Identification and Analysis of a Degradation Product of the Glucocorticoid Desonide in Ointment

Thuy T. Nguyen,[a] Randi Kringstad,[a] Arne J. Aasen[b,*] and Knut E. Rasmussen[c]

[a]Pharmaceutical Quality Assurance, Apothekernes Laboratorium A.S., Box 158, Skøyen, N-0212 Oslo 2, [b]Department of Chemistry, The Agricultural University of Norway, Box 30, N-1432 Ås-NLH and [c]Department of Pharmacy, University of Oslo, Box 1068, Blindern, N-0316 Oslo 3, Norway

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The major degradation product of desonide in a pharmaceutical ointment formulation has been shown to be identical with the *C*-17-carboxylic acid obtained on oxidative cleavage of the α-ketol group of desonide with alkaline hydrogen peroxide. The pK_a value of this acid has been estimated from chromatographic data.

Desonide (**1**) has been used in dermatological therapy for several years. As for other 21-hydroxyglucocorticoids, little is known about its stability in semi-solid dosage forms. More stability data are, however, available on 21-hydroxyglucocorticoids in solutions; examples are prednisolone,[1,2] hydrocortisone,[3,4] triamcinolone acetonide[5] and cloprednol.[6] These papers give useful information for evaluation of results from semi-solid studies. In general, it is accepted that decomposition most likely occurs in the *C*-17,21-dihydroxyacetone side chain. Oxidation as well as non-oxidative reactions have been described.[7–10]

In our laboratories a stability study of desonide is in progress. This paper describes the identification and properties of an acidic degradation product (**2**) of desonide isolated from unbuffered ointment formulations. The acidic degradation product has been shown by spectroscopic and chromatographic data to be identical with the *C*-17-carboxylic acid obtained on oxidative cleavage of the α-ketol group of desonide with alkaline hydrogen peroxide.

Experimental

General. Methanol, hexane, chloroform, ethyl acetate, acetone and acetic acid of analytical grade were obtained from E. Merck (Darmstadt, FRG). Desonide was purchased from Sicor (Milan, Italy). Desonide ointments formulated for research purposes were obtained from *Apothekernes Laboratorium A.S. (Oslo, Norway).*

The liquid chromatograph employed was a model 6000A (Waters Assoc., Milford, MA, USA) equipped with an SP 8780 XR autosampler (Spectra Physics, San José, CA, USA) with a 20 µl sample loop, a UV detector Model 440 (Waters Assoc.) with a fixed wavelength of 254 nm and an SP 4270 integrator (Spectra Physics). Samples were chromatographed on C-18 Spheri-5-Brownlee Labs. MPLC™ cartridges (Santa Clara, CA, USA), and the guard column (30×4.6 mm) was connected directly to the analytical column (100×4.6 mm). The mobile phase was methanol–acetate buffer (60:40) and the flow rate 1.2 ml min^{-1}. The analyses were carried out at ambient temperature. 14 buffer solutions in the pH range 2.2–7.00 were prepared by titrating 0.02 M acetic acid with 1 M NH_3 to the desired pH.

Preparative TLC was carried out on 0.25 mm silica gel plates developed in methanol–chloroform (2:98).

*To whom correspondence should be addressed.

Electron impact mass spectra were obtained with a VG Micromass 7070F mass spectrometer via direct inlet. The probe temperature was 220 °C and the ion source potential was 70 eV. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker WM-400 spectrometer and IR on a Perkin Elmer 598 instrument.

Sample preparation and isolation of degradation products. Desonide and its degradation products were extracted from the ointment by a liquid–solid extraction procedure utilizing Bond Elut™ silica cartridges (Analytichem International, Harbour City, CA, USA). The procedure has been described in detail in an earlier report.[11] The ointment (0.25–0.30 g) was dissolved in hexane–chloroform (80:20, 25 ml) and 0.5 ml was added to the Bond Elut cartridge filled with silica (100 mg). The ointment base was washed from the column with three column volumes of hexane, and desonide and its decomposition products were eluted with methanol (2×0.5 ml). The methanol eluate was injected directly into the liquid chromatograph. Degradation products of desonide were collected for recording of mass spectra. Fractions were collected from 50 injections of the sample, and were concentrated on a rotary evaporator and freeze-dried overnight. The residues were dissolved in methanol and the solutions evaporated to dryness under a stream of nitrogen.

11β,21-Dihydroxy-16α,17α-[(1-methylethylidene) bis(oxy)]pregna-1,4-diene-3,20-dione; desonide (**1**). ^{13}C NMR ($CDCl_3$): δ 17.2, 21.0, 25.5, 26.6, 30.5, 32.0, 34.0, 34.2, 40.6, 44.4, 45.9, 50.0, 55.5, 67.0, 69.7, 81.9, 97.5, 111.4, 122.5, 127.8, 157.1, 171.0, 187.2, 210.8.

11β-Hydroxy-16α,17α-[(1-methylethylidene)bis (oxy)]-3-oxoandostra-1,4-diene-17β-carboxylic acid (**2**). To a solution of desonide (**1**; 124 mg, 0.3 mmol) in methanol (4 ml) and hydrogen peroxide (0.1 ml of a 30 % solution) was added 1 M KOH in methanol (1 ml, 1 mmol) and the mixture stirred at ambient temperature for 2 h. Water (25 ml) was added to the reaction mixture which was then extracted twice with dichloromethane (25 ml). The extract was dried over anhydrous sodium sulfate and concentrated *in vacuo*, furnishing a neutral fraction (see below). The alkaline aqueous fraction was acidified with 1 M HCl to pH 2 and extracted with ethyl acetate (30 ml). The extract was dried over anhydrous sodium sulfate and concentrated to dryness, furnishing a solid residue (105 mg); yield 88 %. M.p. 266–269 °C; $[\alpha]_D^{20}$ +85.6° (*c* 0.9, CH_3OH); *m/z* (%): 402 (M^+, 6), 281 (28), 225 (18), 122 (100), 121 (45), 91 (19), 43 (63), 41 (32); accurate mass determination: 402.2053; calc. for $C_{23}H_{30}O_6$: 402.2042; IR (KBr): 3445 (m), 2930 (m), 1718 (s), 1645 (s), 1598 (s), 1255 (m), 1209 (m), 1163 (m), 1087 (m), 1045 (s) cm^{-1}; ^{1}H NMR (CD_3OD): δ 1.06 (3H, s), 1.22, 1.37 (3H, s), 1.49 (3H, s), 1.5–1.7 (m), 1.83 (2H, m), 2.1–2.3 (m), 2.39 (m), 2.68 (m), 4.39 (1H, m), 5.05 (1H, broad s), 6.01 (1H, s), 6.25 (1H, dd, *J* 1.7 and 10.1 Hz), 7.46 (1H, d, *J* 10.1 Hz); ^{13}C NMR (CD_3OD): δ 18.1, 21.9, 25.9, 27.2, 32.2, 33.4, 35.3, 35.9, 41.3, 46.3, 47.2, 50.8, 57.7, 70.9, 83.7, 95.8, 112.3, 122.9, 128.2, 160.1, 174.6, 175.9, 189.1.

11β-Hydroxy-16α,17α-[(1-methylethylidene)bis (oxy)]-3-oxoandosta-1,4-diene (**3**). The neutral fraction (see above) was subjected to preparative TLC, furnishing unreacted desonide (3 mg) and a less polar fraction (8 mg) which crystallized as prisms from methanol; yield 7.5 % based on the original amount of desonide. M.p. 285–286 °C; *m/z* (%): 358 (M^+, 11), 237 (57), 161 (24), 147 (26), 122 (100), 121 (59), 91 (21), 43 (51), 41 (36); accurate mass determination: 358.2143; calc. for $C_{22}H_{30}O_4$: 358.2144; IR (KBr): 3400 (m), 2890 (m), 1649 (s), 1609 (s), 1202 (m), 1162 (m), 1046 (s) cm^{-1}; ^{1}H NMR (CD_3OD): δ 1.01 (3H, s), 1.24 (3H, s), 1.33 (3H, s), 1.49 (3H, s), 1.5–1.6 (m), 1.69 (m), 2.1–2.2 (m), 2.36 (m), 2.64 (m), 4.02 (1H, d, *J* 5.5 Hz), 4.38 (1H, m), 4.66 (1H, t, *J* 5.4 Hz), 6.00 (1H, s), 6.24 (1H, dd, *J* 1.7 and 10.1 Hz), 7.46 (1H, d, *J* 10.1 Hz); irradiation at δ 4.02 simplified the triplet at δ 4.66 to a doublet; due to poor solubility in CD_3OD the solution was concentrated to dryness and redissolved in $CDCl_3$ prior to the recording of the ^{13}C spectrum. ^{13}C NMR ($CDCl_3$): δ 19.8, 21.1, 23.9, 26.0, 30.5, 32.0, 34.1, 34.2, 41.6, 42.8, 44.1, 47.5, 55.7, 70.54 [C(11)–OH], 70.39 [C(11)–OD; cf. Ref. 12], 79.5, 86.5, 109.7, 122.5, 127.9, 156.0, 169.8, 186.4.

Results and discussion

Degradation products of desonide. Fig. 1 shows a

typical chromatogram of a Bond Elut™ extract of desonide ointment which indicates the presence of two major degradation products of desonide (peaks 2 and 3). The extract was analyzed by HPLC on the C-18 reversed-phase column with methanol–0.02 M acetate buffer (60:40, pH 4.0) as mobile phase. By examining the retention of desonide and its degradation products as a function of the pH of the mobile phase it was observed that the retention of the main degradation product (peak 2) varied greatly between pH 2.2 and 7.0. Fig. 2 shows a plot of the capacity factors vs. pH of the mobile phase. As outlined by Horvath *et al.*,[13] the sigmoidal plot for the major degradation product (peak 2) shows the characteristics of a monoprotic acid. The work was therefore concentrated on the identification and characterization of this component. The component was isolated from the ointment after liquid–solid extraction and preparative HPLC. The collected fractions were pooled and lyophilized, and the isolated compound compared with the authentic C_{23} acid.

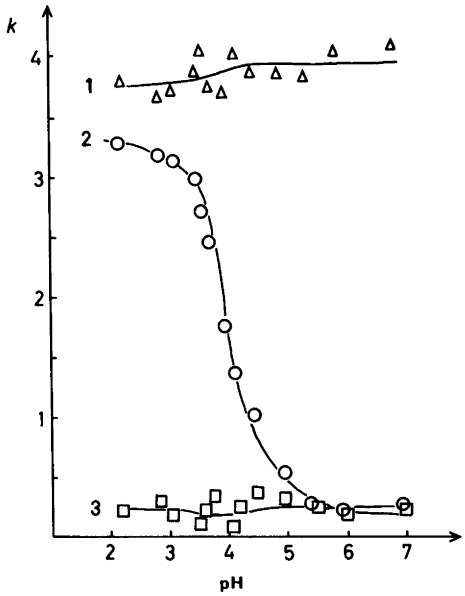


Fig. 2. Plot of capacity factors vs. pH of the mobile phase. 1 = Desonide; 2 and 3 = degradation products.

Synthesis and characterization of the acidic C_{23} degradation product of desonide. Oxidative cleavage of the α-ketol side chain of desonide (**1**; $C_{24}H_{32}O_6$), employing alkaline hydrogen peroxide as outlined by Ogata *et al.*[14] for α-ketols, proceeded smoothly at ambient temperature, furnishing a major product with acidic properties together with a minor neutral compound. The structure of the acidic compound (**2**) was substantiated by its spectral data. High resolution mass spectrometry together with the presence of twenty-three signals in the proton noise-decoupled ^{13}C NMR spectrum established its composition as $C_{23}H_{30}O_6$. The NMR spectra of the acid **2** revealed the expected differences when compared to those of desonide (**1**); one ^{13}C resonance line in the spectrum of **2** at δ 175.9 (C-20; assignment of this signal and that of C-5 at δ 174.6 may be reversed), being characteristic of a carboxyl group, replaced lines at δ 67.0 (C-21) and δ 210.8 (C-20) ascribed to the C_2 side-chain of desonide (**1**).[15] Furthermore, the ^1H NMR signals of the hydroxymethyl group at δ 4.19 and δ 4.67 (AB-system; *J* 19.1 Hz) of desonide (**1**) were absent in the spectrum of the acid **2**. Similar oxidative cleavage of a structurally closely related steroid, 7α-fluorodesonide, has been reported by Gusarova *et al.*,[16] who obtained the corresponding acid employing molecular oxygen in an alkaline solution of the glucocorticoid.

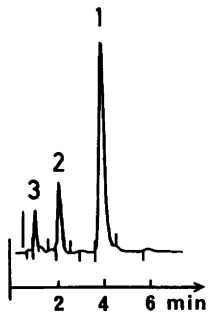
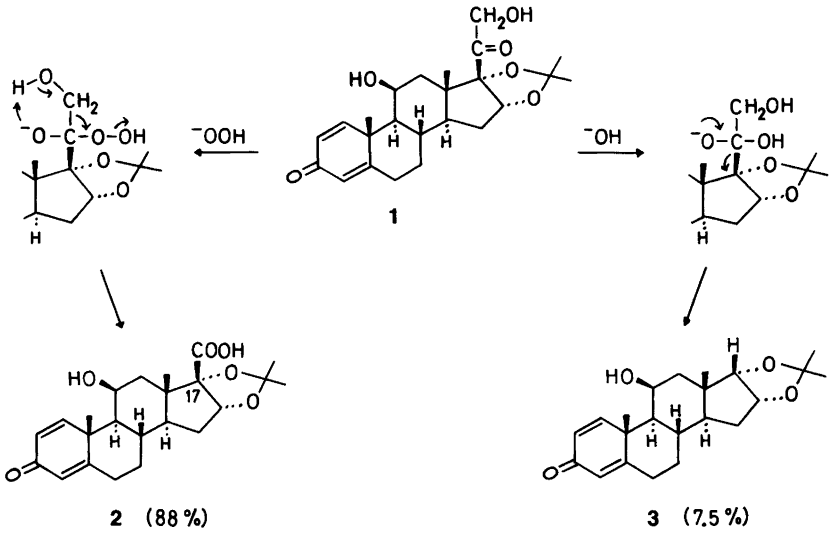


Fig. 1. Chromatogram of a Bond Elut™ extract of desonide ointment. For chromatographic conditions see text. Peak 1 = Desonide; peaks 2 and 3 = degradation products.

HPLC analysis of the *C*-17-carboxylic acid (**2**) showed that its retention time was identical to that of the acidic degradation product of desonide between pH 2.2 and 7.0 in the mobile phase. The mass spectrum of the isolated acidic degradation product in ointment was virtually identical with the mass spectrum of the synthetic *C*-17-carboxylic acid (**2**). The minor neutral product,



Scheme 1.

$C_{22}H_{30}O_4$, exhibited spectral data in agreement with structure **3** depicted in Scheme 1. The loss of the C_2 side-chain of desonide (**1**) was presumably effected by a competing base-catalyzed side reaction analogous to the base-catalyzed α-fission of α-ketols described by Sharp and Miller.[17] This side reaction might have contributed to some extent to the yield of the *C*-17-carboxylic acid **2** by cleavage of the C-20/C-21 bond.

Chromatographic estimation of the pK_a*-value of the C-17-carboxylic acid.* The effect of solute ionization on the retention of weak acids in a reversed phase system has been discussed by Horvath *et al.*[13] For a monoprotic acid the capacity factor can be expressed by:

$$k = \frac{k_o + k_{-1}\dfrac{K_{am}}{[H^+]_m}}{1 + \dfrac{K_{am}}{[H^+]_m}} \qquad (1)$$

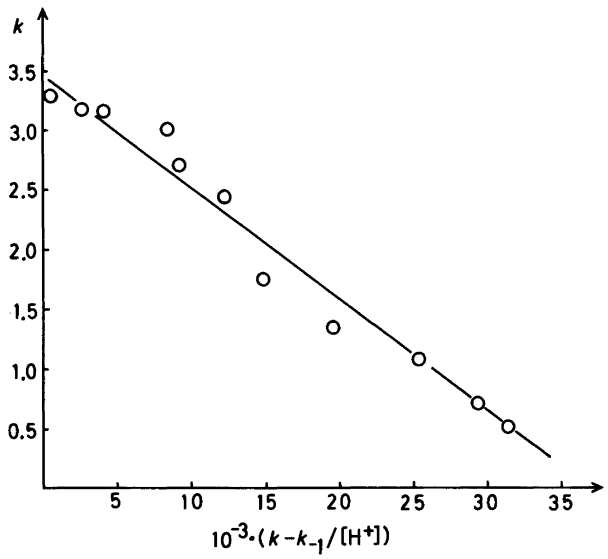


Fig. 3. Plot of k vs. $(k-k_{-1}/[H^+])$ for the *C*-17-carboxylic acid.

where k_o is defined as the capacity factor of the undissociated acid and k_{-1} as the capacity factor of the conjugate base. K_{am} is the acid dissociation constant in the mobile phase. This expression is based on the assumption that the equilibrium constant for the reversible association of the solute and the hydrocarbon ligand is determined only by the solvophobic interactions. Eqn. (1) can be written in a linearized form as eqn. (2).

$$k = k_o - (k - k_{-1}) \frac{K_{am}}{[H^+]_m} \quad (2)$$

which facilitates a graphical evaluation of K_{am} from capacity factors measured over a sufficiently wide pH range. Fig. 3 shows a plot of k vs. $k-k_{-1}/[H^+]$ for the *C*-17-carboxylic acid. According to eqn. (2), a straight line is obtained with intercept k_o and slope $-K_{am}$. The pK_{am} value of the *C*-17-carboxylic acid was estimated by linear regression to be 4.0, which seems to be a reasonable estimate for the pK_a value of the acid.

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