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A variation of Mattox rearrangement mechanism under alkaline condition

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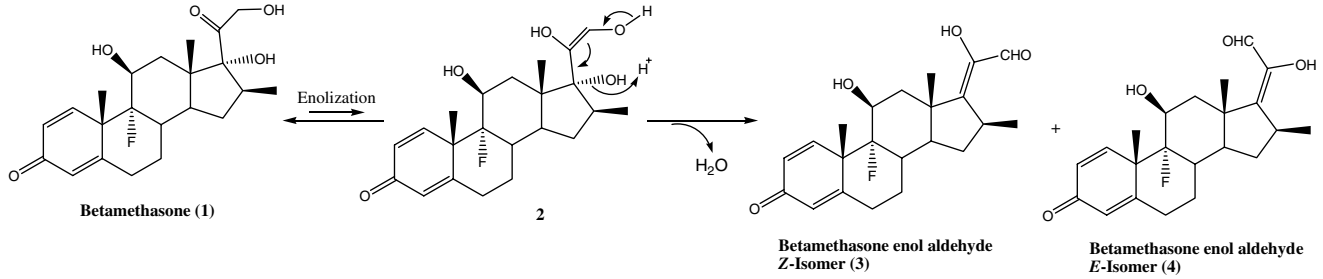
Abstract—A variation of the Mattox rearrangement, a key degradation pathway under acidic conditions for corticosteroids possessing the 1,3-dihydroxyacetone side chain, has been found to occur for the 17,21-diesters of these corticosteroids but under the alkaline condition. The mechanism of this variation of the original Mattox rearrangement is proposed.

Mattox rearrangement is an important chemical transformation that occurs at the 1,3-dihydroxyacetone side chain of a group of corticosteroids such as betamethasone, dexamethasone, cortisol, prednisone, and related compounds. During the Mattox process (Scheme 1),[1] using betamethasone (**1**) as a typical example, the side chain of betamethasone D-ring would undergo dehydration to form the corresponding enol aldehyde [betamethasone 20-hydroxy-17(20)-en-21-aldehyde, which has *Z*- and *E*-isomers (**3** and **4**)] through a presumed enol intermediate (**2**).[2] The process is catalyzed under acidic conditions by either strong acids such as sulfuric acid[3] and methanolic HCl[4] or weak acids such as acetic acid.[5,6] Although the enol aldehyde formed is relatively stable, it can further degrade into various secondary degradants under chemical as well as biochemical conditions. For example, enol aldehyde was proposed as the intermediate leading to the formation of 17-deoxy acid metabolites in patients who were administered cortisol;[5] the mechanism was supported by in vitro metabolism studies using mouse liver by which the *Z*-isomer was converted into 17-deoxy acids (Scheme 2).[7] Therefore, the enol aldehyde, formed via Mattox rearrangement, is a key intermediate not only in chemical degradation of the relevant corticosteroids but also in their biotransformation (metabolism).

During the forced degradation studies of betamethasone and related compounds, we found that betamethasone enol aldehyde can also be generated directly from



Scheme 1. Formation of betamethasone enol aldehyde from betamethasone via Mattox rearrangement under the usual acidic condition.

Keywords: Mattox rearrangement; Corticosteroids; Degradation; Betamethasone dipropionate; Betamethasone enol aldehyde.
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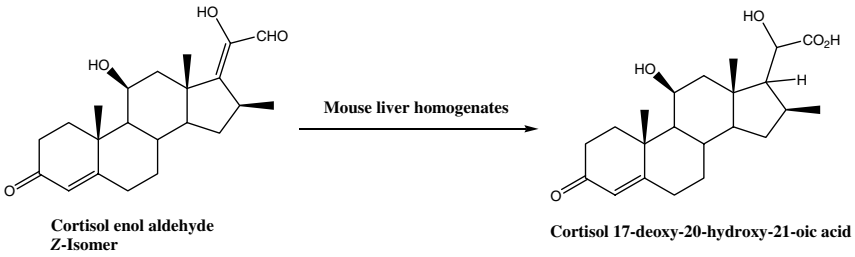


Scheme 2. In vitro metabolization of cortisol enol aldehyde (*Z*-isomer) by mouse liver homogenates according to Singer et al.[7]

betamethasone 17,21-dipropionate under alkaline condition; significant yields of enol aldehyde can be produced instantaneously. This alkaline process, which has not been reported in the literature according to our survey, cannot be explained by the known Mattox rearrangement mechanism. Therefore, formation of enol aldehyde under alkaline condition must occur with a somewhat different mechanism than the original Mattox rearrangement. In this Letter, we propose the mechanism that is a variation of the original Mattox rearrangement based on the evidence obtained from our studies.

When a solution of betamethasone 17,21-dipropionate in acetonitrile was treated with a small aliquot of 1 N NaOH aqueous solution at room temperature, the *Z*- and *E*-isomers of the enol aldehyde were formed immediately and quickly reached yields of ~30% and ~10% within approximately 20 min, respectively, as revealed by LC–MS analysis of the reaction solution.[8] The enol aldehyde isomers thus formed were found to be identical to those produced from betamethasone under acidic conditions.[9] For example, they displayed the two characteristic UV absorbance maxima at ~240 and ~275 nm regions (Fig. 1). In addition, their retention times, MS/MS fragmentation patterns, and NMR spectra were the same as those of enol aldehyde isomers generated under acidic conditions (Fig. 2 and Table 1). Under the same alkaline condition, no enol aldehyde was formed from either of the monoesters of betamethasone, that is, betamethasone 17-propionate and betamethasone 21-propionate, or from betamethasone itself. On the other hand, treatment of betamethasone 17,21-dipropionate under acidic conditions at room temperature for 20 h resulted in the formation of 17-monopropionate, 21-monopropionate, and betamethasone; no sign of enol aldehyde was observed, as revealed by the HPLC monitoring of the reaction (Chromatogram B in Fig. 3).[10] This clearly indicates a sequential degradation pathway under the acidic conditions in which the diester was first hydrolyzed into the two monoesters and then betamethasone. Apparently, the enol aldehyde was not formed until an appreciable amount of betamethasone was built up in the reaction solution, from which point enol aldehyde could start to form in significant quantities from betamethasone. Based on these results, a variation of the Mattox rearrangement mechanism is proposed in Scheme 3. Similar to the original Mattox rearrangement under acidic conditions, a presumed dipropionate enol or enolate intermediate (**6**, Scheme 3) would be a prerequisite for this variation of the Mattox rearrangement. The rearrangement under the alkaline condition would then be triggered by attack of a hydroxyl anion at the carbonyl of 21-propionyl moiety, followed by the rearrangement of the enol double bond from the 20(21)-en position to 17(20)-en position which should result in the departure of 17-oxygen in the form of propionate. In this revised version of Mattox rearrangement, the 21-propionyl apparently activates the molecule, which makes it susceptible to attack by a nucleophile at the carbonyl group, while the 17-propionyl provides a good leaving group for the 17-oxygen/hydroxyl. Based on this mechanism, both propionyl groups are critical for the rearrangement to occur, which can satisfactorily explain

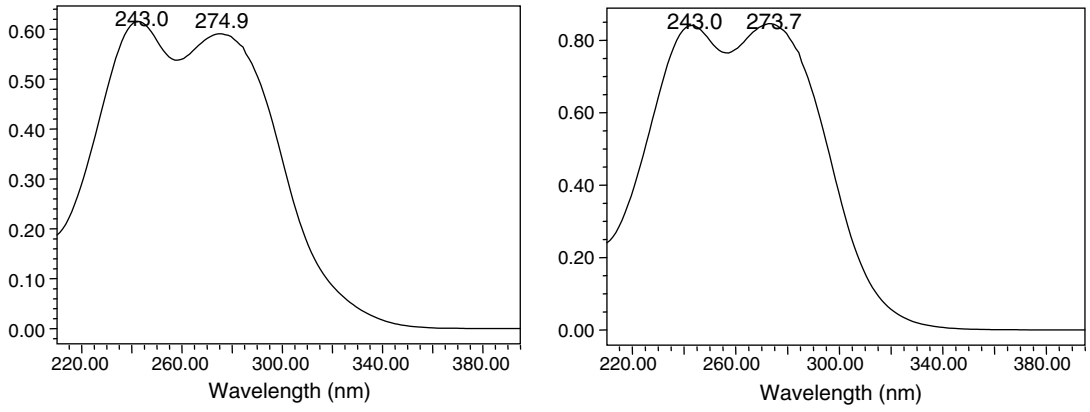


Figure 1. Photodiode array UV scans of the *Z*- and *E*-isomers of betamethasone enol aldehyde (left and right scans, respectively) generated under the acidic stress condition as outlined in the legend of Figure 3. Enol aldehyde isomers generated under the alkaline condition showed identical spectra.

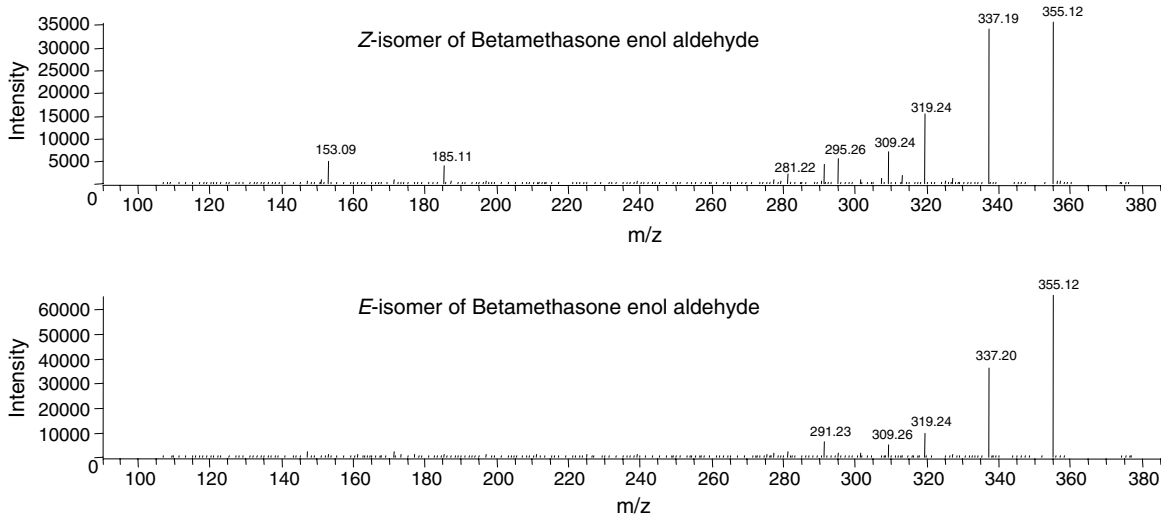
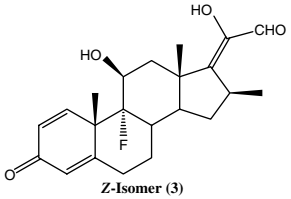
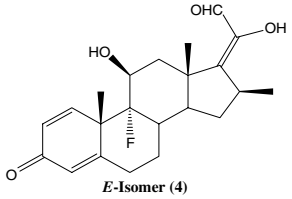


Figure 2. MS/MS fragmentation patterns of the *Z*- and *E*-isomers of betamethasone (upper and lower spectra, respectively) generated under the acidic stress condition as outlined in the legend of Figure 3. Enol aldehyde isomers generated under the alkaline condition showed identical spectra.

Table 1. ^{1}H and ^{13}C NMR Signal assignment for the *Z*- and *E*-isomers of betamethasone enol aldehyde[1]



Z-Isomer (3)



***E*-Isomer (4)**

No.[2]	Proton δ (ppm)	Carbon δ (ppm)	Proton δ (ppm)	Carbon δ (ppm)
1	7.28, d, 10.1 Hz	152.71	7.28, d, 10.1 Hz	152.53
2	6.19, dd, 10.1, 1.9 Hz	129.01	6.20, dd, 10.1, 1.9 Hz	129.01
3		185.22		185.19
4	5.99, dd, 1.9, 1.2 Hz	124.16	5.99, dd, 1.9, 1 Hz	124.16
5		166.84		166.72
6	2.33, 2.63, m, m	30.13	2.33, 2.63 m, m	30.13
7	1.36, 1.85, m, m	27.05	1.36, 1.85 m, m	27.05
8	2.47, m, $^{2}J_{HF} = 30.3$ Hz	33.04, $^{2}J_{CF} = 19.2$ Hz	2.47, m, $^{2}J_{HF} = 30.4$ Hz	32.84, $^{2}J_{CF} = 19.3$ Hz
9		100.60, $^{1}J_{CF} = 174.3$ Hz		100.77, $^{1}J_{CF} = 174.5$ Hz
10		47.78		47.96
11	4.07, d, $^{3}J_{HF} = 10.6$ Hz, 5.38 (OH), dd 4.1, 1.9 Hz	70.97, $^{2}J_{CF} = 37.1$ Hz	4.10, d, $^{3}J_{HF} = 10$ Hz 5.44 (OH), d, 3.8 Hz	70.28, $^{2}J_{CF} = 36.9$ Hz
12	1.61, 2.60, dt 14.3, 3.5, 3.5 Hz; dd 14.3, 2.2 Hz	41.01, $^{3}J_{CF} = 1$ Hz	1.88, 2.29, d, 13 Hz; dd, 13, 2.3 Hz	44.61, $^{3}J_{CF} = 1$ Hz
13		45.37		44.09
14	1.46, m	47.42, $^{3}J_{CF} = 1.5$ Hz	1.48, m	47.72, $^{3}J_{CF} = 1.5$ Hz
15	1.16, 1.96, m, m	33.83	1.12, 1.91, m, m	32.60
16	3.21, m	32.48	2.82, m	36.41
17		152.12		154.06
18	1.23, s	17.84	1.29, s	21.63
19	1.49, s	22.87	1.49, s	22.92
20	7.68 (OH), s	142.55	7.87 (OH), s	143.58
21	9.61, s	187.27	9.71, s	185.34
22	1.26, d, 7 Hz	27.05	1.21, d, 6.9 Hz	19.50

Notes: (1) ^{1}H and ^{13}C NMR spectra were obtained on a Varian Inova 500 spectrometer operating at a proton frequency of 500 MHz (carbon frequency of 125 MHz) with a 3 mm carbon-proton dual probe. All spectra were taken at 25 °C on DMSO-d_6 solutions of the compounds. The 2D experiments, HMQC, HMQC–TOCSY and HMBC were used to establish the connectivity of the proton and carbon nuclei. 2D NOESY experiments were used to establish the stereochemistry of the groups around the C17–C20 double bond. (2) The numbering of the steroid rings follows the usual convention.

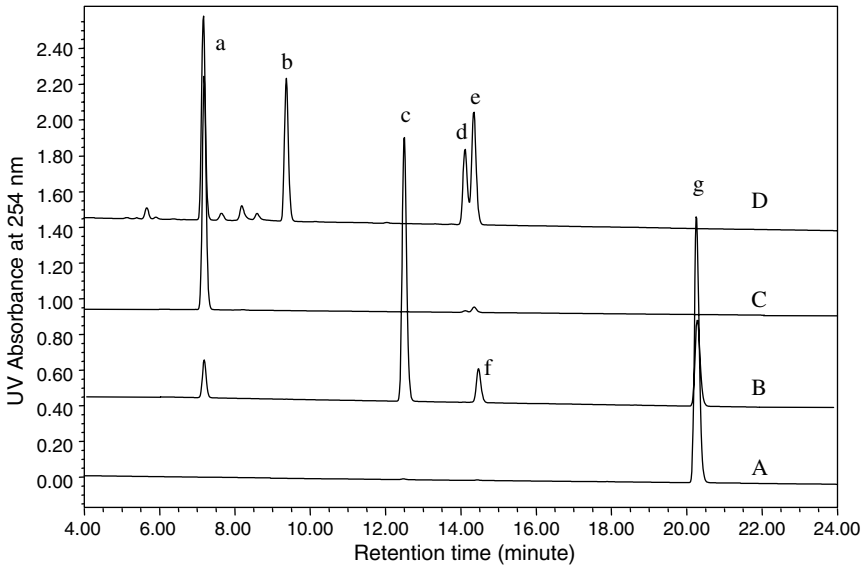
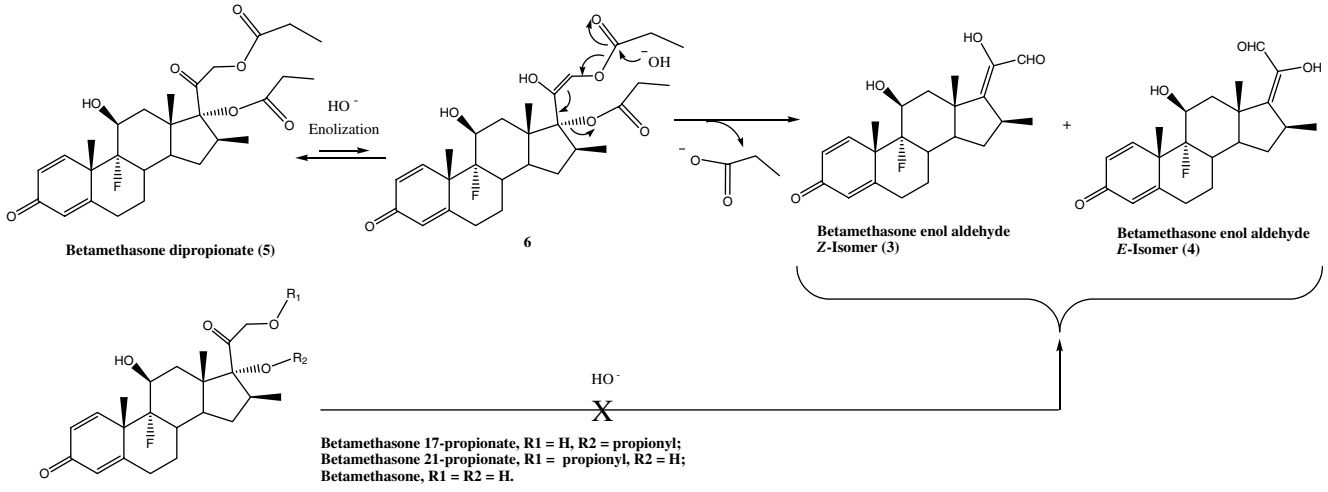


Figure 3. The study for generation of betamethasone enol aldehyde from betamethasone 17,21-dipropionate and betamethasone under acidic conditions: the compound to be stressed was dissolved in a mixture of acetonitrile and water (1/1, v/v) at a concentration of approximately 1 mg/mL. To the above solution was added a 1/10 volume equivalent of concentrated sulfuric acid and the resulting solution was either allowed to stand at room temperature or heated at 60 °C for up to 20 h. An aliquot of the reaction solutions was analyzed by a Waters HPLC system consisting of a Model 2695 Separations Module, a Model 2996 photo diode array detector, and a YMC J'sphere ODS-H80, 150 × 4.6 mm, 3 μm column. Elution was effected with a linear gradient generated between mobile phase A (acetonitrile/water, 25/75, v/v) and mobile phase B (acetonitrile/water, 70/30, v/v); the percentage of mobile phase B was increased from an initial 0% to 100% over a period of 25 min. The flow rate was 1.5 mL/min. *Chromatograms:* (A) Betamethasone 17,21-dipropionate before mixing with H_2SO_4; (B) betamethasone 17,21-dipropionate, 20 h after mixing with H_2SO_4 at room temperature; (C) betamethasone, 20 h after mixing with H_2SO_4 at room temperature; (D) betamethasone 17,21-dipropionate, 20 h after mixing with H_2SO_4 at 60 °C. *Identities of the peaks:* (a) betamethasone; (b) betamethasone sulfate; (c) betamethasone 17-propionate; (d) betamethasone enol aldehyde, *Z*-isomer, (e) betamethasone enol aldehyde, *E*-isomer; (f) betamethasone 21-propionate; (g) betamethasone 17,21-dipropionate. The identity of Peak b, betamethasone sulfate, was established by LC–MS only; its MS spectrum showed a peak at *m*/*z* 473 which corresponds to a presumed covalent adduct between betamethasone and sulfate. Peak f (betamethasone 21-propionate) eluted at approximately the same time as Peak e (betamethasone enol aldehyde, *E*-isomer). However, LC–MS data indicated that Peak f was homogenous (i.e., no betamethasone enol aldehyde).



Scheme 3. Formation of betamethasone enol aldehyde from betamethasone 17,21-dipropionate via the variation of Mattox rearrangement under alkaline condition. Although the intermediate **6** is shown in the enol form, the corresponding enolate form is also possible. No enol aldehyde was formed from either of the monoesters, that is, betamethasone 17-propionate and betamethasone 21-propionate, or from betamethasone itself under the identical alkaline condition.

the fact that none of the monoesters of betamethasone or betamethasone itself undergoes the rearrangement under this alkaline condition.

In summary, we have shown that the Mattox rearrangement, which is a key degradation pathway under acidic conditions for corticosteroids possessing the 1,3-dihy-

droxyacetone side chain, would also occur for the 17,21-diesters of these corticosteroids but under the alkaline condition. This variation of the original Mattox rearrangement proposed in this Letter should facilitate the understanding of the degradation behavior of relevant corticosteroid 17,21-diesters, some of which are used as the active pharmaceutical ingredients in many marketed drug products.

Acknowledgments

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8. A solution of 1.0 mg of betamethasone 17,21-dipropionate in 1.5 mL of acetonitrile was mixed with 75 μL of 1 N NaOH aqueous solution at room temperature. The resulting mixture was allowed to standard at room temperature for 20 min. Aliquots of the reaction solution were injected into a LC–MS system, consisting of a Thermo Electron Surveyor HPLC system coupled with a linear ion trap mass spectrometer, for HPLC-PDA/MS^n analyses. The HPLC system was equipped with a Supercosil ABZ-Plus C18, 250 × 4.6 mm, 5 μm column. Isocratic elution was effected with a mobile phase of acetonitrile/10 mM ammonium acetate (1/1, v/v) at a flow rate of 2 mL/min. The HPLC flow was split at a ratio of 10:1 prior to entering the MS detector; approximately 200 μL/min of the HPLC flow was directed into the ion source of the MS detector. Positive electron spray ionization was used with a spray voltage of 4.5 kV. The capillary temperature was set at 300 °C and the sheath gas flow rate 40 units. A full MS scan was obtained in the mass range of 100–800 Da. Tandem MS/MS experiments were performed using helium as the collision gas with an isolation width of 3.0 Da for the parent ion and normalized collision energy of 28.
9. Acidic conditions for producing enol aldehyde from betamethasone for analytical purpose: A solution of 1.0 mg of betamethasone in 1.5 mL of acetonitrile was mixed with 50 μL of concentrated sulfuric acid at room temperature and allowed to stand at room temperature for up to 30 min. For isolation of both the *E*- and *Z*-isomers of betamethasone enol aldehyde, 2 mL of concentrated sulfuric acid was added into a 20 mL, 0.5 mg/mL betamethasone solution in acetonitrile/water (1/1, v/v). After the resulting solution was heated at 66 °C for 20 h, it was concentrated and portions of the concentrated solution were separated, through a number of injections, on a Waters Alliance HPLC system equipped with a Supelcosil ABZ plus, 250 × 4.6 mm, 5 μm, column and a fraction collector. A linear gradient with a flow rate of 1.5 mL/min was generated between mobile phase A (acetonitrile/water, 25/75, v/v) and mobile phase B (acetonitrile/water, 90/10, v/v); the percentage of solution B was increased from 0% to 35% in 25 min and continuously increased to 100% in the next 10 min. Under this condition, the two isomers, eluting at ~23 and ~24 min with a complete baseline separation, were collected. The collected fractions of the *E*- and *Z*-isomers from multiple runs were combined, respectively, and then evaporated in vacuo. The dried samples of the *E*- and *Z*-isomers were analyzed by ^1H and ^{13}C NMR; the results and assignment are summarized in Table 1. Various 2D experiments were performed to establish the structural connectivity and configurations.
10. The acidic stress conditions used in these experiments to generate the enol aldehyde from betamethasone and its derivatives are described in the legend of Figure 3.