyl-L-threonyl-L-leucyl-L-alanine derivatives gave glycodi-, -tri-, -tetra-, and -pentapeptides corresponding to the sequences 18–19, 18–20, 18–21, and 18–22 of deoxyribonuclease A.

Glycoproteins containing the 2-acetamido-1-*N*-(L-aspart-4-oyl)-β-D-glucosylamine carbohydrate–protein linkage include many of the biologically important proteins, such as hormones, enzymes, plasma proteins, etc.[2] The mechanism of the biosynthesis of the carbohydrate chain and its attachment to the protein backbone are not as yet completely elucidated, because of the difficulty in the separation and identification of the final product, in addition to the instability of the possible carbohydrate intermediates. For this reason, a study of the biosynthesis of glycoproteins based on chemically synthesized peptide acceptors and carbohydrate intermediates[3,4] has been undertaken in this laboratory. Glycoproteins from pancreatic tissues were selected because this tissue has been shown to synthesize rapidly the possible intermediates.

In a preceding paper,[3] we have described the synthesis of glycopeptides derived from beef ribonuclease B and in the present paper we describe the synthesis of glycopeptides derived from beef deoxyribonuclease A. This enzyme exists in bovine pancreatic tissue in three forms, A, B, and C, which differ in the carbohydrate composition of the chain attached to Asn-18 as well as in their amino acid sequences.[5-8] In addition, the amino acid sequence Asn-X-Ser, which is generally assumed to be a prerequisite for the linkage of a carbohydrate chain to an asparagine residue,[2] exists at Asn-103, X being Asp-104, but no carbohydrate chain is linked to Asn-103. Thus, biosynthetic experiments with glycopeptides derived from the Asn-18 and Asn-103 regions could give important information on the role played by the amino acid sequence in the formation and structure of the carbohydrate chain. As model glycopeptides, the synthesis of di-, tri-, tetra-, and pentapeptides related to the sequence 18–22 (Asn-Ala-Thr-Leu-Ala) of deoxyribonuclease A, where a 2-acetamido-2-deoxy-β-D-glycopyranosyl residue is linked to the amide group of the Asn-18 residue, is described.

The present synthesis of the protected glycopeptides Asn(GlcNAc)-Ala (**2** and **3**), Asn(GlcNAc)-Ala-Thr (**11** and **12**), Asn(GlcNAc)-Ala-Thr-Leu (**13**), and Asn(GlcNAc)-Ala-Thr-Leu-Ala (**14**) is based on the synthesis of the peptide chain, unmasking of the terminal amino group, and condensation with 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-1-oyl]-2-deoxy-β-D-glucopyranosylamine (**1**), in a sequence of reactions similar to that described[3] for the synthesis of glycopeptides derived from the region of Asn-34 of beef ribonuclease B. Of the two reagents for peptide synthesis, *N*-ethyl-5-phenylisoxazolium 3'-sulfonate[9] (WRK) and 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline[10] (EEDQ), the latter-named reagent was found to be more efficient for the synthesis of N^4-glycosylasparagine,[11] but less efficient for peptides of high molecular weight.[12] Both reagents were tested for the condensation of the glycopyranosylamine **1** with L-alanine *p*-nitrobenzyl ester to give crystalline 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-1-oyl-(L-alanine *p*-nitrobenzyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine (**2**) in similar yields. The *p*-nitrobenzyl ester group, protective of the C-terminal group, is stable under acid conditions and, thus, is useful for elongation of the peptide chain from the N-terminal group. In order to elongate the chain at the C-terminal group, the methyl ester derivatives, which can be easily converted into reactive hydrazides,[14] were selected. Condensation of **1** with L-alanine methyl ester in the presence of the WRK re-