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Aerial oxidation of the glucocorticoid side-chain under pH control

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ABSTRACT

The outcome of aerial oxidation of the glucocortico-steroid side-chain (as exemplified by dexamethasone) has been shown to be subject to strict pH control. At pH 7.4 the glyoxal is the only product; at pH values of 8 and 9.2 the etioacid is formed, and at pH values of 13 or above the epimeric glycolic acids are produced. The glycolic acid epimer that predominates by a factor of 2 and is more stable has been shown by an X-ray crystal structural analysis to be the 20R compound. The presence of arsenite changes the course of the reaction and only the glycolic acids are yielded at pH values of 8 and above.

1. Introduction

The transformation of the hydroxy-ketone sidechain of glucocortico-steroid hormones and related steroids under alkaline conditions is complex with products depending on the presence or absence of oxygen, the presence or absence of a 17-OH group and the nature of the base or solvent used [1–4]. One aspect of this complicated picture is that aerial oxidation, or oxidation in the presence of oxygen, of the hydroxyketone side-chain has been reported to produce both etioacids and glycolic acids. Although the former were produced with dilute alcoholic NaOH or KOH and the latter with aqueous base, it was not clear to us what governed the reactions or why the outcomes should be different.

Early workers established that oxidative treatment of 21-hydroxy-20-ketosteroids yielded etioacids in the presence of air (or oxygen) and alkali [2,3]. The 21-acetates were studied and only enough alcoholic sodium or potassium hydroxide to saponify the acetate and bring about the cleavage was used (viz., two equivalents). There was no suggestion in their reports of the production of glycolic acids. The reaction was described as an oxidative cleavage and 1 mol of base and 1 mol of oxygen was required for the conversion of 1 mol of 21-hydroxy-20-ketosteroid to 1 mol of etioacid, with the concomitant generation of 1 mol of formate.

Lewbart and Mattox, on the other hand, reported that treatment of the glyoxal 3α-hydroxy-11,20-dioxo-5β-pregnan-21-al with aqueous sodium hydroxide yielded the epimeric pair of

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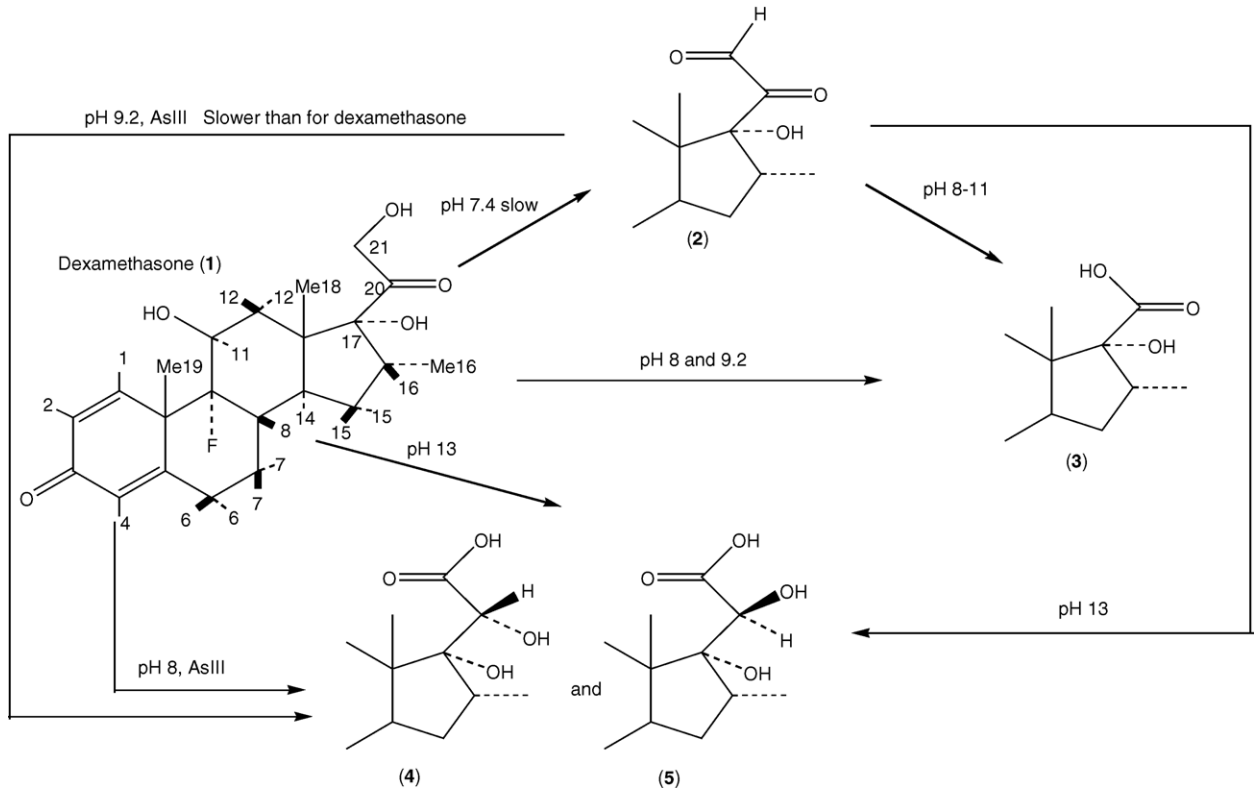


Scheme 1 – Transformations of dexamethasone (1) in aqueous buffers at elevated pH values and in the presence of arsenite. The protons, methyl groups and side-chain carbons in 1 are numbered (see Table 1).

20-hydroxy acids [5]. The same compounds were also obtained by cupric acetate oxidation of the glyoxal. Other papers have reported on non-specific degradation of corticosteroids [6].

Here, we report the results of the oxidation of the hydroxyketone sidechain of dexamethasone **1** (Scheme 1) in buffered aqueous solutions at pH values above neutrality and show that oxidation products are entirely dependent on pH. In addition to this, the recent report [7] that arsenic, specifically arsenite in very low doses, markedly affected glucocorticoid function prompted us to determine any direct interactions between arsenite and glucocorticoid hormones, as represented by **1**. Specifically we studied the effects of arsenite on the aerial oxidation of the dexamethasone side-chain.

2. Experimental

2.1. General

^{1}H NMR spectra were recorded in D_2O on a JEOL ECA 800 spectrometer (JEOL, Tokyo, Japan) operating at 800 MHz. Shifts are reported relative to external TMS. ^{1}H assignments were aided by ^{1}H–^{1}H COSY experiments. Fast atom bombardment mass spectrometry (FAB MS) was carried out on a JEOL JMS-700 GC/MS (JEOL, Tokyo, Japan). Benzoylformic acid, 2-hydroxyacetophenone, and DCl and NaOD solutions were obtained from the Aldrich Chemical Company, Tokyo, Japan. Dexamethasone and all other chemicals and solvents were obtained from Wako Pure Chemical Industries, Osaka, Japan. Meltings points were measured on an As-One ATM-01 melting point apparatus (As-One Co. Ltd., Osaka, Japan) and are uncorrected.

2.2. Buffers

Buffers (0.1 M) were made according to formulas listed in the literature [8,9]. Buffers of pH values 7.4 and 8.0 were made with phosphate, and those of 9.2 and 10.8 with carbonate/bicarbonate. The same quantities of reagents (weights of chemicals, volumes of solvent) were used for making buffers in D_2O as if they were being made in water. No consistent differences were found between readings made for buffers in D_2O and those in water [Piccolo 2 HI 1290 m with amplified pH electrode (Hanna Instruments, Portugal) and a ISFET KS723 solid state meter (Shindengen, Tokyo, Japan)] and the term pH is used here for measurements made in both D_2O and water with no correction for isotope effects.

2.3. Structure determination

The available material was microcrystalline and, data being insusceptible of access with a conventional charged-coupled device area detector, recourse was made to synchrotron instrumentation. A full sphere of data was measured ($\lambda = 0.550$ Å, $2\theta_{max} = 42^\circ$, T ca. 110 K) yielding 29,512 reflections, these merging to 2637 unique after 'empirical'/multiscan 'absorption correction' ($\mu = 0.064\ mm^{-1}$; 'T'$_{min/max} = 0.90$), 2353 being used in the full matrix least squares refinement,

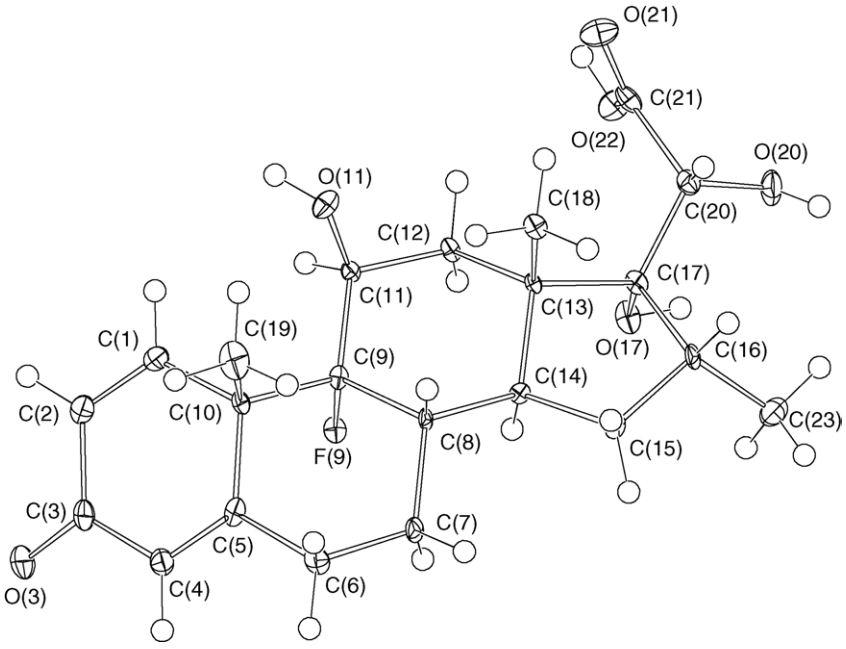


Fig. 1 – Molecular projection of dexamethasone glycolic acid 4.

anisotropic displacement parameters being refined for C, O, F, (x, y, z, U_{iso})$_H$ being constrained at estimates after location of the hydroxylic hydrogen atoms in difference maps. Conventional residuals on $|F|$ at convergence were $R = 0.060$, $R_w = 0.080$ (weights: $(\sigma^2(F) + 0.0004\,F^2)^{-1}$). Neutral atom complex scattering factors were employed within the Xtal 3.7 program system [10], chirality of the structure being assigned from the chemistry. A molecular projection is shown in Fig. 1 (50% probability amplitude displacement ellipsoids for C, O, F, hydrogen atoms being shown with arbitrary radii of 0.1 Å); a full .cif deposition (excluding structure factor amplitudes) has been made with the Cambridge Crystallographic Data Centre, #271377.

2.3.1. *Crystal data*

$C_{22}H_{31}FO_7$, $M = 426.5$. Orthorhombic, space group $P2_12_12_1$ (D_2^4, No. 19), $a = 6.022(5)$, $b = 12.731(5)$, $c = 25.946(5)$ Å, $V = 1989$ Å^3. D_c ($Z = 4$) = 1.42$_4$ g cm^{-3}.

2.3.2. *Comment*

The conformation and geometry of the polycyclic skeleton are essentially similar to those of dexamethasone [11].

The compound crystallizes as a monohydrate, in a structure which displays widespread intra- and inter-molecular hydrogen-bonding from/between the hydroxylic and carbonyl entities (but not the fluorine): $H_O(11)\ldots O(3)(2-x, \frac{1}{2}+y, \frac{1}{2}-z)$ 2.0; $H_O(17)\ldots O(20)$ 2.2; $H_O(20)\ldots O(22)(x-\frac{1}{2}, 1\frac{1}{2}-y, 1-z)$ 2.1; $H_O(22)\ldots O(w)(1+x, y, z)$ 1.7; H(wa)…O(21) 1.9; H(wb)…O(3)($x-1, y+1, z$) 1.9 Å (all est.).

2.4. *Reactions in NMR tubes*

Reactions were initially carried out in NMR tubes and were mainly followed by observation of the 16-methyl signals. Following this, reactions were carried on a sufficient scale to provide product compounds for characterization. After identification of the products, further experiments were carried out in NMR tubes and followed by ^{1}H NMR spectroscopy to refine information on the transformations observed. These included transformations carried out in the presence of arsenite. Sodium meta-arsenite solutions in D_2O were prepared immediately before use. Reactions to be followed by ^{1}H NMR spectroscopy were carried out at 25 °C in capped NMR tubes (5 mm dia), no effort being made to exclude atmospheric oxygen. Saturated solutions of **1** were made by ultrasonication of an excess of the compound in the appropriate buffer in D_2O (2 ml) for 5 min. A portion (1 ml) of the solution was then filtered into an NMR tube. This solution was assumed to be saturated (i.e., 100 μg/ml, 0.25 mM [12]). Experiments in NMR tubes were carried out with phosphate buffer (pH values of 7.4 and 8.0) and carbonate/bicarbonate buffer (pH values 9.2 and 10.8) and in 0.05 M sodium deuteroxide in D_2O. Titrations of chemical shifts (δ values) against pH were carried out with acidic compounds to provide additional information (Fig. 2a–c). pH values of D_2O solutions used in these titrations were adjusted with NaOD and DCl solutions. Preliminary identification of products in these NMR experiments were confirmed by the following syntheses.

2.4.1. *Dexamethasone glyoxal (2)*

A solution of **1** (50 mg, 0.13 mmol) in phosphate buffer (500 ml, 0.1 M, pH 7.4) was left at 25 °C for 28 days. After this time the ^{1}H NMR spectrum showed that **1** had been completely converted to a single compound. The solution was evaporated to dryness (<40 °C) and the residue extracted with methanol. The methanol extract was then subjected to size-exclusion chromatography (SEC) on Sephadex LH-20 (elution with methanol) and dexamethasone glyoxal (**2**, 33 mg, 66%) obtained as a white crystalline powder (Mp 126 °C, lit. 127–130 [13]). ^{1}H NMR (Table 1); FAB + MS (methanol solution) *m*/*z* 423 (hemiacetal, M + 1).

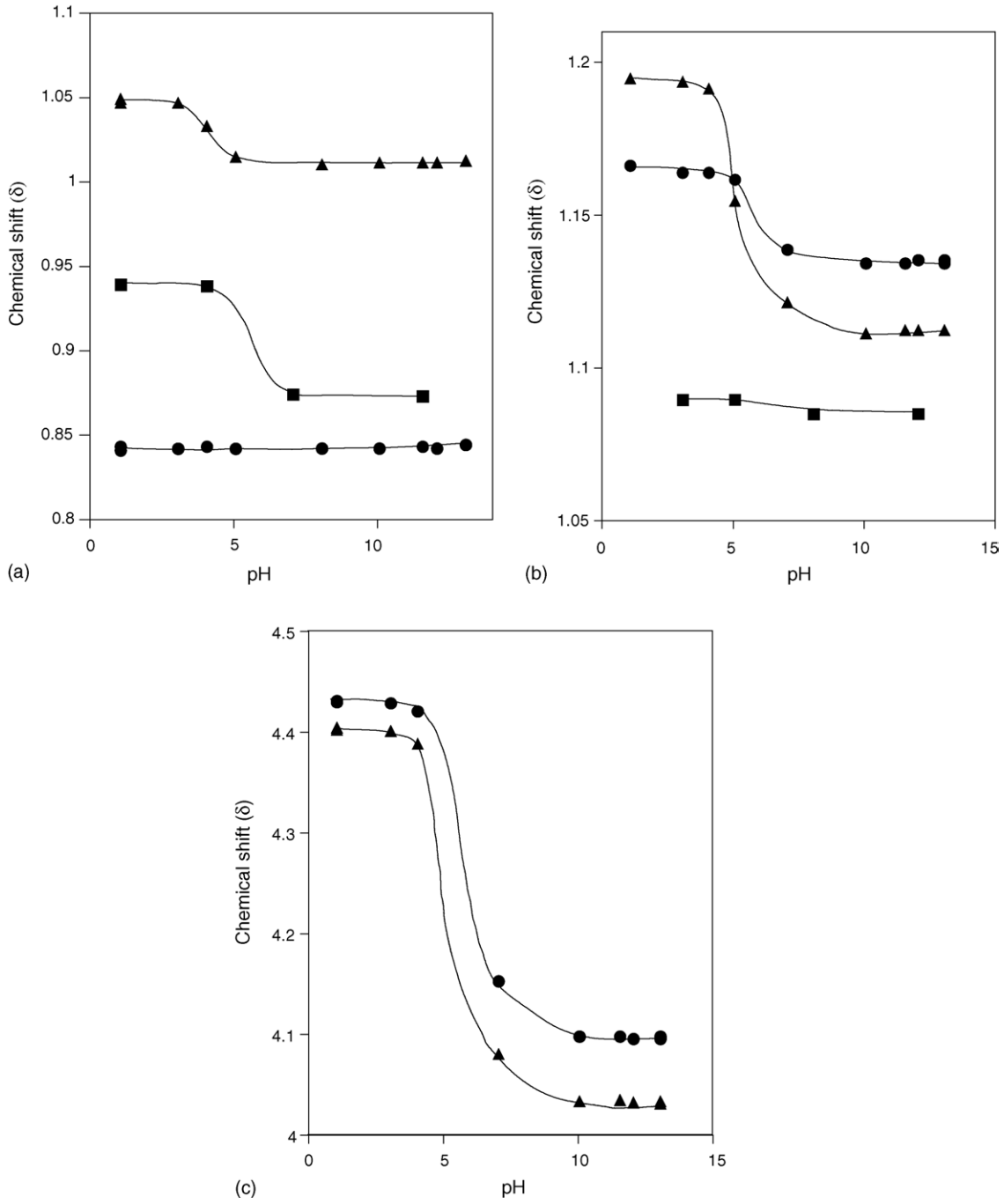


Fig. 2 – Plots of chemical shift (δ) against pH for (a) 16-methyl group protons, (b) 18-methyl group protons, and (c) protons on C20. Squares, etio acid, compound 3; triangles, glycolic acid, compound 4; circles, glycolic acid, compound 5.

2.4.2. Dexamethasone etioacid (3)

A solution of **1** (50 mg, 0.13 mmol) in carbonate/bicarbonate buffer (500 ml, 0.1 M, pH 9.2) was left to stand at room temperature overnight. The solution was then evaporated to dryness (<40 °C), the residue extracted with methanol and subjected to SEC as for glyoxal **2**. Dexamethasone etioacid **3** was obtained as a white powder (34 mg, 71%; recrystallized from acetone/light petroleum to give colorless needles, Mp >260 °C decomp., lit. >258 °C decomp. [14]). ^{1}H NMR (Table 1); FAB-MS *m*/z (M − 1) 377.

2.4.3. Dexamethasone epimeric glycolic acids (4 and 5)

A solution of **1** (100 mg, 0.25 mol) in 0.05 M aqueous sodium hydroxide (1 l) was left at room temperature overnight. The pH of the solution was reduced to 7 (HCl) followed by evaporation to dryness (<40 °C). The residue was subjected to fractional crystallization (aqueous methanol) and the least soluble epimer **4** obtained as colorless needles (37 mg, Mp 254–256 °C); the more soluble epimer **5** obtained as small colorless prisms (7 mg, Mp >300 °C). NMR data for both compounds are given in Table 1. High resolution FAB + MS, compound **4**,

Table 1 – ^{1}H NMR data for dexamethasone (1) and its derivatives

Proton	Compound						
	Dexamethasone, **1**, D_2O	Dexamethasone glyoxal, **2**, D_2O	Dexamethasone etioacid, **3**, D_2O unbuffered	Dexamethasone glycolic acid, **4**, methanol-d_4	Dexamethasone glycolic acid, **5**, methanol-d_4	Dexamethasone 17-ketones	
1	7.543, d, $J_{1,2}$ = 10.1 Hz	7.546, d, $J_{1,2}$ = 11.0 Hz	7.568, d, $J_{1,2}$ = 10.1 Hz	7.419, d, $J_{1,2}$ = 10.1 Hz	7.419, d, $J_{1,2}$ = 10.1 Hz		
2	6.437, dd, $J_{1,2}$ = 10.1 Hz, $J_{2,4}$ = 1.8 Hz	6.442, $J_{1,2}$ = 11.0 Hz, $J_{2,4}$ = 1.8 Hz	6.432, dd, $J_{1,2}$ = 10.1 Hz, $J_{2,4}$ = 1.8 Hz	6.265, dd, $J_{1,2}$ = 10.1 Hz, $J_{2,4}$ = 1.8 Hz	6.267, dd, $J_{1,2}$ = 10.1 Hz, $J_{2,4}$ = 1.8 Hz		
4	6.244, d, $J_{2,4}$ = 1.8 Hz	6.247, d, $J_{2,4}$ = 1.8 Hz	6.239, d, $J_{2,4}$ = 1.8 Hz	6.065, d, $J_{2,4}$ = 1.8 Hz	6.059, d, $J_{2,4}$ = 1.8 Hz		
6_A	2.487	2.478	2.486	2.385	2.381		
6_B	2.735	2.738	2.742	2.706	2.699	2.765	2.765
7_A	1.490	1.490	1.489	1.498	1.512		
7_B	1.985	1.989	1.990	1.880	1.868		
8	2.564	2.578	2.513	2.413	2.411		
11	4.410	4.396	4.365	4.168	4.170		
12_A	1.564	1.615	1.511	1.639	1.747		
12_B	2.188	2.199	2.059	2.015	2.073		
14	2.115	2.174	1.956	2.061	2.008		
15_A	1.289	1.302	1.232	1.127	1.156		
15_B	1.843	1.858	1.808	1.705	1.668		
16	3.049	3.068	2.975	2.483	2.558	2.611	2.152
16Me	0.889, d, 7.3 Hz	0.894, d, 7.3 Hz	0.885, d, 7.3 Hz	1.074, d, 7.3 Hz	0.918, d, 7.3 Hz	1.059, d, 7.4 Hz	1.209, d, 7.4 Hz
18Me	0.989	1.024	1.093	1.176	1.176	1.139	1.233
19Me	1.567	1.566	1.576	1.578	1.584	1.613	1.613
20				4.180	3.925		
21_A	4.687, d, $J_{A,B}$ = 18.7 Hz						
21_B	4.416, d, $J_{A,B}$ = 18.7 Hz						

m/z (M + 1) 409.2013, required for $C_{22}H_{30}FO_6$ 409.2027; compound **5**, *m/z* (M + 1) 409.2015, required for $C_{22}H_{30}FO_6$ 409.2027. Also isolated by SEC from the liquors remaining after crystallization were 9-fluoro-11β-16α-methylandrosta-1,4-diene-3,17-dione and 9-fluoro-11β-16β-methylandrosta-1,4-diene-3,17-dione; ^{1}H NMR (Table 1). FAB-MS *m/z* (M − 1) 331; FAB + MS 333.

2.4.4. *Dexamethasone (**1**) to epimeric glycolic acids (**4**,**5**) in the presence of arsenite*

Sodium meta-arsenite (5 μl of 0.5 M solution in D_2O) was added to a saturated solution of **1** in carbonate/bicarbonate buffer in D_2O (1 ml, 0.1 M, pH 9.2) and the sample transferred to an NMR tube. The arsenite concentration was 2.5 mM, 10 times that of the steroid. ^{1}H NMR spectra were recorded at intervals appropriate to the rate of transformation of **1**. The pH value of 9.2 represents a typical reaction but essentially similar results were obtained in the pH range of 8–10.8.

2.4.5. *Dexamethasone glyoxal (**2**) to etioacid (**3**)*

A saturated solution of **2** in carbonate/bicarbonate buffer in D_2O (1 ml, pH 9.2) was placed in an NMR tube and the conversion of the glyoxal to the etioacid monitored by ^{1}H NMR spectrsocopy. Dissolution with a pH of 8 gave a similar result.

2.4.6. *Dexamethasone glyoxal (**2**) to glycolic acids (**4**,**5**)*

Compound **2** (about 100 μg) was dissolved in 0.05 M NaOD in D_2O and the conversion of the glyoxal to the epimeric glycolic acids monitored by ^{1}H NMR spectroscopy.

2.4.7. *Dexamethasone glyoxal (**2**) to glycolic acids (**4**,**5**) in the presence of arsenite*

Sodium meta-arsenite (5 μl of 0.5 M solution in D_2O) was added to a saturated solution of **2** in carbonate/bicarbonate buffer in D_2O (1 ml, pH 9.2). the arsenite concentration was 2.5 mM, approximately 10 times that of the steroid. The solution was tranferred to an NMR tube and the conversion of the glyoxal to the glycolic acids monitored by ^{1}H NMR spectroscopy. Solutions with pH values of 8 and 10.8 gave essentially the same results.

2.4.8. *2-Hydroxyacetophenone with sodium deuteroxide*

The reactions of a solution of 2-hydroxyacetophenone (0.5 mg) in 0.05 M NaOD in D_2O (1 ml) were monitored by ^{1}H NMR spectroscopy. Signals for 2-hydroxyacetophenone were diminished with the concomitant appearance of peaks readily attributable to benzoic acid and mandelic acid. Fifty percent of the 2-hydroxyacetophenone had been transformed after 10 h at 25 °C. About 70% of the transformed 2-hydroxyacetophenone was converted to benzoic acid and 30% to mandelic acid. Reactions carried out in the dark and in normal laboratory lighting gave the same results.

2.4.9. *Benzoylformic acid with sodium deuteroxide*

A solution of benzoylformic acid (0.5 mg) in 0.05 M NaOD in D_2O (1 ml) was monitored by ^{1}H NMR spectroscopy. No reaction was observed.

3. Results and discussion

Aerial oxidation of **1** in aqueous solution at pH 7.4 was slow (weeks) and yielded the glyoxal (Scheme 1, **2**); the reaction did not proceed further. At pH values of 8 and 9.2 the etioacid **3** was the only product of oxidation of both **1** and **2**. At pH values in excess of 12 the glycolic acids (**4**) and (**5**) were the only products of the oxidation of **1**. An NaOH concentration of 0.05 M was sufficient for the reaction to be completed in 0.5 h. As these reactions were followed by monitoring the ^{1}H NMR spectra, in particular of the 16- and 18-Me groups of **1** and its transformation products, we carried out a full examination of the dependence of the chemical shifts of the resonances attributed to these groups on pH. The results are plotted in Fig. 2a for the 16-methyl groups on the etio acid **3** and the glycolic acids **4** and **5**, and in 2b for the 18-methyl groups on the three compounds. In addition, the chemical shifts of the protons at C20 were diagnostic in the identification of compounds **4** and **5**, and Fig. 2c shows the dependence of the chemical shift of these protons on pH for the two compounds. The approximate p*K* values (typical of carboxylic acids at around 5) can be read from the plots.

The two glycolic acid epimers (**4**) and (**5**) were not produced in equal quantity; the major product contributed about 65% of the total (see below). Exposure of an aqueous solution of **1** to a pH of 11 yielded a mixture of both the etioacid **3** and the glycolic acids **4** and **5** with the latter compounds again appearing in a 2:1 ratio.

It was proposed [5] that the conversion of hydroxyketone (via the glyoxal) to the epimeric pair of glycolic acids involved an intramolecular Cannizzaro reaction. Support for this proposal came from the observation that 17-OH steroids, in which 17(20) enolization is not possible, underwent the reaction at a greater rate and under milder conditions (1.25 M alkali, 0.5 h) than 17-deoxy compounds (4 M alkali, 8 h). We have found that **1**, in saturated solution in 0.05 M aqueous NaOH, is fully converted to the epimeric glycolic acids **4** and **5** in 0.5 h. Although these are mild conditions for a Cannizzaro reaction, evidence for the essential hydride shift was provided by carrying out the reaction in D_2O/NaOD. That the C20s in the new compounds bore hydrogens rather than deuteriums was evident from the ^{1}H NMR spectra. The involvement of a Cannizzaro-type reaction would require that the first stage to be the conversion of the hydroxyketone steroid to its glyoxal and, indeed, **2** (made by aerial oxidation of **1** at pH 7.4) yielded **4** and **5** under base treatment at the same rate as did **1** itself.

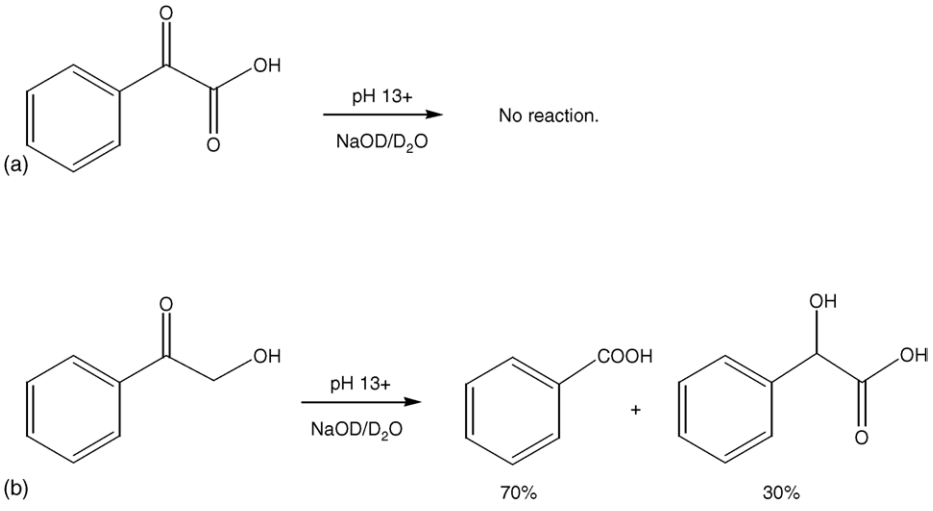
The epimers **4** and **5**, synthesized on a preparative scale in 0.05 M NaOH, were separated by fractional crystallisation and an X-ray structure analysis showed that the major compound **4** had the stereochemistry shown in Scheme 1 and Fig. 1 (20R). Simple modelling indicated that formation of the 20R compound was likely to be favoured over the 20S epimer because of steric factors. Isolation and crystallization of the minor glycolic acid **5** was problematical; the compound was apparently labile—much more so than its epimer **4**—with degradation by loss of the side-chain to yield further compounds, provisionally identified as 9-fluoro-11β-16α-methylandrosta-1,4-diene-3,17-dione and 9-fluoro-11β-16β-methylandrosta-1,4-diene-

3,17-dione (by ^{1}H NMR and FAB MS of an unseparated mixture and by analogy with the 3(α),17(α),21-trihydroxypregnane-11,20-dione series [4]). These 17-ketones have previously been identified as impurities in commercial samples of dexamethasone sodium phosphate [15]. It was not clear to us why this should occur under the reaction conditions employed, although it has been reported that the hydroxyketone side-chain of glucocorticoid steroids undergo retrograde aldol reactions to yield such ketones when treated with alkali in the absence of air. Nor was it clear why the 20S epimer should be so much less stable than its 20R epimer. It was previously noted that the formation of the methyl esters of the analogous glycolic acids (by cupric acetate in methanol) in the prednisolone series favours the formation of one epimer over the other [16]. However, those authors reported, with no obvious basis, that the 20S compound predominated [16]. A later publication from the same group described the conversion of **1**, as well as prednisolone, to methyl esters of their glycolic acids [17]. Both epimers were synthesized but only the 20S compound was further reacted; it was hydrolyzed to the free acid with methanolic KOH. Possibly the epimer considered to be the 20R compound was omitted from this step because of its lability. Again no reason was given for the assignment of the stereochemistry of the 20 carbon. We believe, on the basis of the X-ray crystal structural determination of **4** that the stereochemical assignments [16,17] in both the prednisolone and dexamethasone series need revision; the predominant and stable epimer in the dexamethasone series is the 20R compound **4** and it likely to be so in the prednisolone series also. With regard to the stability of steroidal glycolic acids of this type and their methyl esters, it is also noteworthy that Lewbart and Mattox [5] made no mention of any instability of either of the epimeric 20-hydroxyacids derived from 3α-hydroxy-11,20-dioxo-5β-pregnan-21-al (i.e., an analogous compound but lacking a 17-hydroxyl group) by treatment with aqueous NaOH.

We next turned our attention to possible effects of arsenite on these transformations and found that the addition of a 10 times molar excess of arsenite to aqueous solutions of **1** at pH values of 8–11 caused the course of the oxidation to change completely, the glycolic acids **4** and **5** being the only products; the etioacid **3** was not produced at all.

In order to explain the manner in which arsenite subverts the course of the oxidative cleavage reaction that yielded **3**, we sought a more detailed explanation of its mechanism. Several possibilities were considered including the decarboxylation or decarbonylation of an intermediate ketoacid and a photochemical cleavage. 2-Hydroxyacetophenone served as a model compound for this conversion but was rather less reactive than the steroid; at pH values of 12 or less reaction was very slow and at pH 13 or higher it yielded about 65% benzoic acid and 35% mandelic acid with reaction complete in a few hours at room temperature (Scheme 2), offering parallels, then, with the steroid conversion to both the etioacid and the glycolic acids. However, benzoylformic acid proved to be stable in aqueous solution at pH values in excess of 13 (Scheme 2) and therefore could not be an intermediate in the transformation of 2-hydroxyacetophenone to benzoic acid. Thus the possibility of a ketoacid intermediate in the generation of the etioacid was eliminated. We then investigated the possible reaction of singlet oxygen with the double bond of the hydroxy-keto side-chain in enolate form, and considered whether the enolate might function as a photosensitizer and possibly provide an adequate chromophore for laboratory lighting. However, the conversion of 2-hydroxyacetophenone to benzoic acid/mandelic acid at pH 13+ occurred at the same rate in the dark as in normal laboratory light, so the involvement of singlet oxygen was also shown to be unlikely. Details of the mechanism remain obscure.

How then does arsenite cause the production of glycolic acids rather than the etioacid at pH values of 8 and 9.2? We considered the possibility that the oxidation of arsenite to arsenate exhausts the available oxygen in the capped NMR tubes in which the reactions were carried out, and thereby renders the production of the etioacid (which requires oxygen) impossible, and opens the pathway to the glycolic acids. However the reported [18] rate of oxidation of of arsenite to



Scheme 2 – The reactions of benzoylformic acid and 2-hydroxyacetophenone with aqueous sodium deuteroxide.

arsenate at the relevant pH values would be much too slow to support such an explanation. Possibly arsenite complexes in some way with the oxygen functions of the glucocorticoid sidechain and promotes the reaction to the glycolic acids and/or hinders the production of the etioacid. There are two observations that may be relevant. First, at pHs 8 and 9.2 in the presence of arsenite the glycolic acids **4** and **5** are produced in exactly equal amounts (as opposed to the 2 to 1 ratio observed when **1** was subjected to pH 13+). And second, subjection of the glyoxal **2** to arsenite at pH 9.2 produced **4** and **5** at a slower rate than did **1** itself when subjected to the same conditions. This implies that, in the presence of arsenite at pH values of 8 and 9.2, the route to the glycolic acids is not through its glyoxal and raises the unexpected possibility that the mechanism for the production of **4** and **5** from **1** at pH 13+, and at pH values 8 and 9.2 in the presence of arsenite, might be different – through its glyoxal in the former case but not in the latter. However, when the reaction in the presence of arsenite is carried out in D_2O a proton rather than a deuteron is still gained at C20, so that a hydride shift is involved in the transformation.

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