Affinity-Labelling Corticoids I. Synthesis of 21-Chloroprogesterone, Deoxycorticosterone 21-(1-Imidazole) Carboxylate, 21-Deoxy-21-Chloro Dexamethasone, and Dexamethasone 21-Mesylate, 21-Bromoacetate, and 21-Iodoacetate.

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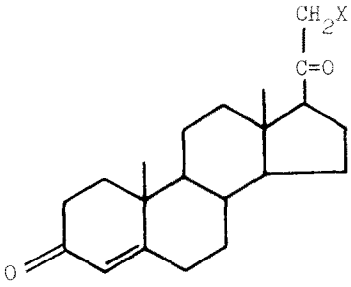
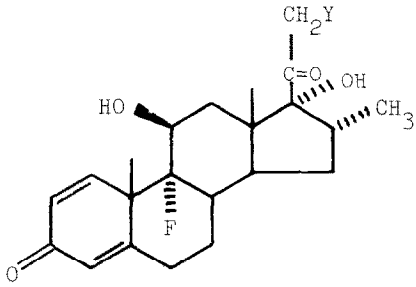
ABSTRACT

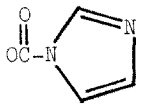
The efficient and unambiguous preparation of several C-21 substituted affinity-labelling corticoids are described. Included is an improved procedure for the preparation of 21-chloroprogesterone and the first reported synthesis of deoxycorticosterone 21-(1-imidazole) carboxylate. Dexamethasone derivatives prepared include separate and unambiguous synthesis of 21-deoxy-21-chlorodexamethasone and dexamethasone 21-mesylate, as well as the first reported synthesis of dexamethasone 21-bromoacetate and dexamethasone 21-iodoacetate.

A study has been undertaken to determine the molecular and chemical aspects of steroid hormone action mediated by the glucocorticoid receptor from lactating goat mammary tissue. These studies require the preparation of affinity-labelling steroid derivatives as potential candidates for covalent attachment to the steroid binding site. Suitable steroid derivatives are required to have high binding activity and contain a leaving group proximal to a proposed nucleophilic amino acid residue in or near the receptor binding site. Preliminary binding studies suggested dexamethasone (1) as the steroid of choice due to its high affinity for the cytosolic glucocorticoid receptor from lactating goat mammary tissue [2]. Additional evidence with the glucocorticoid receptor from other tissues suggested the possibility of a nucleophilic amino acid in the vicinity of the C-21 substituted glucocorticoids independently by Simons et al [3,4] and by ourselves. In this paper we report the synthesis and characterization of C-21 substituted derivatives of deoxycorticosterone (2) and of dexamethasone (1). Included is an improved procedure for the preparation of 21-chloroprogesterone (3),

and the first reported synthesis of deoxycorticosterone 21-(1-imidazole) carboxylate (4). Dexamethasone derivatives described herein include separate and unambiguous synthesis of 21-deoxy-21-chlorodexamethasone (5) [21-chloro-17,11β-dihydroxy-9-fluoro-16α-methyl-1,4-pregnadiene-3,20-dione], and dexamethasone 21-mesylate (6), as well as the first reported synthesis of dexamethasone 21-bromoacetate (7) and dexamethasone 21-iodoacetate (8).





	X
2	OH
3	Cl
4	

	Y
1	OH
5	Cl
6	OSO_2CH_3
7	$OCCH_2Br$
8	$OCCH_2I$

21-Chloroprogesterone (3) was initially prepared by the method of Counsell et al using methanesulfonyl chloride in pyridine, and in our hands the yield was only 27% (on a 0.3 mmol scale) after crystallization [5]. However, when dimethylformamide was used instead of pyridine the yield increased to 85%. This result parallels that of Evans et al,

who converted primary hydroxyl groups of pyranosides to the corresponding chlorides using similar conditions [6]. The other progesterone derivative, deoxycorticosterone 21-(1-imidazole) carboxylate (4), was prepared in excellent yield from deoxycorticosterone (2) using N,N'-carbonyldiimidazole and sodium imidazolide in tetrahydrofuran at room temperature. Both compounds 3 and 4 are expected to undergo nucleophilic displacements at C-21, from which additional 21-substituted progesterones can be prepared and used for receptor binding studies.

The preparation of analogous 21-dexamethasone derivatives was also undertaken, with special attention focused on achieving selective reaction at the C-21 hydroxyl group in the presence of other hydroxyl groups. 21-Deoxy-21-chlorodexamethasone (5) was first reported by Simons et al as a trace product in their synthesis of the 21-mesylate 6 [3]. We prepared chloride 5 by either of two methods both of which provide only chloride 5, uncontaminated by the mesylate 6. The yields of these reactions are not yet optimized. Thus using the procedure of Counsell et al [5], chloride 5 could be prepared directly from dexamethasone (1) or using pyridinium hydrochloride in dimethylformamide, mesylate 6 was converted to chloride 5. In our hands, mesylate 6 was prepared directly from dexamethasone (1) using methanesulfonyl chloride in pyridine. After recrystallization from acetone-hexane analytically pure mesylate 6 was obtained [3,7].

The 21-bromoacetate and 21-iodoacetate derivatives 7 and 8 were prepared by condensation of dexamethasone (1) with the respective haloacids using dicyclohexylcarbodiimide and pyridine in methylene chloride. The moderate yields (30-45%) obtained thus far are suggested to be a result of product decomposition during purification. These dexamethasone derivatives 7 and 8 are predicted to bind to the glucocorticoid receptor in a similar fashion to that found for cortisone 21-iodoacetate whose affinity-labelling to 20β-hydroxysteroid dehydrogenase has been studied by Warren et al [8].

Compounds 4-8 were fully characterized by ^{1}H NMR, IR, and low resolution chemical ionization or high resolution field desorption mass spectrometry. It is interesting to note that the ^{1}H NMR spectra of derivatives 4 and 5 show a non-equivalence of the C-21 protons

whereas the others (2-3, 6-8) show only a singlet for the C-21 protons. The HRFD (high resolution field desorption) spectra of compounds 4-8 gave the exact mass of the molecular ion.

EXPERIMENTAL

^1H NMR spectra were obtained on a Varian XL-100 spectrometer. Infrared spectra were recorded on a Perkin-Elmer 281 grating spectrometer. Low resolution chemical ionization mass spectra were determined on a Hewlett Packard 5985 GCMS. High resolution field desorption mass spectra were obtained in the mass spectrometry laboratory, School of Chemical Sciences, University of Illinois. The UV spectra were obtained on a Cary 118 UV spectrometer and the rotations measured on a Perkin Elmer 241 polarimeter. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Thin layer chromatography was performed on Merck pre-coated TLC plates (silica gel 60/Kieselguhr F 254) and visualized with I_2 or H_2SO_4 charring. All solvents were dried and distilled before use.

The following abbreviations are used. DMF = N,N-dimethylformamide, DCC = dicyclohexylcarbodiimide, THF = tetrahydrofuran, TLC = thin layer chromatography, and HRFD = high resolution field desorption.

21-Chloroprogesterone (3).

A solution of deoxycorticosterone (2) (701 mg, 2.1 mmol), methanesulfonyl chloride (485 mg, 4.2 mmol) and anhydrous DMF (5 ml) was heated at 65° under N_2 with stirring for 17 h. TLC indicated that the reaction was complete after 5 h. The mixture was cooled and evaporated *in vacuo* to give crude chloride 3 (665 mg, 91%) which was identical to that reported on the basis of TLC, IR, and ^1H NMR [5]. The crude product was recrystallized from acetone-ethanol to give pure chloride 3 as colorless crystals [550 mg, 71%, m.p. 202-205° (dec.)], lit. 201-203° (dec.) [5].

Deoxycorticosterone 21-(1-Imidazole) Carboxylate (4).

Imidazole (6.8 mg, 0.076 mmol) and Na (1.41 mg, 0.023 mmol) in THF (10 ml) were refluxed for 2 h and cooled to 25°. This mixture was added to a solution of deoxycorticosterone (2) (150.1 mg, 0.45 mmol) and N,N'-carbonyldiimidazole (76.7 mg, 0.47 mmol) in THF (15 ml). After stirring for 2 h at 25°, the mixture was evaporated *in vacuo*, the residue taken up in CH_2Cl_2 (40 ml) and washed successively with H_2O, 2.5% $KHCO_3$ and H_2O, and finally dried over Na_2SO_4. Evaporation of the CH_2Cl_2 afforded pure compound 4 (171.5 mg, 90%, m.p. 75-78°). Data for compound 4: R_f = 0.75 (6:4 benzene: ethyl acetate), ^1H NMR ($CDCl_3$) δ 0.79 (s, C-18), 1.28 (s, C-19), 4.66 - 4.90 (m, $-CH_2OCON$), 5.73 (s, C-4), 7.09 (s, C-4 of imidazole), 7.45 (s, C-5 of imidazole), and 8.18 (s, C-2 of imidazole); IR (Nujol) 1810 cm^{-1} ($O\underset{\delta}{C}-N$), 1720 ($C\underset{20}{=}O$), 1600 cm^{-1}, (Δ^4-3-ketone); MS (HRFD) M^+ calc. 424.2364, found 424.2370; UV (ethanol) λ max 240 nm, ε 23,000; $[\alpha]_D^{25}$ + 142° (c 0.001, ethanol).

21-Deoxy-21-Chlorodexamethasone (5) from dexamethasone (1).

To a solution of dexamethasone (1) (75 mg, 0.19 mmol) in pyridine

(0.8 ml) at 0° under N_2 was added methanesulfonyl chloride (39.6 mg, 0.29 mmol). After stirring 26 h at 0°, the reaction mixture was poured onto ice-H_2O (10 ml). The resulting brown residue was filtered and dissolved in ethanol. The ethanol solution was decolorized twice with Norite, evaporated *in vacuo* to give crude chloride 5. Recrystallization from CH_3OH-acetone three times afforded pure chloride 5 (29.9 mg, 35%): R_f = 0.58 (9:1 $CHCl_3$:CH_3CH_2OH); ^1H NMR (acetone-d_6) 0.86 (s, C-19), 1.28 (s, C-18), 1.60 (s, C-4); IR (KBr) 1718 cm^{-1} (C_{20}=O), 1660 cm^{-1} (Δ^4-3-ketone); MS (CI/CH_4) 451 (8%, M+$C_3H_5^+$), 439 (12%, M+$C_2H_5^+$), 411 (63%, M^+), 391 (100%, MH^+ - HF), 375 (79%, MH^+ - $2H_2O$), 373 (33%, MH^+ - H_2O - HF), 355 (87%, MH^+ - $2H_2O$ - HF); MS (CI, C_4H_{10}), 411 (55%, MH^+); UV (ethanol) λ max 238 nm, ε 17,500; $[\alpha]_D^{25}$ +97° (c 0.001, ethanol). Compound 5 from this procedure was identical to that prepared below on the basis of TLC, melting point, and all spectroscopic data.

21-Deoxy-21-Chlorodexamethasone (5) from mesylate 6.

A solution of mesylate 6 (11.3 mg, 0.024 mmol) in DMF (0.2 ml) was added to a solution of pyridinium hydrochloride (11.6 mg, 0.096 mmol) in DMF (0.3 ml) previously cooled to -60° under N_2. The resulting mixture was stirred for 3 additional h at -40 to -20°, while TLC indicated it to be complete after 1.5 h. The mixture was warmed to room temperature, allowed to stand overnight, evaporated to dryness, and added to ice-H_2O (0.5 ml). The resulting precipitate was filtered and dried to afford pure chloride 5 [3.9 mg, 40%, m.p. 243-247° (dec.)], (lit. 235.5-242°) [3]. Data for compound 5: R_f = 0.82 (2:3 benzene: ethyl acetate); NMR, IR, UV, $[\alpha]_D^{25}$ same as above; MS (HRFD)M^+ calc. 410.1661 found 410.1667.

Dexamethasone 21-Mesylate (6) [7].

To a solution of dexamethasone (1) (52.8 mg, 0.13 mmol) in pyridine (1 ml) at 0° under N_2 was added methanesulfonyl chloride (31.2 mg, 0.27 mmol). After stirring for 12 h at -4° under N_2, ice-H_2O (20 ml) was added. The precipitate was filtered, washed with H_2O, and dried to give crude mesylate 6 as a colorless crystalline solid [43.2 mg, 67%, m.p. 212-214° (dec.)]. Recrystallization from acetone-cyclohexane gave pure mesylate 6 [39.0 mg, 60%, m.p. 225-228°, (dec.)], lit. 231-232° (dec., as THF solvate) [3]. Data for compound 6: R_f = 0.68 (2:3 benzene:ethyl acetate); ^1H NMR (pyridine-d_5) δ 1.07 (s, C-16 CH_3), 4.91 (s, $CH_2OSO_2CH_3$), 5.64 (s, C-4); IR (Nujol) 3580, 3400 cm^{-1} (OH), 1730 cm^{-1} (C_{20}=O), 1660 cm^{-1} (Δ^4-3-ketone), 1620 cm^{-1} (C=C), 1110 cm^{-1} (OSO_2CH_3); MS (HRFD) M^+ calc. 470.1775 found 470.1742; UV (ethanol) λ max 238 nm, ε 17,500; $[\alpha]_D^{25}$ + 89° (c 0.001, ethanol).

Dexamethasone 21-Bromoacetate (7).

To a solution of dexamethasone (1) (98.2 mg, 0.25 mmol) in CH_2Cl_2 (15 ml) at 0° under N_2 was added successively bromoacetic acid (85 mg, 0.6 mmol) in CH_2Cl_2 (2 ml), pyridine (62.2 mg, 0.75 mmol) in CH_2Cl_2 (1 ml) and DCC (136.8 mg, 0.65 mmol) in CH_2Cl_2 (2 ml). The reaction mixture was stirred at 0° for 2 h then at room temperature for 1 h. The unreacted DCC was quenched by addition of 0.04 ml glacial acid and stirred for 10 min. After evaporation *in vacuo* the solid residue was stirred with acetone (10 ml) and dicyclohexylurea filtered off. The

filtrate was poured into ice-H_2O (20 ml) and the resulting precipitate was filtered and dried to give crude bromoacetate 7 as a colorless crystalline solid (61.2 mg, 48%, m.p. 152-155°). Recrystallization twice from ethanol afforded pure bromoacetate 7 (56.3 mg, 44%, m.p. 151-154°). Data for compound 7: R_f = 0.74 (9:1 benzene:CH_3CH_2OH); ^1H NMR (acetone-d_6) δ 1.04 (s, C-16), 1.28 (s, C-18), 1.39 (s, C-19), 2.96 (m, CH_2O), 4.15 (s, $OCOCH_2Br$), 6.00 (s, C-4). IR (KBr): 3380 cm^{-1} (OH), 1748 cm^{-1} ($OCOCH_2Br$), 1718 cm^{-1} ($C\overline{\underline{=}}O$), 1655 cm^{-1} (Δ^4-3-ketone); MS (HRFD) M^+ calc. 512.1210, found 512.1206, MH^+ calc. 513.1210, found 513.1254; UV (ethanol) λ max 238 nm, ε 19,200; $[\alpha]_D^{25}$ +64° (c 0.001, ethanol).

Dexamethasone 21-Iodoacetate (8).

The above procedure was followed in which dexamethasone (1) was treated with iodoacetate acid to give crude iodoacetate 8 as a colorless crystalline solid (23.1 mg from 40.3 mg 1, 45%). Recrystallization once from ethanol afforded pure iodoacetate 8 (17.1 mg, 30%, m.p. 132-135°). Data for compound 8: R_f = 0.65 (9:1 $CHCl_3$:CH_3OH); ^1H NMR (acetone-d_6) δ 1.04 (s, C-16), 1.29 (s, C-18), 1.39 (s, C-19), 2.96 (m, CH_2O), 3.93 (s, $OCOCH_2I$), 6.00 (s, C-4). IR (KBr): 3450 cm^{-1} (OH), 1740 cm^{-1} ($OCOCH_2I$), 1700 ($C\overline{\underline{=}}O$), 1655 cm^{-1} (Δ^4-3-ketone); MS (HRFD) M^+ calc. 561.1072 found 561.1135; UV (ethanol) λ max 242 nm, ε 19,200; $[\alpha]_D^{25}$ +64° (c 0.001, ethanol).

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