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A comparative study of enol aldehyde formation from betamethasone, dexamethasone, beclomethasone and related compounds under acidic and alkaline conditions

Bin Chen, Min Li, Mingxiang Lin, Gilbert Tumambac, Abu Rustum*

Global Quality Services – Analytical Sciences, Schering-Plough Corporation, 1011 Morris Avenue, Union, NJ 07083, USA

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ABSTRACT

Enol aldehydes are one type of key degradation and metabolic intermediates from a group of corticosteroids containing the 1,3-dihydroxyacetone side chain on their D-rings, such as betamethasone, dexamethasone, beclomethasone, and related compounds. The formation of enol aldehydes from these corticosteroids is via acid-catalyzed β-elimination of water from the side chain, a process known as Mattox rearrangement. It was recently reported by our group that enol aldehydes could also be formed directly from the corresponding 17,21-diesters of these corticosteroids but only under alkaline condition, which was proposed to follow a variation pathway of the original Mattox rearrangement. In this paper, we report the results of a comparative study of enol aldehyde formation from these structurally similar corticosteroids (under the original acidic Mattox condition) and their 17,21-diesters (under the alkaline Mattox variation condition), respectively. In general, enol aldehydes were found to be formed under both conditions; however, the ratios of the *E*- and *Z*-isomers of the enol aldehyde were different in each case. The only exception was beclomethasone 17,21-diester under the alkaline condition, where a competing elimination of HCl from the 9,11-positions became predominant. These results can be explained by their structural differences with regard to the Mattox mechanism and its variation pathway. Lastly, solvent effect under acidic condition was studied between an aprotic and a protic solvent and the result suggests that enol aldehyde formation is greatly favored in an aprotic environment.

1. Introduction

Corticosteroids containing the 1,3-dihydroxyacetone side chain on their D-rings, such as betamethasone, dexamethasone, cortisol and related compounds (Fig. 1), are potent anti-inflammatory agents that have been formulated into various pharmaceutical dosage forms [1–3]. One of the main degradation pathways of these corticosteroids in the formulated drug products is the formation of enol aldehyde via β-elimination of water from the D-rings containing the 1,3-dihydroxyacetone side chain, a process known as Mattox rearrangement (Scheme 1) [4]. During the Mattox process, using betamethasone (**1**) as an example, the side chain of betamethasone D-ring would undergo dehydration to form the corresponding betamethasone enol aldehyde, which has *E*- and *Z*-isomers (**3** and **4**), through a presumed enol intermediate (**2**) [5]. The process is catalyzed by acid which can be either strong acids such as sulfuric acid [6] and methanolic HCl [7] or weak acids such as acetic acid and certain Lewis acids [8,9]. Recently we reported that betamethasone enol

* *Corresponding author*. Tel.: +1 9088203391; fax: +1 9088206359.
E-mail address: min.li@spcorp.com (M. Li).
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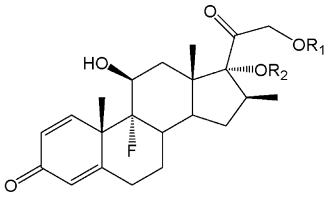
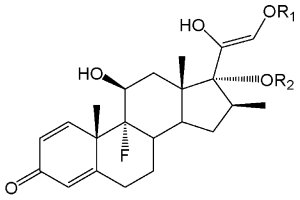
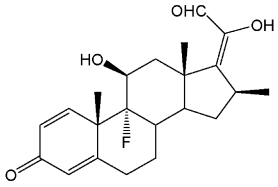


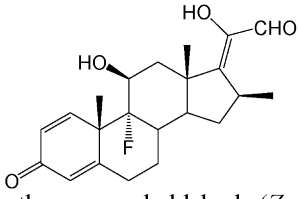
Betamethasone (**1**), $R_1 = R_2 = H$; Betamethasone 17, 21-dipropionate (**5**), $R_1 = R_2 = -COCH_2CH_3$



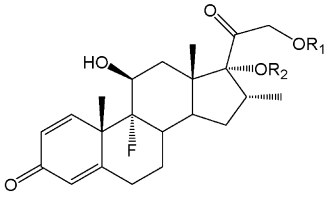
Proposed intermediate in Mattox rearrangement (**2**), Scheme 1, $R_1 = R_2 = H$; Proposed intermediate in the revised Mattox rearrangement (**6**), Scheme 2, $R_1 = R_2 = -COCH_2CH_3$



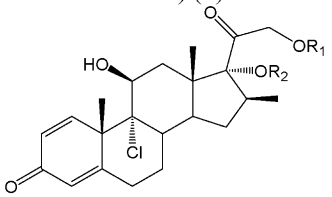
Betamethasone enol aldehyde (*E*- or trans-isomer) (**3**)



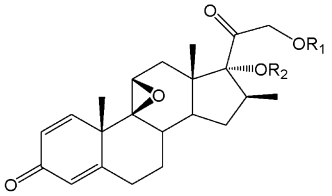
Betamethasone enol aldehyde (*Z*- or cis-isomer) (**4**)



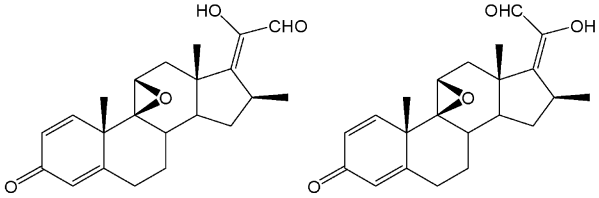
Dexamethasone (**7**), $R_1 = R_2 = H$; Dexamethasone 17, 21-dipropionate (**8**), $R_1 = R_2 = -COCH_2CH_3$



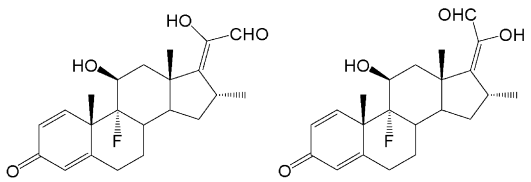
Beclomethasone (**9**), $R_1 = R_2 = H$; Beclomethasone 17, 21-dipropionate (**10**), $R_1 = R_2 = -COCH_2CH_3$



Betamethasone 9, 11-epoxide 17, 21-dipropionate (**11**), $R_1 = R_2 = -COCH_2CH_3$

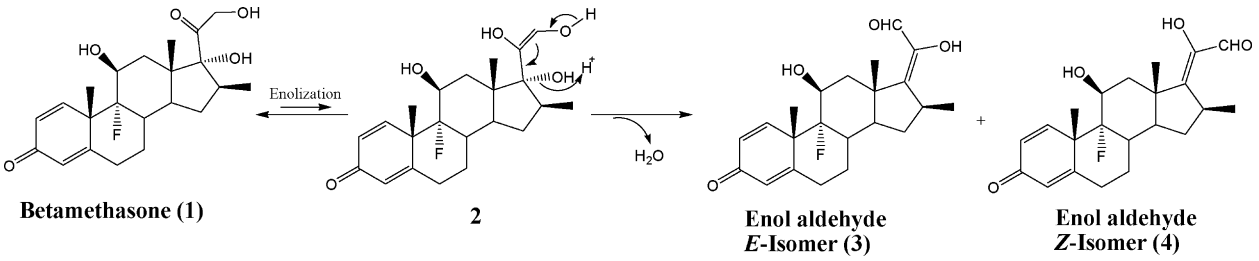


Betamethasone 9, 11-epoxide enol aldehydes (**12**)

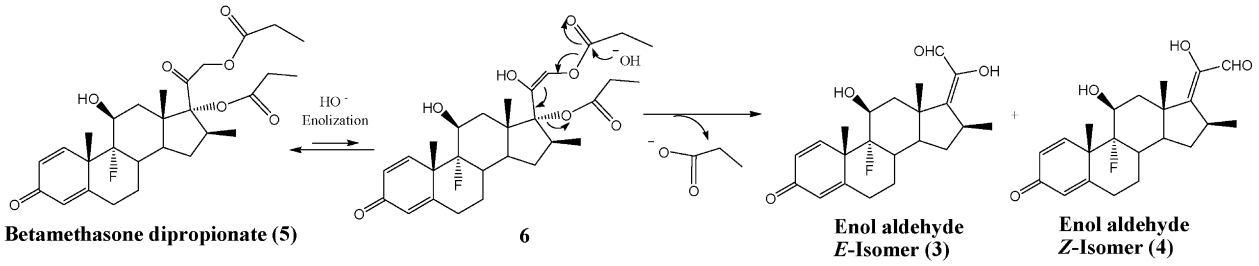


Dexamethasone enol aldehydes (**13**): *Z*- or cis-isomer (**13a**, left) and *E*- or trans-isomer (**13b**, right)

Fig. 1 – Structures of the molecules mentioned in this study.



Scheme 1 – Formation of betamethasone enol aldehyde (3 and 4) from betamethasone (1) via Mattox rearrangement under the usual acidic condition.



Scheme 2 – Formation of betamethasone enol aldehyde (3 and 4) from betamethasone dipropionate (5) via the revised Mattox rearrangement under alkaline condition.

aldehyde can also be generated from betamethasone dipropionate (5) under alkaline condition, which was proposed to follow a revised Mattox rearrangement mechanism (Scheme 2) [10]. In this variation of Mattox rearrangement, both the 17- and 21-acyl groups are required to effect a concerted elimination leading to the formation of enol aldehyde; none of the mono esters (betamethasone 17-propionate and betamethasone 21-propionate) produces enol aldehyde under this condition.

Enol aldehydes are one type of the key degradants of betamethasone and other corticosteroids with similar D-ring structures, which can further degrade into a number of secondary degradants dependent upon the formula and/or storage condition of a particular drug product [11]. Enol aldehydes are also important metabolic intermediates; enol aldehydes and some of its further degradants were reported as metabolites for some of the corticosteroids mentioned above [12]. In this paper, we report the results of a comparative study on the formation of enol aldehydes from several structurally similar corticosteroids under acidic condition (the original acidic Mattox condition) and from their 17,21-dipropionate esters under alkaline condition (the alkaline Mattox variation condition), respectively. In addition, solvent effect on the formation of enol aldehydes from

Table 1 – Summary of the HPLC and/or LC–MS analysis conditions used to generate data in this study.

No.	Instrumentation	HPLC or LC–MS analysis conditions[a]	Data generated
1	A Waters HPLC system equipped with a Model 2695 separation module and a Model 2996 photo diode array (PDA) detector	Column: YMC J'sphere ODS-H80, 150 × 4.6 mm, 3 μm. Mobile phase A: acetonitrile/water, 25/75, v/v. Mobile phase B: acetonitrile/water, 70/30, v/v; Gradient: linear, 0% B to 100% B in 25 min. Flow rate: 1.5 mL/min.	Fig. 7A
2	A Thermo Electron Surveyor HPLC system equipped with a PDA detector, coupled with a linear ion trap mass spectrometer	Column: Supercosil ABZ-Plus C18, 250 × 4.6 mm, 5 μm. Isocratic elution: 1: 1 acetonitrile: 10 mM ammonium acetate at a flow rate of 2 mL/min.	Fig. 3A and B; Fig. 5A and B; Fig. 7B.
3	A Thermo Electron Surveyor HPLC system equipped with a PDA detector, coupled with a linear ion trap mass spectrometer	Column: YMC Basic, 150 × 4.6 mm, 3 μm. Mobile phase A: acetonitrile/water, 35/65, v/v. Mobile phase B: acetonitrile/water, 90/10, v/v; Gradient: 0–5 min, 0% B; 5–20 min, 0% B to 100% B. Flow rate: 1.5 mL/min.	Fig. 7C and D
4	A Waters HPLC system equipped with a Model 2695 separation module and a Model 2996 photo diode array (PDA) detector	Column: YMC Hydrosphere C-18, 50 × 4.6 mm, 3 μm. Mobile phase A: water. Mobile phase B: acetonitrile/methanol, 8/25, v/v; Gradient: 0–21 min, 33% B; 21–35 min, 33% B to 63% B. Flow rate: 2.0 mL/min. Column temp: 35 °C.	Fig. 5D

[a] Unless otherwise noted, all gradients are linear and column temp is ambient.

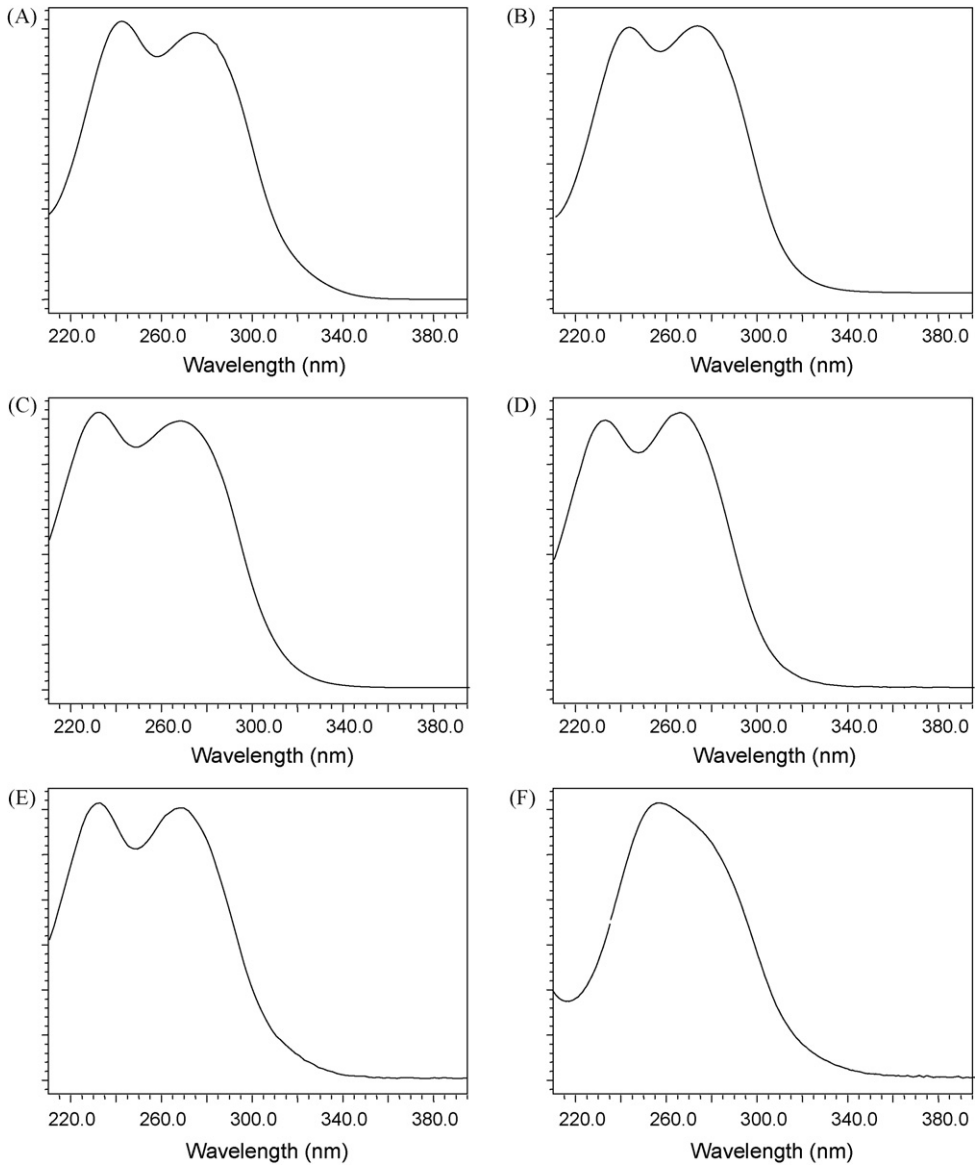


Fig. 2 – UV spectra of the peaks of interest. (A) Betamethasone enol aldehyde, *Z*-isomer; (B) Betamethasone enol aldehyde, *E*-isomer; (C) Dexamethasone enol aldehyde; (D) Beclomethasone enol aldehyde, *E*-isomer; (E) Beclomethasone enol aldehyde, *Z*-isomer; (F) Betamethasone 9,11-epoxide enol aldehyde (mixture of the *E*- and *Z*-isomers).

betamethasone under acidic condition was also investigated.

2. Experimental

2.1. Materials

All the steroids and related compounds used as starting materials for the degradation studies (**1**, **5**, **7** to **10**, and **11**) were produced in house with certified purities of greater than 99%; all the starting materials except for **11** are also commercially available. Solvents (methanol, acetonitrile, and water) were of HPLC grade and purchased from Fisher Scientific. Sulfuric acid (certified ACS plus) and 1 N NaOH (certified) were also supplied by Fisher Scientific.

2.2. Instrumentation

A variety of HPLC and LC–MS instruments were used under various conditions for the HPLC and LC-PDA/UV-MS^n ($n = 1$ to 3) analyses performed in this study, which are summarized in Table 1. Various 1D and 2D NMR experiments were performed either on a Varian Inova 500 NMR spectrometer operating at a proton frequency of 500 MHz (carbon frequency of 125 MHz) or on a Varian Inova 600 NMR spectrometer operating at a proton frequency of 600 MHz (carbon frequency of 150 MHz with a 3 mm carbon–proton dual probe.

2.3. Structure identification/characterization

LC-PDA/UV-MS^n ($n = 1$ to 3) was the major analytical tool used in this study. NMR was also used in cases where LC-PDA/UV-

Table 2 – Reaction conditions used to generate enol aldehyde in this study[a].

No.	Reaction conditions	Results
1	1/10 volume of conc. sulfuric acid was added to betamethasone solution in 1:1 acetonitrile:water (v/v). The resulting solution was allowed to stand for ca 20 h.	Fig. 7A
2	1/20 volume of conc. sulfuric acid was added to betamethasone solution in acetonitrile. The resulting solution was allowed to stand for ca 50 min.	Fig. 3A
3	1/10 volume of conc. sulfuric acid was added to betamethasone solution in methanol. The resulting solution was allowed to stand for ca 30 min and 3 d.	Fig. 7C and D
4	1/20 volume of 1 N NaOH aqueous solution was added to betamethasone dipropionate solution in acetonitrile. The resulting solution was allowed to stand for ca 20 min.	Fig. 5A
5	1/20 volume of conc. sulfuric acid was added to dexamethasone solution in acetonitrile. The resulting solution was allowed to stand for ca 30 min.	Fig. 3B
6	1/20 volume of 1 N NaOH aq solution was added to dexamethasone dipropionate solution in acetonitrile. The resulting solution was allowed to stand for ca 40 min.	Fig. 5B
7	1/20 volume of conc. sulfuric acid was added to beclomethasone solution in acetonitrile. The resulting solution was allowed to stand for ca 40 min.	Fig. 3C
8	1/20 volume of 1 N NaOH aq. solution was added to beclomethasone dipropionate solution in acetonitrile. The resulting solution was allowed to stand for ca 20 min.	Fig. 5C
9	1/20 volume of 1 N NaOH aq. solution was added to beclomethasone 9,11-epoxide dipropionate solution in acetonitrile. The resulting solution was allowed to stand for ca 15 min.	Fig. 5D

[a] Unless otherwise noted, concentration of the starting material is ca 1 mg/mL. All reactions were carried out at room temp.

MS^n could not provide unambiguous structure assignment. Peak identification of the reaction products was based on previous studies by our group [10] and comparison of their LC–MS^n fingerprinting data (retention times, molecular weights and LC–MS^n fragmentation patterns) [13] and UV spectra with those of the corresponding authentic samples.

Enol aldehydes displayed characteristic UV absorption profiles (Fig. 2) as compared to the corresponding steroids. All the

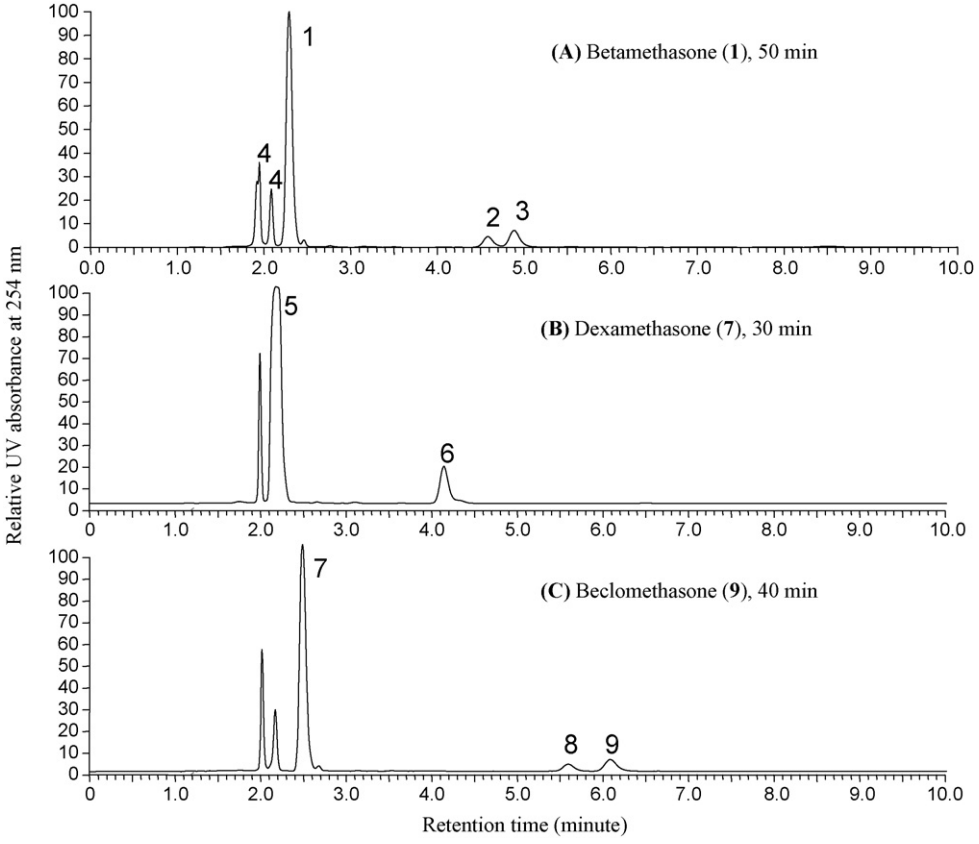


Fig. 3 – The study for generation of enol aldehyde in acetonitrile under acidic condition from 1 (A), 7 (B), and 9 (C). The reactions were carried out at room temp and were monitored by LC–MS. For detailed analysis and reaction conditions, refer to Tables 1 and 2, respectively.

***Peak identities*: (1) 1; (2) 4; (3) 3; (4) Betamethasone sulfates; (5) 7; (6) Dexamethasone enol aldehyde; (7) 9; (8) Beclomethasone enol aldehyde, *Z*-isomer; (9) Beclomethasone enol aldehyde, *E*-isomer.**

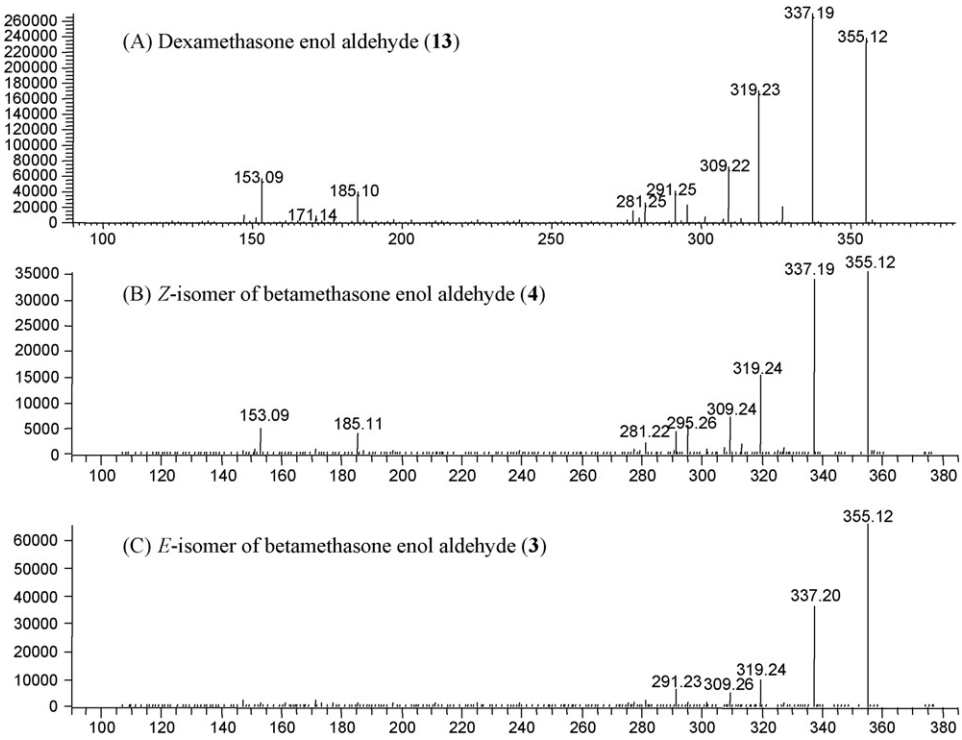


Fig. 4 – Comparison of the LC–MS^2 fragmentation pattern of dexamethasone enol aldehyde generated from the acidic stress study with those of the Z- and E-isomers of betamethasone enol aldehyde.

enol aldehydes generated in this study exhibit very similar UV absorption profiles (Figs. 2A through 2E), i.e., two absorption maxima at approximately 240 and 270 nm, respectively, except for the enol aldehydes of betamethasone 9,11-epoxide (**12**) in which case the two absorption maxima appear to have merged together (Fig. 2F). The exception observed in the latter case is apparently due to the fact that the UV absorption maximum for betamethasone 9,11-epoxide is at 250 nm, which is closer to the UV absorption maximum at ~270 nm for the enol aldehyde chromophore. On the other hand, betamethasone (**1**), dexamethasone (**7**), and beclomethasone (**9**) all have a UV absorption maximum at ~240 nm.

2.4. *Reaction conditions*

All reaction conditions used to generate enol aldehydes from the compounds of interest in this study are listed in Table 2.

3. Results and discussion

There appear to be very few detailed studies in the literature regarding the formation of enol aldehydes from betamethasone and its closely related analogs such as dexamethasone and beclomethasone. Other than the study results published by our group recently [10,14], the only in-depth study regarding the formation of betamethasone enol aldehydes (**3** and **4**) under acidic condition appears to be the degradation study of betamethasone reported by Hidaka et al. [15]. Although the acidic conditions used in the two studies were somewhat different, both **3** and **4** were isolated by the two groups. In the first part of the current study, we compared the formation of enol aldehydes from dexamethasone (**7**) and beclomethasone (**9**), respectively, with that of betamethasone (**1**) under acidic condition where the original Mattox mechanism should be followed. In the second part of the current study, we compared the formation of enol aldehydes from the 17,21-dipropionate esters of dexamethasone and beclomethasone (**8** and **10**), respectively, with that of betamethasone 17,21-dipropionate (**5**) under alkaline condition where the Mattox variation mechanism should be operative as we reported recently [10].

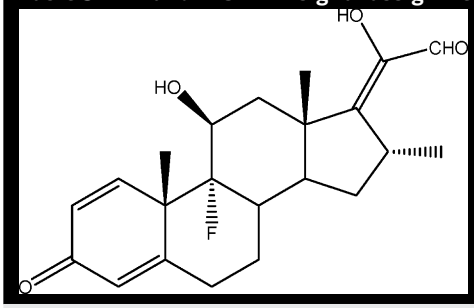
3.1. *Comparative study of enol aldehyde formation from betamethasone (1), dexamethasone (7), and beclomethasone (9) under acidic condition*

The structures of all the relevant compounds are shown in Fig. 1. Of these compounds, dexamethasone (**7**) is the epimer of betamethasone (**1**). The only structural difference between the two molecules lies in the orientation of the methyl group bonded to C-16 in the D-ring (β-position for betamethasone and α-position for dexamethasone). The only structural difference between betamethasone and beclomethasone (**9**) is that 9-fluoro in betamethasone is replaced by 9-chloro in beclomethasone.

3.1.1. *Dexamethasone (7) vs. betamethasone (1) under acidic condition*

Formation of enol aldehydes from betamethasone (**1**) under acidic condition is known to proceed via the Mattox mechanism [4]. In this study, when dexamethasone (**7**) was treated under the same acidic condition as that used for betametha-

Table 3 – ^{1}H and ^{13}C NMR signal assignment for the Z-isomer of dexamethasone enol aldehyde (13a)[a]



No.[b]	Proton δ (ppm)	Carbon δ (ppm)
1	7.30, d, 10.1 Hz	152.83
2	6.22, dd, 10.1, 1.9 Hz	129.05
3		185.32
4	6.01, dd, 1.9, 1.2 Hz	124.23
5		166.94
6	2.34, 2.65, m, m	30.20
7	1.41, 1.86, m, m	27.30
8	2.48, m, $^{2}J_{HF}$ = 34.4 Hz	32.71, $^{2}J_{CF}$ = 19.2 Hz
9		101.65 $^{1}J_{CF}$ = 174.6 Hz
10		48.02, $^{2}J_{CF}$ = 22.4 Hz
11	4.09, d, $^{3}J_{HF}$ = 12.2 Hz, 5.44 (OH), d, 4.0 Hz	71.20, $^{2}J_{CF}$ = 37.9 Hz
12	1.68, 2.62, dt 13.9, 2.2, 3.8 Hz; dd 13.9, 2.2 Hz	41.15
13		45.55
14	1.72, m	44.18, $^{3}J_{CF}$ = 1.5 Hz
15	1.34, 1.72, m, m	33.24
16	3.32, m	31.52
17		152.69
18	1.17, s	17.38
19	1.51, s	22.80, $^{3}J_{CF}$ = 5.3 Hz
20	7.81 (OH), s	143.60
21	9.59, s	187.37
22	1.12, d 7.2 Hz	26.54

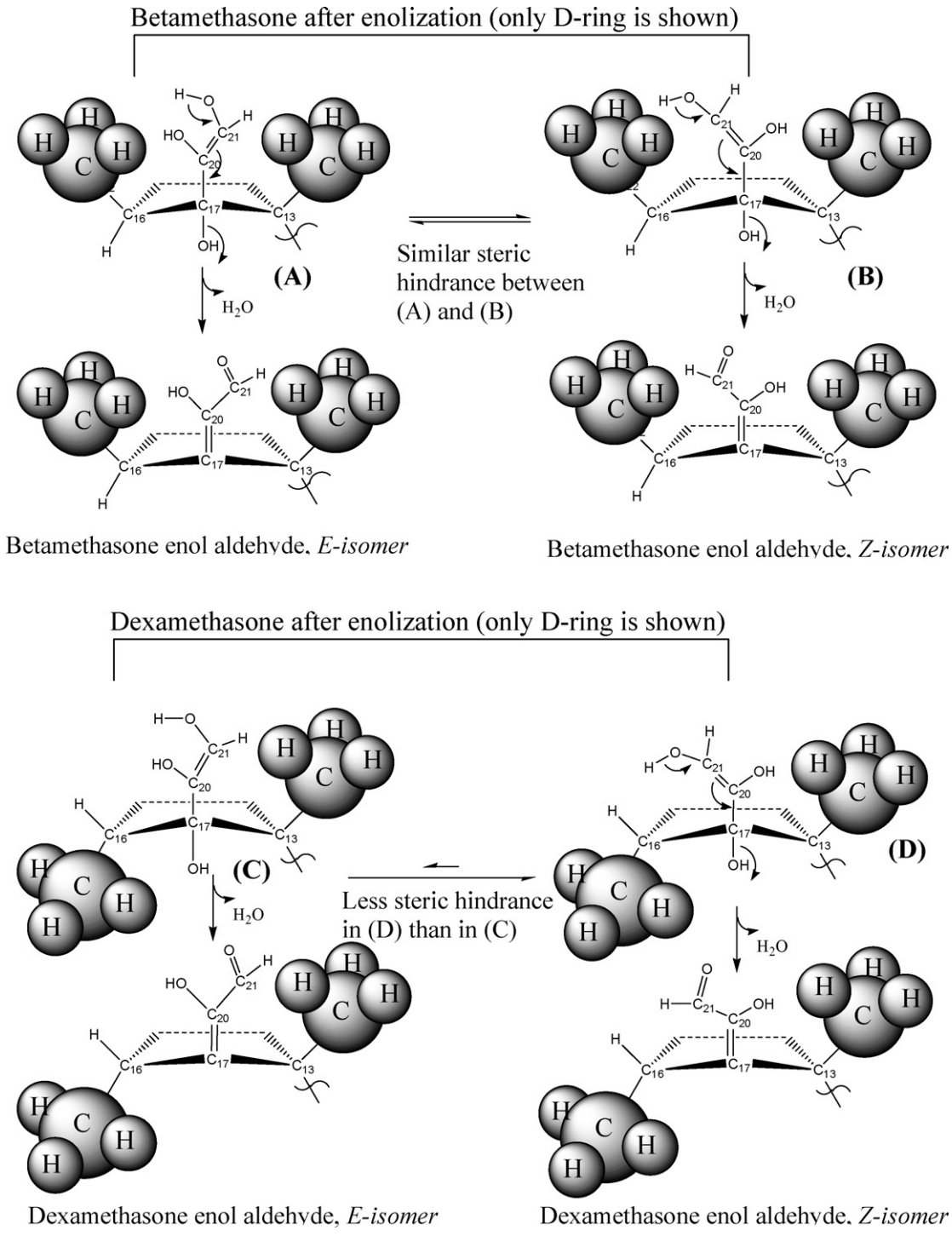
[a] ^{1}H and ^{13}C NMR spectra were obtained on a Varian Inova 600 spectrometer operating at a proton frequency of 600 MHz (carbon frequency of 150 MHz) with a 3 mm carbon–proton dual probe. All spectra were taken at 25 °C on DMSO-d_6 solutions of the compounds. The 2D experiments, HSQC, HSQCTOXY, HMBC and NOESY 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 C-17–C-20 double bond.

[b] The numbering of the steroid rings follows the usual convention.

sone (Table 2), dexamethasone enol aldehyde was generated (Fig. 3B). The dexamethasone enol aldehyde (**13**) formed was found to be mostly the Z-isomer based on the comparison of its LC–MS^2 fragmentation pattern [13] with those of the Z- and E-isomers of betamethasone, respectively (Fig. 4). Upon isolation of the dexamethasone enol aldehyde peak, NMR analysis (Table 3) showed that the Z-isomer was formed predominantly (~90%), while the E-isomer was formed as a minor product (~10%) [16]. This product distribution is quite different from the Mattox rearrangement of **1** in which the ratio of the Z-isomer (**4**) vs. the E-isomer (**3**) formed from **1** is approximately 40% vs. 60% (Fig. 3A). The high preference in the formation of the Z-isomer of dexamethasone enol aldehyde (**13a**) over the E-isomer (**13b**) can be well explained stereochemically (Scheme 3). The enolized side chain [20,21-dihydroxy-20(21)-en] attached to the D-ring could have two orientations which would lead to the formation of the Z-isomer and E-isomer of the corresponding enol aldehydes, respectively (Scheme 3). In the case of betamethasone (**1**), the two orientations of the enolized side chain appear to encounter similar steric hindrance from the β-oriented (upward) methyl group attached to C-16 and the β-oriented methyl group attached to C-13. Consequently, both the Z-isomer (**4**) and E-isomer (**3**) of betamethasone enol aldehyde are formed in comparable yields. In the case of dexamethasone (**7**), since the methyl group attached to C-16 is now α-oriented (downward), the enolized side chain would preferably take an orientation that is less sterically hindered, which is the orientation that should lead to the formation of the Z-isomer of the enol aldehyde as shown in Scheme 3. Therefore, the Z-isomer of dexamethasone enol aldehyde (**13a**) was generated as the predominant product from **7** under acidic condition.

3.1.2. *Beclomethasone (9) vs. betamethasone (1) under acidic condition*

Beclomethasone (**9**) is structurally identical to betamethasone (**1**) except that 9-fluoro in **1** is replaced by 9-chloro in **9**. From the above stereochemical discussion, it can be predicted that both the Z-isomer and the E-isomer of beclomethasone enol aldehyde should be generated in comparable yields from beclomethasone (**9**) under acidic condition. Indeed, after **9** was stressed under the same acidic condition as used for stress-



Scheme 3 – Proposed stereochemical explanation for the formation of enol aldehydes under acidic condition: betamethasone (1) vs. dexamethasone (7) (only the D-ring is shown for each molecule). In the case of betamethasone, the two conformations (A and B) of the presumed intermediate (after enolization) leading to the formation of the enol aldehyde E- and Z-isomers appear comparable with regard to steric hindrance. Therefore, both the E- and Z-isomers of betamethasone enol aldehyde (3 and 4) were generated in comparable yields from betamethasone under acidic condition. Whereas in the case of dexamethasone, the conformation (C) of the intermediate leading to the formation of enol aldehyde Z-isomer is highly favored over the other conformation (D) due to the much less steric hindrance encountered by conformation (C). Therefore, the Z-isomer of dexamethoasone enol aldehyde (13a) was generated predominantly. Similar explanations can also be applied to the generation of enol aldehyde from betamethasone 17,21-dipropionate (5) and its dexamethasone counterpart (8) under alkaline condition.

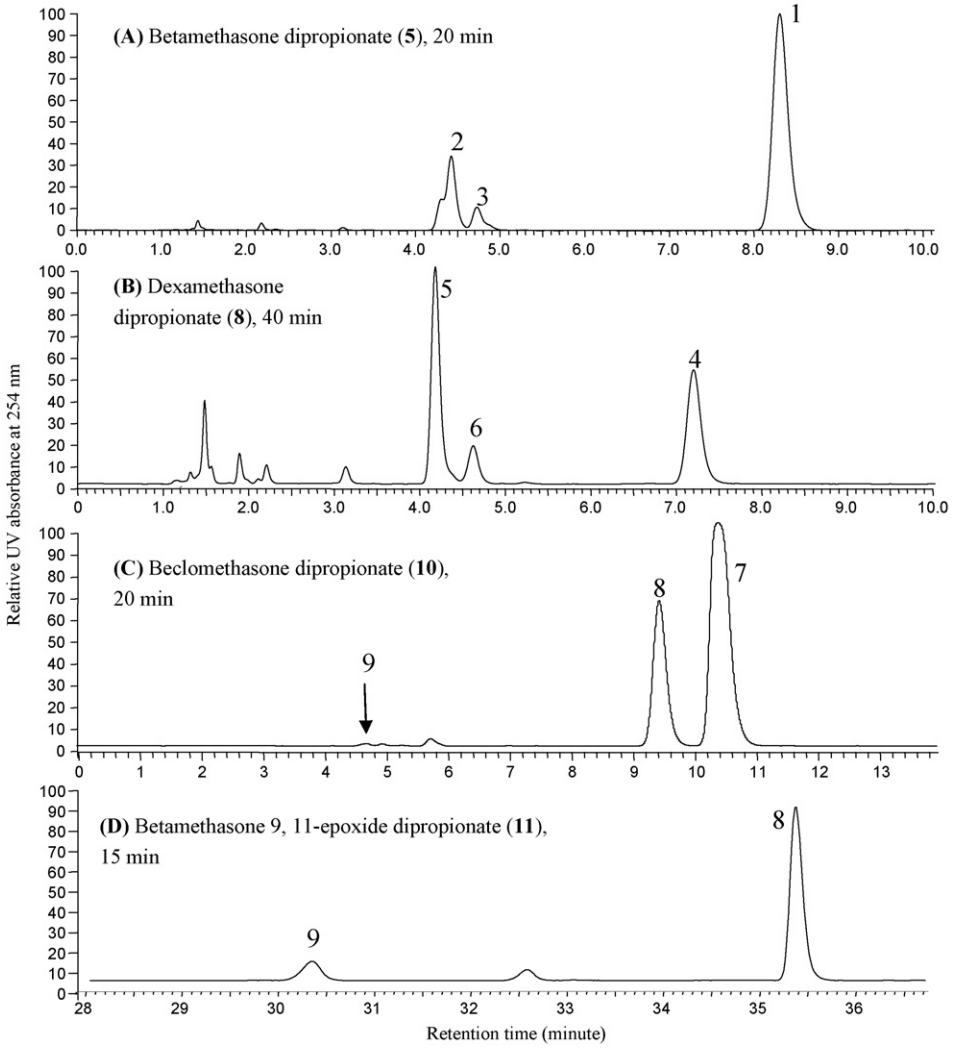


Fig. 5 – The study for generation of enol aldehyde in acetonitrile under alkaline condition from 5 (A), 8 (B), 10 (C), and 11 (D). The reactions were carried out at room temp and were monitored by LC–MS; the chromatograms were obtained under different conditions. For detailed analysis and reaction conditions, refer to Tables 1 and 2, respectively. *Peak identities*: (1) 5; (2) 4; (3) 3; (4) 8; (5) Dexamethasone enol aldehyde (13); (6) Dexamethasone 21-monopropionate; (7) 10; (8) 11; (9) 12 (both Z- and E-isomers).

ing betamethasone (**1**) and dexamethasone (**7**), the Z- and E-isomers of beclomethasone enol aldehyde were generated in a ratio very similar to what has been observed for **1** under the same reaction condition (Fig. 3C and A).

3.2. *Comparative study of enol aldehyde formation from betamethasone dipropionate (5), dexamethasone dipropionate (8), and beclomethasone dipropionate (10) under alkaline condition*

It has been recently reported by our group that betamethasone enol aldehyde can be readily generated directly from betamethasone 17,21-dipropionate (**5**) at room temperature under alkaline condition [10]. In this revised version of Mattox rearrangement, the process appears to be concerted: 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. Approximately 20–30% of **5** was converted to both isomers of betamethasone enol aldehyde (**3** and **4**) within 20 min at room temperature (Fig. 5A). Interestingly, the ratios of the two betamethasone enol aldehyde isomers formed under the alkaline condition are different from that under the acidic condition. While the E- and Z- isomers of betamethasone enol aldehyde (**3** and **4**) were formed in comparable yields from betamethasone (**1**) under the acidic condition (Fig. 3A), the Z-isomer of betamethasone enol aldehyde (**4**) was more favored from **5** under the alkaline condition, with the ratio of the Z-isomer (**4**) vs. the E-isomer (**3**) being ~3:1 (Fig. 5A).

3.2.1. *Dexamethasone dipropionate (8) vs. betamethasone dipropionate (5) under alkaline condition*

It appears that the same stereochemical consideration illustrated in Scheme 3 can also be applied to the generation of

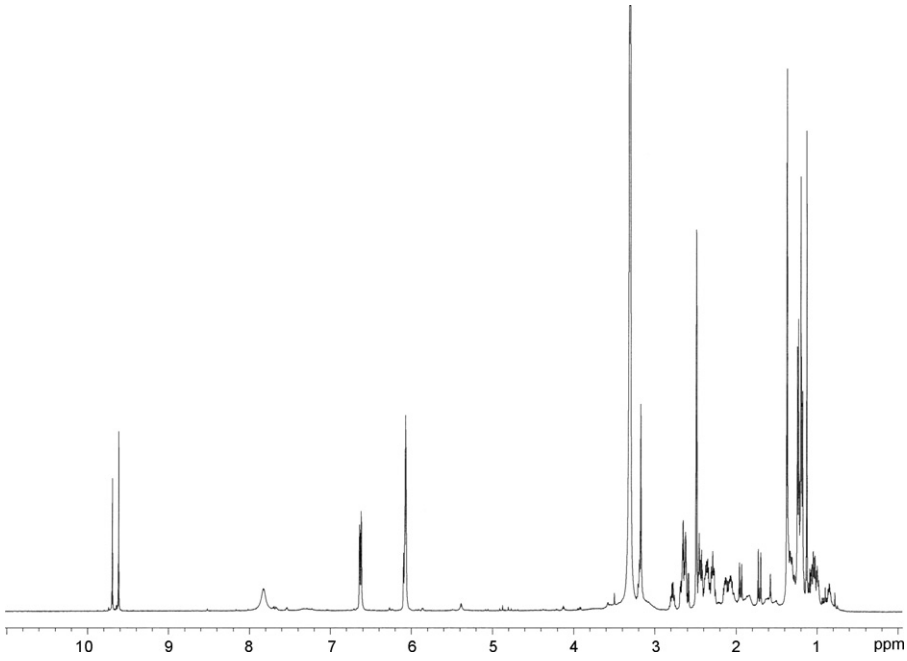


Fig. 6 – ^{1}H NMR spectrum of betamethasone 9,11-epoxide enol aldehyde (both *Z*- and *E*-isomers).

the enol aldehydes from **8** and **5** under the alkaline condition. Not surprisingly, the *Z*-isomer of dexamethasone enol aldehyde (**13a**) was generated from **8** (Fig. 5B) predominantly, while the *E*-isomer of betamethasone enol aldehyde (**3**) formed accounted for a more significant portion in the two isomers of betamethasone enol aldehyde (**3** and **4**) generated from **5** (Fig. 5A).

3.2.2. *Beclomethasone dipropionate (**10**) vs. betamethasone dipropionate (**5**) under alkaline condition*

When beclomethasone dipropionate (**10**) was treated under the same alkaline condition, nonetheless, no beclomethasone enol aldehyde was formed. Instead, only a trace amount of betamethasone 9,11-epoxide enol aldehyde (**12**) appeared to be produced (Peak 9 in Fig. 5C). The initial structure assignment was based on the molecular weight (Mw 354) expected of **12** and its characteristic UV profile. The predominant degradation product of **10** under the alkaline condition was betamethasone 9,11-epoxide 17,21-dipropionate (**11**, Peak 8 in Fig. 5C), which was formed from the 9,11-elimination of HCl from **10**. It became evident that **11** would further degrade via the variation of the Mattox rearrangement to produce **12**. Therefore, when a pure sample of **11** was subjected to the same alkaline condition, **12** was formed in ~13% yield within 15 min (Fig. 5D). Based on the stereochemistry of betamethasone 9,11-epoxide with regard to the β-orientation of its two methyl groups attached to the C-13 and C-16 positions, respectively (refer to Scheme 3 for similar stereochemistry), both the *Z*- and *E*-isomers of the enol aldehyde are expected to form. Nonetheless, only a single peak was observed which could be attributed to **12** based on its molecular weight and UV profile (Fig. 5D). One possibility would be that the *Z*- and *E*-isomers of **12** co-elute under the LC–MS condition used in this study. Thus, the enol aldehyde peak was isolated via semi-preparative HPLC and sent for NMR analysis. Indeed, the NMR results confirmed that the single peak contains both the *Z*- and *E*-isomers in a ratio of ~55:45 as shown in Fig. 6 [17].

3.3. *Solvent effect on the reaction rate of enol aldehyde formation from betamethasone under acidic condition*

The solvent effect on enol aldehyde formation under acidic condition was also investigated (Fig. 7). The reaction rates varied significantly in 1:1 acetonitrile:water, acetonitrile, and methanol. At room temperature, the reaction was very slow when betamethasone was dissolved in an acidified 1:1 (v/v) mixture of acetonitrile and water. Less than 5% of betamethasone enol aldehyde was generated after 20 h of reaction (Fig. 7A). When betamethasone was dissolved in an acidified acetonitrile solution, betamethasone enol aldehyde was generated at a much faster rate even though a much less amount of sulfuric acid was added to the reaction solution. The yield of betamethasone enol aldehyde reached ~15% within an hour at room temperature (Fig. 7B). On the other hand, when acetonitrile was replaced with methanol, there was essentially no reaction at room temperature, even after 3 days (Fig. 7C and D). These results suggest that an aprotic environment favors the formation of betamethasone enol aldehyde.

In summary, the comparative study of enol aldehyde formation from betamethasone, dexamethasone, and beclomethasone under the original acidic Mattox rearrangement condition and from their 17,21-diesters under the alkaline variation of Mattox condition has shown that the two mechanisms (the original Mattox and its variation) should be generally applicable for corticosteroids containing the 1,3-dihydroxyacetone side chain on their D-rings and their 17,21-diesters, respectively. Certain deviation and variation from the mechanisms can be explained by the subtle structural differences between these steroids. A clear understanding of the degradation pathways of these steroids and

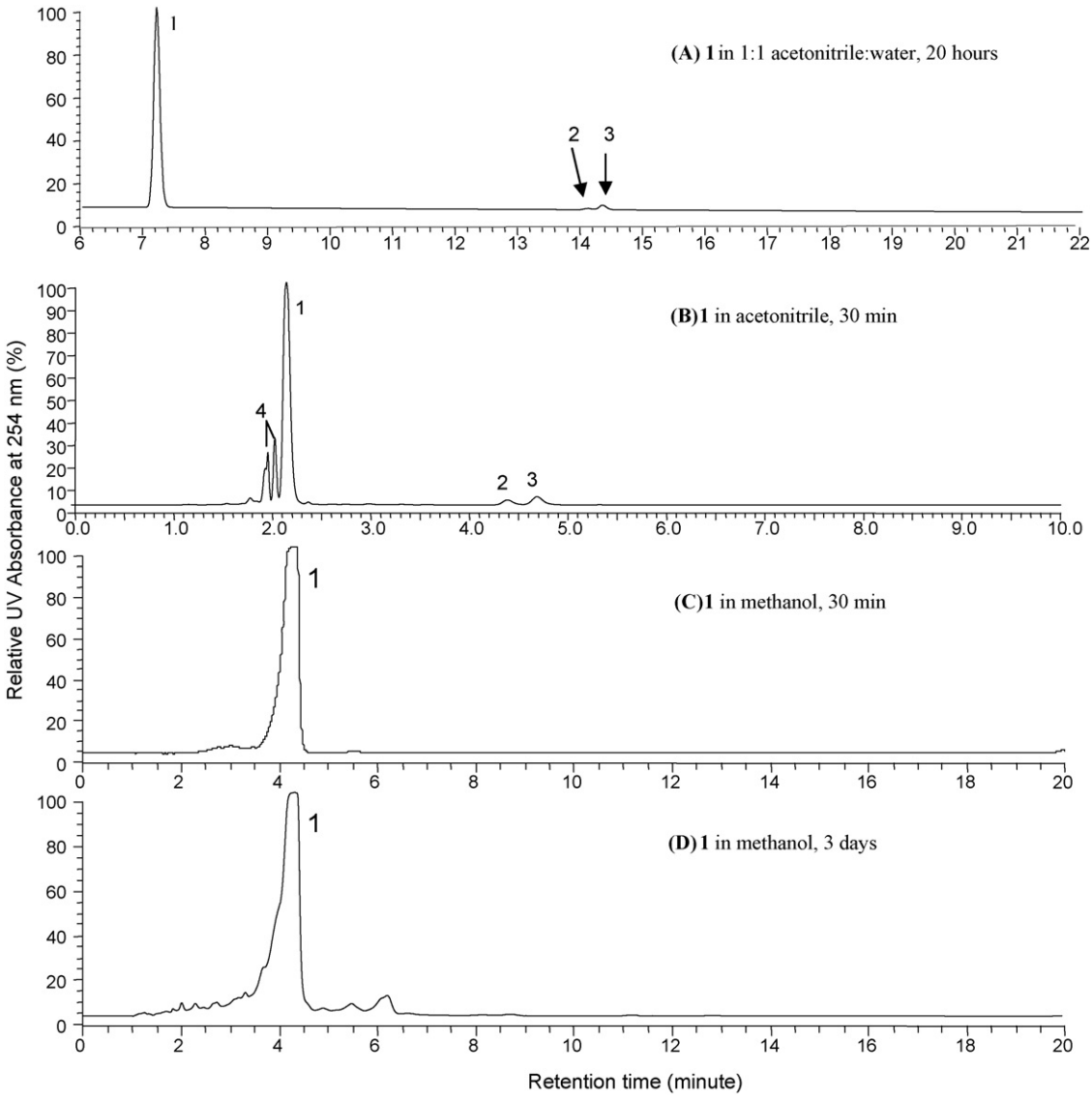


Fig. 7 – The study of solvent effect on generation of betamethasone enol aldehyde (3 and 4) from betamethasone (1) under acidic condition at room temp. (A) in 1:1 acetonitrile:water, 20 h; (B) in acetonitrile, 30 min; (C) in methanol, 30 min; (D) in methanol, 3 days. Chromatograms were obtained under different conditions. For detailed analysis and reaction conditions, refer to Tables 1 and 2, respectively. *Peak identities*: (1) Betamethasone (1); (2) Betamethasone enol aldehyde, *Z*-isomer (4); (3) Betamethasone enol aldehyde, *E*-isomer (3); (4) Betamethasone sulfates.

their 17,21-diesters is very important in maintaining and improving the quality of the drug products formulated with these drug substances.

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[17] The structures of both isomers were confirmed by ^1H NMR, ^{13}C NMR, 2D HSQC, and 1D NOE experiments. The determination of the ratio between the two isomers is based on the peak area of the Z- aldehyde signal (s, 9.62 ppm) vs. that of the *E*-aldehyde signal. (s, 9.70 ppm).