SYNTHESIS OF SOME STEROID SPIN LABELS

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ABSTRACT

An improved synthesis of spin-labeled cortisol, cortisone, and testosterone using N,N'-cyclohexylcarbodiimide (DCC) is reported. The spin labeled steroids are shown to result from the esterification at the most reactive C(21) and C(17) hydroxyl groups. A mild and high yield oxidation of cortisol spin label to cortisone spin label by chromium trioxide-pyridine is also discussed.

INTRODUCTION

Earlier we reported the synthesis of spin-labeled cortisol and testerone in 16 and 8 percent yields, respectively, using N,N'-thionyldiimidazole as the coupling reagent (2). The present work was undertaken to improve the yields of these spin labels and to confirm the tentative structural assignments made earlier. Spin labeled analogs of steroids are known to be valuable tools in studying steroid-protein interactions (3,4,5). The present spin labels were synthesized for use in a proposed study of the binding of cortisol and related steroids to corticosteroid binding globulin (CBG) in blood using ESR spectroscopy.

RESULTS AND DISCUSSION

Reaction of cortisol 1 and 3-carboxyl-2,2,5,5-tetramethyl-pyrrolidin-1-yloxyl 5 in dry pyridine in the presence of DCC afforded a mixture of two compounds which were separable by column chromatography. Elution with benzene gave a yellow solid which was found to be the adduct 7 of spin label acid 5 with DCC similar to that

reported for carboxylic acids (8). Continued elution with benzene-acetone yielded a light yellow compound which was purified by crystallization from acetone to afford 2, m.p. 223°-224° (35 percent) (mixture of two enantiomers).

The IR spectrum of 2 showed absorption at 3420, 1743, 1727, and 1668 cm^{-1}. The NMR spectrum of 2 exhibited only broad peaks as expected because of the paramagnetic center in the molecule. A sharp NMR spectrum was obtained, however, in chloroform containing a few mg of phenylhydrazine (9). In this case, the phenylhydrazine present reduced the nitroxide moiety to the corresponding hydroxylamine, which, not being paramagnetic, afforded sharp NMR resonance. Distinct resonances were observed for the methyl groups on the pyrrolidine moiety and also for the steroid nucleus. Additional peaks were also present, however, at δ 6.5-7.2 and δ 4.4-5.5 due to the phenyl protons and aminoprotons in phenylhydrazine. The mass spectrum of this compound exhibited the molecular ion peak at m/e 530.

Even though there are three hydroxyl groups in cortisol, only one spin label ester was formed in detectable amounts in the reaction. The esterification is expected to take place at the least hindered primary hydroxyl group at C(21) (10). To confirm this expectation, cortisol bis methylenedioxy (cortisol BMD) 6 was prepared using the reported procedure (6) and was then subjected to esterification using each of the two reagents, DCC and N,N'-thionyldiimidazole. In the first case, the DCC adduct 7 was obtained as the only product along with recovered cortisol BMD. In the second case, no reaction had occurred and cortisol BMD was recovered unchanged.

Further evidence for esterification at the C(21) hydroxyl group

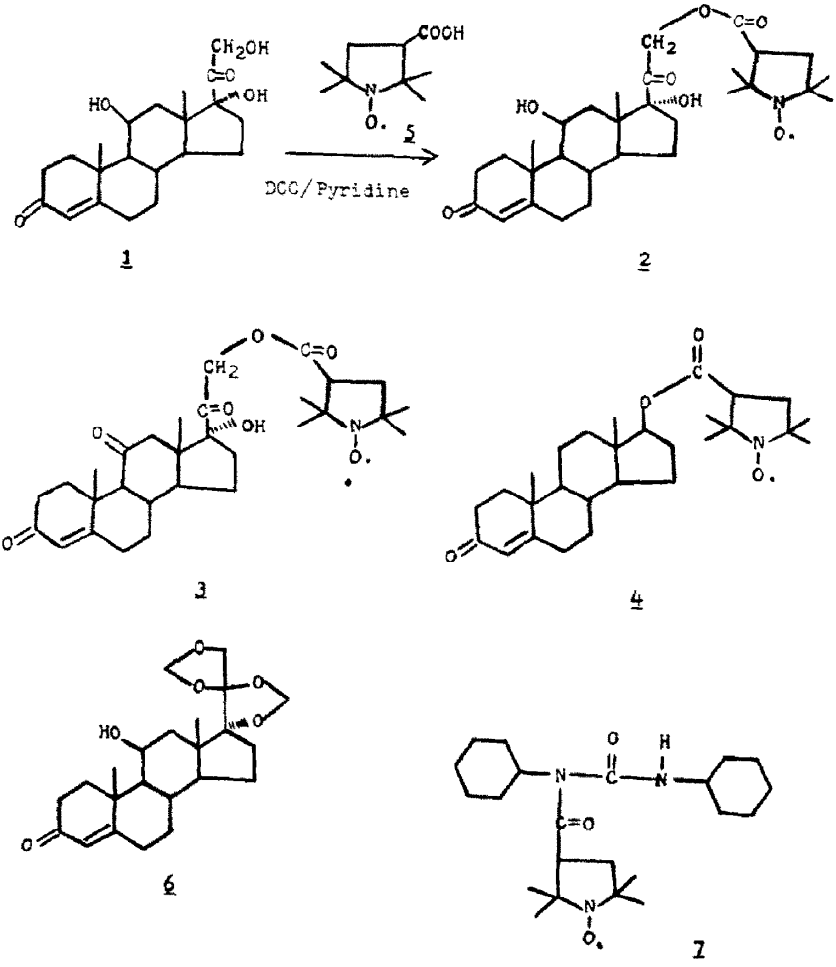

Chart 1. Synthesis of steroid spin labels

is advanced by the conversion of the cortisol ester 2 to the cortisone ester 3. Although nitroxide radicals are sensitive to many synthetically useful reagents, we nevertheless carried out the chromium trioxide-pyridine complex oxidation of the cortisol ester 2 with the hope of achieving only oxidation of the 11-hydroxyl group. This oxidation afforded a single product which was found to be identical (mp, mixed mp, IR, NMR, TLC, and Rf value) with the cortisone spin label ester 3 prepared directly (vide infra). The oxidation product exhibited IR absorption bands at 1743, 1730, 1710, and 1665 cm^{-1}. The appearance of an additional carbonyl absorption at 1710 cm^{-1} is in consistent with oxidation of the 11-hydroxyl group having taken place. The ESR spectrum indicated that the nitroxide moiety remained intact. On the basis of the above evidence, the steroidal ester formed in each condensation reaction is shown to be the 21-ester.

Condensation of cortisone with spin label acid 5 in the presence of DCC in dry pyridine yielded an ester in addition to the DCC adduct 7. The crude material upon chromatography gave the cortisone ester 3, which was purified by crystallization from acetone, mp 237°-238° (32 percent). Compound 3 exhibited IR absorption bands at 1743, 1730, 1710, and 1665 cm^{-1}. The NMR of this compound was recorded after reduction of the nitroxide moiety using phenylhydrazine in an NMR tube; the NMR spectrum exhibited signals for six methyl groups (δ 1.1. to 1.5) and one vinylproton (δ 5.75). The mass spectrum exhibited the molecular ion peak at m/e 528.

Similarly, reaction of testosterone with the spin label acid 5 in the presence of DCC in dry pyridine afforded mainly one compound which was purified by chromatography over silica gel followed by

fractional crystallization from acetone. The testosterone 17-ester 4 was isolated as light yellow crystals in 59 percent yield (mp 205°-206°). This compound exhibited IR bands at 1725, 1665, and 1185 cm^{-1}. The purified compound after reduction with phenylhydrazine in an NMR tube exhibited signals for six methyl groups (18H at δ 0.9, 1.05, 1.2, 1.25, and 1.4) and one vinyl proton (δ 5.75).

These new steroid spin labels should be useful for a variety of spin label studies.

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian EM-360 or JEOL-100 spectrometer using $CDCl_3$ as solvent and TMS as the internal standard. The NMR spectrum of each paramagnetic compound was recorded after its *in situ* reduction to the corresponding hydroxylamine compound in an NMR tube using 5 to 10 mg of phenylhydrazine. IR spectra were recorded on a Perkin-Elmer Model 257 grating infrared spectrophotometer. ESR spectra were recorded on a Varian E-104 spectrometer, and UV spectra were recorded on a Cary-14. Preparative thin layer chromatography (TLC) was carried out on Silicar TLC 7GF (Mallinckrodt) coated plates.

Cortisol 21-(2,2,5,5-tetramethylpyrrolidin-1-yloxy)-3-carboxylate (2)

A mixture of cortisol (1.35 g) and 0.7 g of 3-carboxyl-2,2,5,5-tetramethyl-1-pyrrolidin-1-yloxyl (5) in dry pyridine was evaporated to dryness three times using fresh portions of dry pyridine. The residue was taken up in dry pyridine (5 ml) and DCC (3.1 g) was added to it; the resulting mixture was shaken well and then kept at room temperature in a dry atmosphere for six days. The reaction mixture was cooled and water was added; the resulting mixture was then diluted with acetone and agitated well. The precipitated cyclohexyl urea was filtered off, and the remaining solution was then extracted with chloroform and ethyl acetate. The extract was dried, and the solvent was removed *in vacuo*. The crude material was chromatographed on a silica gel column (35 g, column length 16 cm). Elution with benzene and benzene-acetone (9:1) afforded the DCC adduct 7, (mp 179°-180°). Further elution with benzene-acetone (9:1 and 9:2) gave the cortisol ester 2 which was purified by crystallization from acetone to give a light yellow powder, m.p. 223°-224° (0.635 g, 35 percent).

NMR ($CDCl_3$): (δ 0.96, 1.08, 1.1, 1.14, 1.22 (s), 18H, 6 CH_3), 4.95 (s, 2H, O-CH_2), and 5.68 (s, 1H, vinyl H). IR ($CHCl_3$): 3420, 1743, 1727, 1668, and 1100 cm^{-1}. UV (ethanol): λ max 248-252 (ε 1550). ESR (THF): A_N 14.3 (10^{-3}M). MS m/e 530 (M+).

Anal. Calcd for $C_{30}H_{44}NO_7$; C, 67.90; H, 8.36; N, 2.74. Found: C, 67.79; H, 8.53; N, 2.64.

Oxidation of Cortisol-21-ester (2) to Cortisone-21-ester (3)

A solution of pyridine (2.0 ml) in CH_2Cl_2 (25 ml) was stirred at 18°-20°. Chromium trioxide (0.2g) was added to it slowly, and the mixture was stirred at room temperature for 15 minutes. A solution of cortisol ester 2 (130 mg in 10 ml of CH_2Cl_2) was added to the complex in one lot. The mixture was stirred at room temperature for one hour. The organic layer was decanted and the residue was washed with methylene chloride. The combined organic layer was washed with one percent NaOH (two times), 5 percent HCl (two times), 5 percent sodium bicarbonate (once), water and then dried. Removal of the solvent gave a crude product (115 mg) which was purified by preparative TLC on silica gel plates and developed in the solvent system benzene-ethanol (10:1). The major band was cut and eluted with $CHCl_3$-acetone. It was purified by crystallization from acetone: mp 236°-237° (0.093 g, 72 percent). A mixed melting point determination with authentic cortisone 21-ester (3) did not show any depression. This product was identical (TLC, NMR, IR, melting point) with the ester prepared from the condensation of cortisone and spin label acid 5 (vide infra).

Cortisone 21-(2,2,5,5-tetramethylpyrrolidin-1-yloxyl)-3-carboxylate (3)

A mixture of cortisone (0.72 g, 20 mmol) and 3-carboxyl-2,2,5,5-tetramethyl-pyrrolidin-1-yloxyl (5, 0.37 g, 20 mmol) in dry pyridine was evaporated to dryness three times using fresh portions of dry pyridine. The residue was taken up in dry pyridine (5 ml), and DCC (2.4 g, 0.1 mmol) was added to it. The reaction mixture was kept at room temperature in the absence of moisture for six days. The reaction was worked up as described in the case of (2). The crude product was chromatographed over silica gel. Elution of the column with benzene gave the DCC adduct 7 (0.31g). Further elution with benzene-acetone (20:1 and later 10:1) gave the cortisone ester (3) which was purified by crystallization from acetone: mp 237°-238°, 0.42 g, 37 percent.

NMR ($CDCl_3$): (δ 1.1, 1.15, 1.2, 1.4 (s, 18H, CH_3), 1.6-3 (m, CH_2, CH, and OH), 4.9 (bs, 2H, O=C-CH_2-O) and 5.7 (s, 1H, vinyl H). IR ($CHCl_3$): 3440 (OH), 1740, 1730, 1710, 1665 (C=O) and 1040 cm^{-1}. UV (ethanol): λ max 226 (ε 1600). ESR (THF): A_N 14.3 (10^{-3}M). MS m/e 528 (M+). Anal: Calcd for $C_{30}H_{42}NO_7$; C, 68.18; H, 7.95, N, 2.65. Found: C, 67.81; H, 8.26; N, 2.75.

Testosterone 17-(2,2,5,5-tetramethylpyrrolidin-1-yloxyl)-3-carboxylate (4)

A mixture of testosterone (0.86 g, 3 mmol and 3-carboxyl-2,2,5,5-tetramethyl-pyrrolidin-1-yloxyl (5, 0.51 g, 3 mmol) in pyridine was evaporated to dryness three times using fresh portions of dry pyridine. The residue was taken up in dry pyridine (5 ml), and DCC (1.03 g,

5 mmol) was added. The resulting mixture was kept at room temperature for 6 days and worked up as described in the case of (2). The crude product was chromatographed over silica gel (40g, column length 18cm). The column was eluted with benzene and benzene-chloroform (9:11 and 9:2). The benzene-chloroform eluant contained mainly testosterone ester. The solvent was removed and the crude product was crystallized from acetone to afford purified product 4 mp 205°-206°, 0.83 g, 59 percent.

NMR ($CDCl_3$): (δ 0.90, 1.05, 1.2, 1.25, 1.4 (s, 18H, CH_3), and 5.75 (s, 1H, vinyl H). IR ($CHCl_3$: 1735, 1665, and 1185 cm^{-1}. UV (ethanol); λ_{max} 238 (ϵ = 1200). ESR (THF): A_N 14.1 (10^{-3} M). Anal: Calcd for $C_{28}H_{42}NO_4$; C, 73.65; H, 9.27; N, 3.07. Found: C, 73.88; H, 9.36; N, 3.10.

Reaction of Cortisol BMD with Spin-Label Acid 5

A mixture of cortisol BMD (6, 0.42g) and spin label acid 5 (0.19g) in pyridine was evaporated three times using fresh portions of dry pyridine. The residue was taken up in dry pyridine (3 ml) and DCC (0.25g) was added to the solution. The resulting mixture was then kept at room temperature for five days and worked up as usual. The product was found to be a mixture of cortisol BMD and the DCC adduct 7, using TLC, IR, and HPLC.

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REFERENCES AND NOTES

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