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Air oxidation of 17-hydroxycorticosteroids catalyzed by cupric acetate: formation of hemiacetal dimers

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Abstract

Hydrocortisone, cortexolone, hydrocortisone-17-butyrate, and budesonide were oxidized into α-ketoaldehydes by air exposure in the presence of $Cu(OAc)_2$. When free hydroxyl functions were present at position 17, hydrocortisone and cortexolone, the formed oxidation products, were identified as hemiacetal dimeric structures involving the free hydroxyl functions at position 17 and the newly formed aldehydes at position 21. Dimeric structures were established by using $^1H\{^{13}C\}$ correlations (HSQC and HMBC) and ^1H–^1H correlations (COSY and ROESY). The hemiacetal function was further confirmed by reaction of the dimer formed from hydrocortisone with two equivalents of 3-methyl-2-benzotriazolinone hydrazine (MTBH), giving quantitatively two equivalents of the 3-methyl-2-benzotriazolinone hydrazone of 21-dehydrohydrocortisone. When no free hydroxyl function was present as in the case of hydrocortisone-17-butyrate and budesonide, the expected α-ketoaldehydes were obtained.
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Keywords: Corticosteroids; Hydrocortisone; Air oxidation; Cupric acetate; α-Ketoaldehyde; Cyclic hemiacetal

1. Introduction

Due to their strong anti-inflammatory and immunosuppressive properties, corticosteroids are used for many therapeutic indications. Despite their beneficial effects, corticosteroids applied topically have also been associated with allergic contact dermatitis (ACD) reactions [1,2]. The prevalence of corticosteroid related ACD in eczematous patients has been estimated between 3 and 6% in northern European countries where the use of topical corticosteroids is high [1]. This side effect, which can be severe, results from the formation of reactive intermediates during skin metabolism, which are able to modify the side chain of nucleophilic amino acids, and therefore, lead to the formation of modified proteins recognized as foreign by the skin immune system [3]. In this respect, it has been suggested that corticosteroid molecules could be oxidized into 21-dehydro derivatives and that these α-ketoaldehydes, which are potentially strong electrophiles toward nucleophilic amino acids, such as arginine [4,5], could lead to the formation of modified proteins [3]. The processing of these modified proteins by Langerhans cells, the main antigen presenting cells of the epidermis, results in the selection and activation of T-lymphocytes with an appropriate T-cell receptor (TcR) able to recognize peptidic sequences modified by the drug metabolite or hapten [6]. This hypothesis was supported by several metabolism, biochemical, and clinical studies showing that these metabolites, even minor, could be formed in the skin and be involved in the skin sensitization process [7–9].

In order to investigate this hypothesis and try to better characterize interaction mechanisms of 21-dehydrocorticosteroids with proteins, we have oxidized different corticosteroids, such as hydrocortisone (**1**), cortexolone (**2**), hydrocortisone-17-butyrate (**3**), and budesonide (**4**) (Fig. 1), into their 21-dehydro derivatives. The classical oxidation of such molecules, as reported in the literature, is based on air exposure in the presence of cupric acetate, leading smoothly to α-ketoaldehydes in good yields [10]. Nevertheless, these intermediates have been poorly characterized, and we have therefore reinvestigated this reaction. We now report our results, which showed that when a free hydroxyl group was present at position 17 (hydrocortisone and cortexolone), oxidation products could not be isolated as α-ketoaldehydes, but as hemiacetal dimeric structures.

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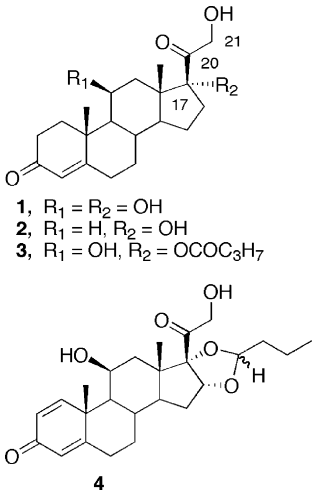


Fig. 1. Chemical structures of hydrocortisone (**1**), cortexolone (**2**), hydrocortisone-17-butyrate (**3**), and budesonide (**4**).

2. Experimental

Caution: Skin contact with 21-dehydrocortisone derivatives must be avoided. As potential sensitizing substances, these compounds must be handled with care.

2.1. Chemistry

^{1}H and ^{13}C NMR spectra were recorded on Bruker AC 200 or ARX 500-MHz spectrometers in CD_3OD unless otherwise specified. Chemical shifts are reported in ppm (δ) with respect to TMS, and $CHCl_3$ was used as an internal standard ($\delta = 7.26$ ppm). Multiplicities are indicated by s (singlet), d (doublet), t (triplet), and m (multiplet). Infrared spectra were obtained on a Perkin–Elmer FT-IR 1600 spectrometer; peaks are reported in reciprocal centimeters. Ultraviolet spectra were determined on a Uvikon 810 spectrometer, and mass spectra (FAB−) were obtained using a ZAB-HF mass spectrometer. Melting points were determined on a Buchi Tottoli 510 apparatus and are uncorrected. Dried solvents were freshly distilled before use. Methylene chloride was dried over P_2O_5 before distillation. All air- or moisture-sensitive reactions were conducted in flame-dried glassware under an atmosphere of dry argon. Chromatographic purifications were conducted on silica gel columns according to the flash chromatography technique.

2.2. Dimer of 21-dehydrohydrocortisone (5)

$Cu(OAc)_2$ (0.09 g, 0.5 mmol, 0.5 equiv.) in methanol (38 ml) was added to a solution of hydrocortisone (**1**) (0.35 g, 0.95 mmol) in methanol (22 ml). The reaction mixture was stirred under an air atmosphere for 5 h, hydrolyzed with a saturated solution of NH_4Cl (30 ml), and extracted with CH_2Cl_2 (4 × 30 ml). Combined organic layers were dried over Na_2SO_4, filtered, concentrated under vacuum, and the crude product was purified by column chromatography on silica (dichloromethane 96%, ethanol 3.5%, eau 0.6%) to give (**5**) (0.33 g, 0.92 mmol, 97% yield). Yellow solid m.p. 100–102 °C. The 500 MHz proton and the 125 MHz carbon NMR data are listed in Table 1. IR ($CHCl_3$, cm^{-1}) ν: 3685 (OH); 3605 (OH); 1721 (C=O); 1662 (C=O); 1613 (C=C). UV λ_{max} 242 nm (ε 29860 in MeOH). $[\alpha]_D = +110$ (c 2.0, MeOH). Mass spectra (FAB−) m/z 719 ($2M^+-1$), 360 (M^+).

MTBH derivative: yellow solid m.p. 160–162 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.89 (s, 3H, H_{18}), 1.04–1.25 (m, 4H), 1.43 (s, 3H, H_{19}), 1.59–2.47 (m, 13H), 2.90 (m, 1H, $H_{16\beta}$), 3.60 (s, 3H, $H_{5'}$), 4.47 (m, 1H, $H_{11\alpha}$), 5.57 (s, 1H, OH_{17}), 5.68 (s, 1H, H_4), 7.19 (dd, 1H, $J = 7.6$ Hz, $H_{2'}$ or $H_{3'}$), 7.21 (dd, 1H, $J = 7.6$ Hz, $H_{2'}$ or $H_{3'}$), 7.39 (dd, 1H, $J = 7.6$ Hz, $H_{1'}$ or $H_{4'}$), 7.51 (dd, 1H, $J = 7.6$ Hz, $H_{1'}$ or $H_{4'}$). ^{13}C NMR (50 MHz, CD_3OD) δ: 18.2, 20.9, 24.0, 31.6, 31.8, 32.2, 32.9, 33.9, 35.0, 39.3, 41.7, 47.3, 51.8, 55.9, 68.8, 92.9, 110.7, 122.3, 122.6, 123.6, 123.8, 127.2, 140.5, 150.7, 171.9, 172.3, 197.7, 199.5, 206.9. IR ($CHCl_3$, cm^{-1}) ν: 3400 (OH); 1662 (C=O); 1503 (C=N). UV λ_{max} 379 nm (ε 1270 in EtOH). $[\alpha]_D = +27$ (c 0.6, $CHCl_3$).

2.3. Dimer of 21-dehydro-cortexolone (6)

The same procedure as for the synthesis of (**5**) was used, except starting from cortexolone (**2**) (0.056 g, 0.016 mmol) to give (**6**) (0.05 g, 0.08 mmol, quantitative yield). Solid m.p. 110–112 °C. ^{1}H NMR (200 MHz, CD_3OD) δ: 0.65 (bs, 6H, H_{18} and $H_{18'}$), 0.89–1.13 (m, 4H), 1.19 (s, 6H, $H_{19'}$ and $H_{19'}$), 1.24–2.73 (m, 32H), 5.11 (s, 1H, H_{21} or $H_{21'}$), 5.23 (s, 1H, H_{21} or $H_{21'}$), 5.68 (bs, 2H, H_4 and $H_{4'}$). ^{13}C NMR (50 MHz, CD_3OD) δ: 15.1, 15.4, 17.7, 21.8, 24.5, 31.7, 33.5, 34.0, 34.4, 34.7, 35.3, 36.9, 37.0, 40.0, 51.6, 51.9, 55.0, 89.9, 93.8, 94.5, 175.1, 202.3, 207.5. IR ($CHCl_3$, cm^{-1}) ν: 3508 (OH), 1720 (C=O), 1663 (C=O). $[\alpha]_D = +102$ (c 0.4, MeOH). Mass spectra (FAB−) m/z 687 ($2M^+ - 1$), 344 (M^+).

2.4. 21-dehydrohydrocortisone-17-butyrate (7)

The same procedure as for the synthesis of (**6**) was used, except starting from the dehydrocortisone-17-butyrate (**3**) (0.91 g, 2.10 mmol) and $Cu(OAc)_2$ (0.43 g, 2.37 mmol, 1.1 equiv.) in ethanol (200 ml) to give (**7**) (0.90 g, 2.10 mmol, quantitative yield). Solid m.p. 181–183 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.92 (t, 3H, $J = 7.5$ Hz, $H_{4'}$), 0.92 (s, 3H, H_{18}), 0.96–1.27 (m, 2H, NA), 1.44 (s, 3H, H_{19}), 1.60 (qt, 2H, $J_1 = J_2 = 7.5$ Hz, $H_{3'}$), 1.71–2.21 (m, 14H, NA), 2.27 (t, 2H, $J = 7.5$ Hz, $H_{2'}$), 2.38–2.60 (m, 1H, NA), 3.07 (m, 1H, $H_{16\beta}$), 4.50 (m, 1H, $H_{11\alpha}$), 5.69 (s, 1H, H_4), 9.17 (s, 1H, H_{21}). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 13.6, 17.4, 18.1, 21.0, 23.9, 31.6, 32.0, 32.7, 33.2, 33.8, 35.0, 36.2, 39.2, 41.2, 47.5, 53.6, 55.7, 68.3, 94.0, 122.5, 171.7, 174.8, 187.0, 199.5, 201.6. IR ($CHCl_3$, cm^{-1}) ν: 3460 (OH), 1739 (C=O), 1716 (C=O), 1662 (C=O). $[\alpha]_D = +66$ (c 1.0, $CHCl_3$).

Table 1
^{1}H and ^{13}C NMR data of the dimeric 21-dehydrohydrocortisone (**5**)

Atom	δ ^{13}C[a]	m[b]		δ ^{1}H[c]
CH2-1	35.8	4	2.20 m	1.86 ddd (4.3/13.7/13.7)
CH_2-2	34.3	4	2.50 ddd (13.7/13.7/16.8)	2.30 ddd (4.3/4.3/16.8)
C-3	202.0	0		
CH-4	122.5	2		5.65 bs
C-5	176.6	0		
CH_2-6	33.3	4	2.55 ddd (1.7/5.4/14.2)	2.25 dddd (1.7/4.3/14.2/14.2)
CH_2-7	34.1	4	2.05 m	1.10 m
CH-8	32.9	2		2.05 m
CH-9	57.5	2		0.98 dd (2.3/11.1)
C-10	40.7/40.9	0		
CH-11	68.7/68.8	2		4.40 bs
CH_2-12	40.7/40.8	2	1.96 dd (3.5/13.9)	1.77 m
CH_2-12′	40.7/40.8	2	1.96 dd (3.5/13.9)	1.68 dd (2.6/13.7)
C-13	49.8	0		
CH-14	53.2/53.2	2		1.77 m
CH_2-15	24.6	4	1.77 m	1.38 ddd (3.9/5.9/11.6)
CH_2-16	34.6	4	2.67 m	1.51 ddd (5.9/8.9/14.8)
C-17	89.9/90.0	0		
CH_3-18	17.6/17.9	3		0.89 s
CH_3-18′	17.6/17.9	3		0.91 s
CH_3-19	21.5	6		1.47 s
C-20	207.3/207.6	0		
CH-21	93.8/94.6	1		5.17 s
CH-21′	93.8/94.6	1		5.27 s

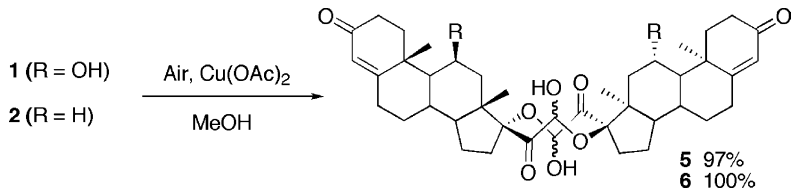
[a] Based on $^{1}H\{^{13}C\}$ correlation experiments.
[b] Multiplicity deduced from DEPT 135 experiments.
[c] Based on $^{1}H\{^{13}C\}$ and ^{1}H–^{1}H COSY correlation experiments.

2.5. 21-dehydrobudesonide (8)

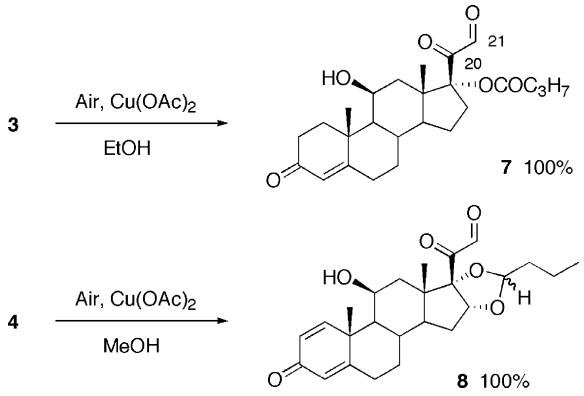
The same procedure as for the synthesis of (**6**) was used, except starting from the budesonide (**4**) (0.11 g, 0.25 mmol, mixture 50/50 of diastereomers) to give (**8**) (0.98 g, 0.23 mmol, 92% yield) as a mixture of diastereomers. Solid m.p. 81–85 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.87–1.02 (m, 12H, 2 × H_{18} et 2 × $H_{5'}$), 1.07–1.36 (m, 6H, NA); 1.44 (s, 6H, 2 × H_{19}); 1.52–2.16 (m, 28H, NA), 2.57 (m, 4H, NA), 3.33 (dd, 2H, $J_1 = 9.7$ Hz, $J_2 = 3.3$ Hz, 2 × $H_{1'}$), 4.46 (s, 2H, 2 × $H_{11\alpha}$), 5.46 (m, 2H, 2 × $OH_{11\beta}$), 6.03 (s, 2H, 2 × H_4), 6.27 (d, 2H, $J = 9.95$ Hz, 2 × H_2), 7.27 (d, 2H, $J = 9.95$ Hz, 2 × H_1), 9.51 (s, 1H, H_{21}), 9.54 (s, 1H, H_{21}). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 14.0 (2C), 17.2 (2C), 21.0 (2C), 30.5, 31.2, 31.9 (2C), 32.9 (2C), 33.3 (2C), 34.1 (2C), 35.0 (2C), 37.1 (2C), 41.3 (2C), 44.1 (2C), 55.3 (2C), 65.0 (2C), 70.1 (2C), 83.1, 84.0, 97.4, 98.2, 104.8 (2C), 108.5 (2C), 122.4 (2C), 127.8 (2C), 156.5 (2C), 170.2 (2C), 186.8 (2C), 190.1 (2C), 199.9 (2C). IR ($CHCl_3$, cm^{-1}) ν: 3504 (OH), 1723 (C=O), 1660 (C=O). $[\alpha]_D = +82$ (c 1.3, $CHCl_3$).

3. Results

Hydrocortisone (**1**), cortexolone (**2**), hydrocortisone-17-butyrate (**3**), and budesonide (**4**) were oxidized in very high yields into their expected α-ketoaldehyde derivatives using a catalytic amount of cupric acetate in methanol. The structures of the oxidized products were established by a combination of ^{1}H and ^{13}C NMR experiments, and unexpected results were observed in the case of hydrocortisone (**1**) and cortexolone (**2**). Thus, the ^{1}H NMR spectrum of the hydrocortisone oxidation product (**5**) showed the absence of an aldehyde H_{21} proton expected at about 9.8 ppm together with the appearance of two non expected signals at 5.17 and 5.27 ppm, respectively. Therefore, a complete assignment was performed using $^{1}H\{^{13}C\}$ correlations (HSQC and HMBC) and ^{1}H–^{1}H correlations (COSY and ROESY) for this derivative (**5**). Several NMR signals, like methyl-18, appeared doubled as due to the formation of a dimer (Table 1). ^{13}C NMR data confirmed the absence of an aldehydic carbon, the presence of two unexpected signals at 93.8 and 94.6 ppm, respectively, and the presence of eight doubled carbon signals. The mass spectrum (FAB−) of compound (**5**) showed a molecular peak at 719 ($2M^{+}-1$) in accordance with a dimeric form and a peak at 360, which could correspond to the monomeric form (M^{+}). In addition, the ultraviolet absorption spectrum of (**5**) ($\lambda_{max} = 242$ nm; $\varepsilon = 29860$ in MeOH) was similar to that reported in the literature for 21-dehydrohydrocortisone, when prepared by other routes, but with a two times increased molar extinction coefficient [10–13]. This was in accordance with the presence of two α,β-unsaturated carbonyl systems and thus, with the formation of a dimer.



Scheme 1.



Scheme 2.

These data were consistent with a dimeric hemiacetal structure (Scheme 1) formed between the free hydroxyl goups at position 17 and the aldehydic functions at position 21. The hemiacetal nature of (**5**) was further confirmed by reaction with two equivalents of 3-methyl-2-benzotriazolinone hydrazine (MTBH) in CH_2Cl_2 or ethanol, giving quantitatively two equivalents of the 3-methyl-2-benzotriazolinone hydrazone of 21-dehydrohydrocortisone.

Similar results were obtained with cortexolone (**2**), while the same reaction carried out on corticosteroids without a free hydroxyl function at position 17, such as hydrocortisone-17-butyrate (**3**) or budesonide (**4**) (Scheme 2), gave quantitatively the expected α-ketoaldehydes as confirmed by the presence of aldehydic signals at 9.17 and 9.51 ppm, respectively.

4. Discussion

The hydroxyl function at position 21 in corticosteroids is highly sensitive to oxidation, and it has been reported in the literature that these molecules could be converted smoothly into their α-ketoaldehyde derivatives by a simple air exposure of a methanolic solution in the presence of $Cu(OAc)_2$ [10]. Air oxidation of hydrocortisone (**1**) using a catalytic amount of cupric acetate in methanol resulted in the quantitative formation of an oxidation product (**5**) with analytical data not in accordance with the expected structure. The main discrepancy was the lack of aldehydic signals in both ^1H and ^{13}C NMR spectra associated with the presence of some doubled signals. This lack of aldehydic signals following air oxidation of hydrocortisone in the presence of $Cu(OAc)_2$ was already reported by Orr and Monder in 1975 [14], who associated these results with a rapid equilibrium between the aldehydic and the predominant gem-diol form. Additional analytical data were more in favor of the formation of a dimer with MS (FAB−) showing a molecular peak at 719 ($2M^+-1$) and the UV spectrum showing a two times increased molar extinction coefficient compared with data obtained using other preparation methods [10–13]. All of our analytical data were, thus, consistent with a hemiacetal, dimeric structure (**5**) that could be formed by reaction of the free hydroxyl functions at position 17 with the newly formed aldehydic functions at position 21. The hemiacetal nature of this dimer was further confirmed by chemical reaction with two equivalents of MTBH, leading to the quantitative formation of two equivalents of MTBH-21-dehydrohydrocortisone.

Formation of a eight member ring between two hydroxy-aldehydes was not expected to be very favorable, but could be assisted by the presence of cuprous ions that could play a role as templates. The ring structure was further supported by the *n* Oe observed between the C-18 methyl groups at 0.89 and 0.91 ppm, respectively, with hemiacetal protons at 5.17 and 5.27 ppm, respectively. Interestingly, quenching of the oxidation reaction medium with EDTA, which could contribute to the chelation of cupric ions, seemed to favor monomeric structures [10].

These results were further confirmed by the oxidation of cortexolone (**2**), which also has a hydroxyl function at C-17, and this also led to the formation of a dimeric structure (**6**). The determinant role of the free hydroxyl function at position 17 was confirmed with results obtained with hydrocortisone-17-butyrate (**3**) and budesonide (**4**) for which the oxidation with air/cupric acetate led to the formation of the classical α-ketoaldehydes (**7**) and (**8**), respectively (Scheme 2).

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