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Corticosteroid Decomposition via a Mixed Anhydride

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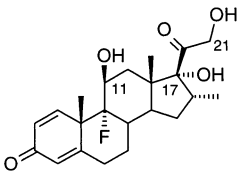
Abstract: Oxidation of dexamethasone in an aqueous suspension by air during prolonged storage produces the 17α-formyloxy-17*β*-carboxylic acid **4**. A pathway to **4** is proposed that involves Baeyer−Villiger-type oxidation of keto aldehyde **1** to mixed anhydride **5**, followed by intramolecular formyl transfer. Synthetically, acid **3** was reacted with *N*,*N*′-carbonyldiimidazole followed by triethylammonium formate in order to generate the transient anhydride **5** en route to an authentic sample of **4**.

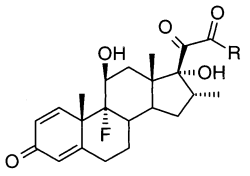
In the course of HPLC/MS assay of expired lots of Maxidex (0.1% dexamethasone ophthalmic suspension USP), we detected a single MW 406 component, denoted Degradant F, having relative retention time (RRT) of 0.82 (dexamethasone = 1). We had identified an accompanying degradant of MW 390 as keto aldehyde **1**[1] in accordance with a longstanding precedent.[2] We surmised that Degradant F was keto acid **2**[3] and set out to synthesize an authentic sample.

Keto aldehyde **1** was prepared by Cu^{II}-catalyzed air oxidation of dexamethasone in methanol.[1,4] The product was obtained largely in the form of the methyl hemiacetal.[4] By HPLC/MS, the $[MH]^+$ ion produced from free **1** was observed and matched that of the MW 390 degradant. To prepare keto acid **2**, we oxidized a sample of **1** using Ag_2O suspended in 0.01 N NaOH[5] with THF added as a cosolvent. A mixture of two MW 406 compounds was obtained in low yield: A (RRT = 0.82), which matched Degradant F, and B (0.92). A small amount of acid **3**[6] (0.76) was also identified in this mixture, and in the degradant mixture as well. Sodium chlorite[7,8] proved to be superior for the oxidation of **1** to **2**. The product was easily purified, by virtue of its relatively low pK_a, by aqueous−organic partitioning at pH 5. Unexpectedly, **2** matched compound B.

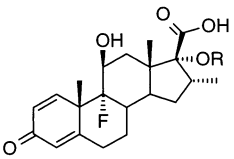
Upon review of the ^{1}H NMR spectrum of the Ag_2O product mixture, we took note of a singlet at δ 8.2. We



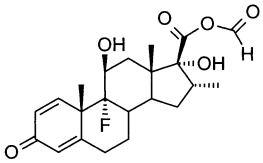
Dexamethasone
MW 392



1: R = H
2: R = OH



3: R = H
4: R = CHO



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inferred that compound A, thus presumably Degradant F, was a formate of **3**. We envisioned C-21 as the source of the formyl group, via Baeyer−Villiger-type oxidation of **1** to the mixed anhydride **5** followed by isomerization to the 17-formate **4**. Relevant to the hypothesized oxidation **1**→**5**, the major products of the reaction of methylglyoxal with H_2O_2 in buffered neutral aqueous solution are formate and acetate.[9] The proposed acyl transfer **5**→**4** is precedented generally as a tactic for esterifying an otherwise resistant 17α-OH group by means of conventional acylating agents in nonaqueous media.[4a,6,10,11]

To prepare a sample of **4**, we devised a complementary approach to the transient anhydride **5** by activating the carboxyl group of **3** and then introducing the formate ion. Accordingly, **3** was reacted in THF solution with 1.1 equiv of *N*,*N*′-carbonyldiimidazole (CDI).[10,11] After CO_2 evolution ceased, 3 equiv of 96% formic acid was added, followed by 3 equiv of Et_3N. Aqueous workup afforded, in 100% yield, an 88:12 mixture of **4** (RRT = 0.82) and **3**. Recrystallization from aqueous EtOH afforded, in 65% yield, **4** of 98% purity. Use of 1.5 equiv of CDI decreased the proportion of **3** to 7% but also gave rise to 5% of the 11,17-diformate, which apparently resulted from the action of *N*-formylimidazole and which was not removed by recrystallization. In an experiment where Et_3N was omitted, HPLC/MS showed slow formation of **4** plus a lipophilic isomer presumed to be **5**, which was not otherwise observed.

The structures of synthetic **2** and **4** were confirmed by a comprehensive series of NMR experiments. Complete assignment of ^{1}H signals was made with the aid of COSY and HETCOR spectra. In **2**, the COOH and 17-OH signals are very broad due to intramolecular exchange. The ^{1}H assignments led to ^{13}C assignments for proton-attached carbons by HETCOR. Quaternary carbon signals were assigned with a long-range HETCOR spectrum.

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The characteristically large coupling constant, $J = 231$ Hz, between the formate C and H of **4** was measured from the ^{13}C satellites of the well-isolated proton signal and is reflected in the DEPT spectra as well. A correlation between C-17 and the formate H was also observed in the long-range HETCOR spectrum.

Characterization of synthetic **4** by HPLC/MS using atmospheric pressure chemical ionization (APCI) yielded $[MH]^+$ at *m*/*z* 407 as the base peak. Under negative ion conditions, the base peak occurred at *m*/*z* 405, consistent with the presence of the carboxylate function. The positive ion MS^2 (MS/MS) spectrum obtained following collisionally induced dissociation (CID) of the 407 ion was dominated by an *m*/*z* 387 fragment, indicating the facile loss of HF. Subsequent CID of the 387 ion yielded a key MS^3 spectrum with structurally informative fragments at 369 ($-H_2O$), 341 ($-$HCOOH), 323 ($-$HCOOH $-$ H_2O), 313 ($-$HCOOH $-$ CO), and 295 ($-$HCOOH $-$ H_2O $-$ CO). Through a comprehensive series of MS^n experiments, it was confirmed that the 323, 313, and 295 ions are products of multiple eliminations.

Characterization of Degradant F by HPLC/MS^n yielded spectra indistinguishable from those of **4** and distinct from those of **2**. When a sample of Degradant F (1 mL HPLC fraction) was treated with 2−3 mg of sodium carbonate and allowed to stand at 23 °C for several hours, it underwent conversion to **3**. A sample of synthetic **4** behaved likewise.

These observations provide compelling evidence for a decomposition pathway leading from dexamethasone to formate **4** via the generation and isomerization of mixed anhydride **5** in an aqueous medium. The concurrent formation of **3**, an established degradant type,[2,3a] can be explained as the consequence of competing intermolecular reaction of **5** with water or other nucleophilic components in solution. These results bear upon the oxidative behavior of corticosteroids in general and reasonably extend to the doxorubicin group of antitumor antibiotics and to other substances having a similarly constituted side chain.

Experimental Section

General Methods. HPLC analyses were performed using a Waters Symmetry C8 column (3.9 × 150 mm) with a mobile phase of 10 mM NH_4OAc in H_2O/MeOH (7:3 to 2:8). Excitation energies in CID/MS^n experiments were selected manually to reduce the selected ion intensity to 1−10% of the base fragment intensity. NMR spectra were obtained at 600 MHz (^1H) and 150 MHz (^{13}C). Melting points are uncorrected. Elemental analyses were performed by Atlantic Microlab, Norcross, GA.

11*β*,17α-Dihydroxy-9α-fluoro-16α-methyl-20-oxo-1,4-pregnadien-21-al (1)[1] Methyl Hemiacetal. The method of Lewbart and Mattox was used[4] with a modified workup procedure. $Cu(OAc)_2 \cdot H_2O$ (0.24 g, 1.20 mmol) was added to a suspension of dexamethasone (6.00 g, 15.3 mmol) in MeOH (120 mL). The mixture was stirred vigorously, open to the air, giving at first a purple-blue color. After 1.5 h, the clear blue-green solution was diluted with 240 mL of EtOAc and filtered through a Florisil pad, eluting with another 240 mL of EtOAc. Concentration in vacuo afforded 6.76 g of **1** as a foam. ^1H NMR ($CDCl_3$) showed 10% of the aldehyde (δ 9.5, s, 1H) and 70% of the methyl hemiacetal (δ 3.5, two s, 3H): HPLC/MS (APCI) *m*/*z* 391 (MH^+ of aldehyde, major), 423 (MH^+ of methyl hemiacetal, minor). This material was used in the following experiment without purification.

11*β*,17α-Dihydroxy-9α-fluoro-16α-methyl-20-oxo-1,4-pregnadien-21-oic Acid (2). To a solution of **1** (methyl hemiacetal, 5.80 g, 13.7 mmol) in 45 mL of *t*-BuOH was added a 2.0 M solution of 2-methyl-2-butene in THF (45 mL, 90 mmol) and 30 mL of saturated aqueous KH_2PO_4 (pH 4.5). A solution of $NaClO_2$ (80%, 1.74 g, 15.5 mmol) in 15 mL of water was added, and the two-phase mixture was stirred rapidly. Within 5 min, the temperature had increased from 25 to 40 °C. A water bath was applied to cool the mixture to 25 °C. After 16 h, the mixture was diluted with EtOAc and extracted with water (three times). (The organic solution retains any unreacted **1** plus side products, including ca. 5% each of **3** and **4**.) The combined aqueous solution (pH 5) was acidified to pH 2 with 1 M aqueous $NaHSO_4$. The precipitate was extracted into EtOAc, and this solution was washed twice with water and brine, dried ($MgSO_4$), filtered, and concentrated. The product was triturated with 25 mL of EtOAc yielding a white solid, which was dried in vacuo to give 2.90 g (52%) of **2**: mp 204−206 °C dec; $[\alpha]^{23}_D$ +66.0° (*c* 0.46, MeOH); IR (KBr) ν 3600 (s), 3520 (s), 3455 (s), 2950, 1748, 1709, 1658, 1596, 1578, 1390, 895 cm^{-1}; ^1H and ^{13}C NMR spectra are tabulated in Supporting Information; HPLC/MS (APCI) *m*/*z* 407 (MH^+). Compound **2** retained 15 mol % EtOAc (by ^1H NMR) after extended drying in vacuo at 50 °C. Anal. Calcd for $C_{22}H_{27}O_6F \cdot 0.15$ EtOAc: C, 64.68; H, 6.77. Found: C, 64.56; H, 6.78; unchanged after further drying in vacuo for 24 h at 100 °C.

11*β*,17α-Dihydroxy-9α-fluoro-16α-methyl-1,4-androstadien-17*β*-carboxylic Acid (3).[6] H_5IO_6 (3.92 g, 17.2 mmol) was added to a stirred suspension of dexamethasone (6.00 g, 15.3 mmol) in 60 mL of EtOH and 24 mL of water. After 30 min, a clear solution resulted, which was stirred for an additional 16 h, forming a white solid. Water (300 mL) was added, and stirring was continued for 1 h. The suspension was filtered, and the solid was washed well with water and dried in vacuo at 70 °C to yield 5.62 g (97%) of **3**: mp 274−277 °C dec (lit.[6] >258 °C dec), $[\alpha]^{23}_D$ +52.4° (*c* 0.48, MeOH); lit.[6] $[\alpha]_D$ +46.6° (dioxane); IR (KBr) ν 3534 (s), 3000−2300 (br), 1689, 1655, 1598, 1264 cm^{-1}; ^1H and ^{13}C NMR spectra are tabulated in Supporting Information; HPLC/MS (APCI) *m*/*z* 379 (MH^+). Anal. Calcd for $C_{21}H_{27}O_5F$: C, 66.64; H, 7.19. Found: C, 66.67; H, 7.29.

17α-Formyloxy-11*β*-hydroxy-9α-fluoro-16α-methyl-1,4-androstadien-17*β*-carboxylic Acid (4). CDI (97% pure by ^1H NMR, 5.05 g, 30 mmol) was added over 5 min to a stirred solution of **3** (10.4 g, 27.5 mmol) in anhydrous THF (64 mL) at 20−25 °C under N_2 (CO_2 evolved). After 1 h, the solution was cooled in ice, and formic acid (96%, 4% water, 3.2 mL, 82 mmol) was added at <15 °C. Triethylamine (11.6 mL, 83 mmol) was then added at <25 °C. The solution was stirred at 25 °C for 16 h. Water (800 mL) was added, and the solution (pH 8.5) was washed with EtOAc (discarded), acidified to pH 3 with 150 mL of 1 M aqueous $NaHSO_4$, and extracted twice with EtOAc. These extracts were combined and washed with water and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo to give 11.1 g (100%) of an 88:12 mixture of **4** and **3**. This material was dissolved in 110 mL of boiling 2:1 (v/v) EtOH−H_2O. The solution was allowed to cool to 25 °C, and seed crystals of **4** (10 mg) were added to initiate crystallization. Crystallization was completed at −10 °C. The solid was collected by filtration and dried in vacuo at 50 °C to afford 7.25 g (65%) of **4**: mp 228−232 °C dec; $[\alpha]^{23}_D$ +27.6° (*c* 0.47, MeOH); IR (KBr) ν 3500−2400 (br), 1741, 1707, 1663, 1596, 1225, 1202 cm^{-1}; ^1H and ^{13}C NMR spectra are tabulated in Supporting Information; HPLC assay (dried basis) **4** 98.5%, **3** 1.5%; HPLC/MS (APCI) *m*/*z* 407 (MH^+). Compound **4** proved to be hygroscopic, as varying microanalytical values indicated hydration ranging up to 2 mol of H_2O per mol of **4**. A sample was dried in vacuo for 24 h at 100 °C. Anal. Calcd for $C_{22}H_{27}O_6F$: C, 65.01; H, 6.70. Found: C: 64.57; H, 6.79.

Supporting Information Available: Tabulated ^1H and ^{13}C NMR spectra of **2**−**4** with peak assignments and MS^3 of **4** and Degradant F. This material is available free of charge via the Internet at http://pubs.acs.org.

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