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Estimation of impurity profiles of drugs and related materials Part 18. Impurities and degradation products of mazipredone[1]

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Abstract

Reversed-phase HPLC methods using C-18 and C-8 columns as well as various isocratic and gradient systems with aqueous ammonium acetate, methanol and acetonitrile are described for the separation of the impurities of mazipredone (11β,17-dihydroxy-21-(4-methyl-1-piperazinyl)-pregna-1,4-diene-3,20-dione hydrochloride). These methods were used also for the estimation of the hydrolytic and oxidative degradation pathways of mazipredone in 0.1 M hydrochloric acid and sodium hydroxide at 80°C. With the aid of HPLC-(APCI)-MS and HPLC-diode-array UV techniques 15 impurities and degradation products have been identified.

Keywords: Mazipredone; Impurity profiling; Drug degradation; High-performance liquid chromatography; Mass spectrometry; HPLC-MS

1. Introduction

Mazipredone (11β, 17-dihydroxy-21-(4-methyl-1-piperazinyl)-pregna-1,4-diene-3,20-dione hydrochloride), a water soluble glucocorticoid derivative was synthesized and introduced into the therapy as an anti-inflammatory and anti-allergic agent by the Chemical Works of Gedeon Richter, Budapest. It is marketed under the trade name Depersolone® as injection, nasal, otic and ophthalmic solutions as well as ointment. The synthesis of mazipredone (Fig. 1) begins with the regioselective mesylation of prednisolone (**6**) to form its 21-mesylate (**11**). This is reacted with *N*-methylpiperazine to form mazipredone base, the monohydrochloride of which is precipitated to form mazipredone [2].

Related organic impurities in bulk drug substances originate (a) from the starting material of the synthesis, (b) from the synthetic steps and (c) from the transformations of the finished product during its preparation and isolation [3]. These result in a rather complex impurity profile of mazipredone. The starting material of the synthesis is prednisolone, the industrial synthesis of which involves two fermentation steps (introduc-

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[1] This paper is part 53 in the series 'Analysis of steroids'. For part 52 and part 17 of this series see [1].

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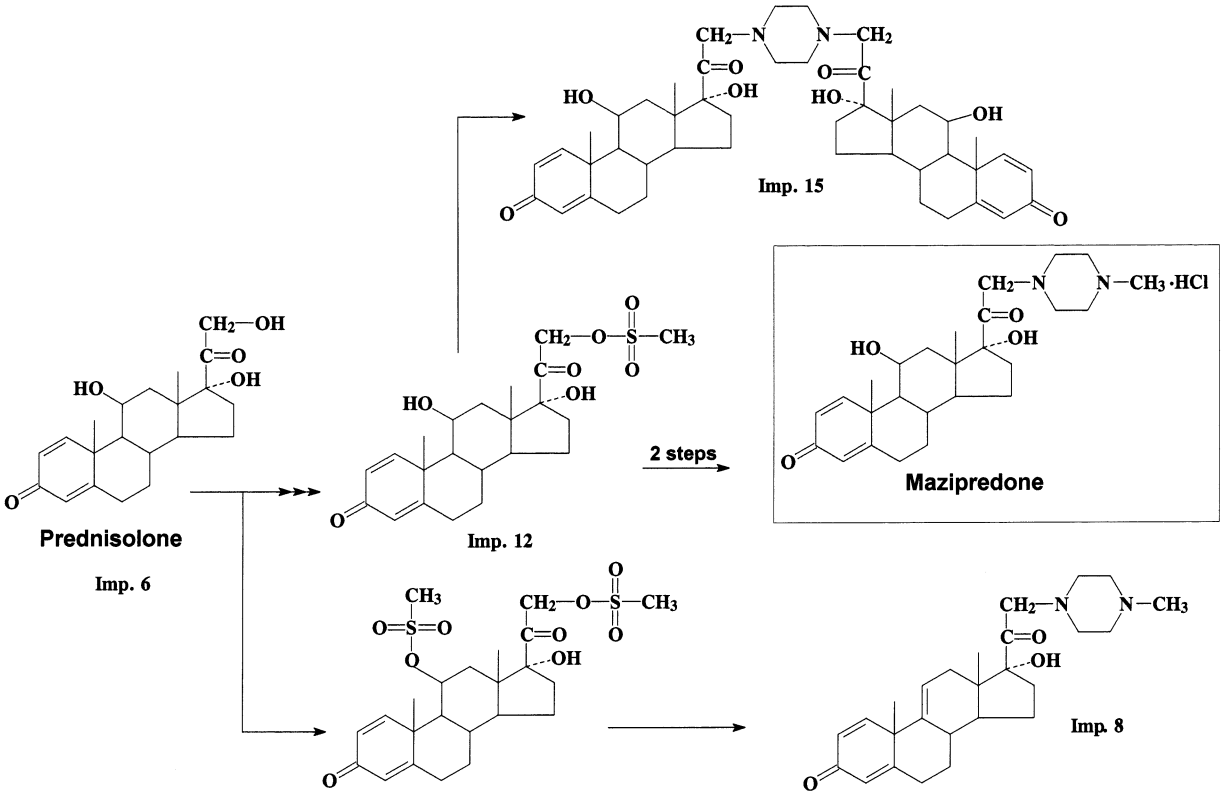

Fig. 1. Reaction scheme of the synthesis of mazipredone with two side reactions.

tion of the 11β-hydroxy group and the Δ^1-double bond). For this reason 21-methylpiperazinyl derivatives of typical impurities of prednisolone originating from the fermentation steps (11-keto, 1,2-dihydro, 11-deoxy derivatives; **4**, **5**, **9**) are likely to appear as impurities in mazipredone.

In addition to impurities originating from the subsequent synthetic steps the instability of mazipredone is another source of impurities. The dihydroxyacetone side chain of corticosteroids is sensitive to atmospheric oxygen and susceptible to various intramolecular oxidoreduction reactions [4]. The very thorough investigation of the various degradation pathways of prednisolone leading to a variety of products has been reviewed [5]. Since mazipredone is an α-aminoketone derivative and these are known to be even more sensitive to oxidation, acidic and basic media than the 21-hydroxy analogs [4,6–9], identical or similar degradation products are likely to be impurities in the bulk drug material. The first aim of this study was the detection, separation and identification of impurities in mazipredone making use of the modern methods available for drug impurity profiling, especially HPLC-MS technique.

The degradation of mazipredone under various conditions was studied at the time of the introduction of the drug more than thirty years ago using the methods available at that time. Volumetric [11], indirect gravimetric [8] and spectrophotometric [10–12] measurements enabled only the determination of the unreacted drug substance. Later on thin-layer chromatography and high-performance liquid chromatography enabled the two main degradation products also to be estimated [13]. The second aim of this study was the estimation of the degradation pathways of mazipredone in acidic and alkaline media in the presence of atmospheric oxygen making use of the modern chromatographic and spectroscopic methods.

Table 1
Retention data of mazipredone, its impurities and degradation products. For the HPLC system see Section 2.3.1

Derivative	Number	Formula in figure	Retention time (min)	Relative retention time
17α-Hydroxy-17-oic acid	**1**	7	11.3	0.52
17α,20-Dihydroxy-21-oic acid	**2**	7	13.8	0.64
17-Deoxy-20-keto-21-hydroxy-21-(4-methyl-1-piperazinyl)	**3**	7	18.7	0.87
11-Keto-mazipredone	**4**	—	21.0	0.97
1,2-Dihydro-mazipredone	**5**	—	21.6	1.00[a]
Mazipredone		1,4,7	21.6	1.00
Prednisolone	**6**	1	23.0	1.06
17α-Hydroxy-20-keto-21,21-dihydroxy	**7**	7	24.6	1.14
$\Delta^{9(11)}$-Dehydro-mazipredone	**8**	1	25.5	1.18
11-Deoxy-mazipredone	**9**	—	26.6	1.23
17-Ketone	**10**	4,7	27.1	1.25
17-Deoxy-20-keto-21,21-dihydroxy	**11**	4	32.8	1.52
Prednisolone-21-mesylate	**12**	1	33.0	1.53
20-Hydroxy-17(20)*E*-ene-21-al	**13**	4	34.7	1.61
20-Hydroxy-17(20)*Z*-ene-21-al	**14**	4	36.7	1.70
Piperazine-bridged mazipredone dimer	**15**	1	53.7	2.49

[a] 0.97 in system 2, Section 2.3.2.

2. Experimental

2.1. Apparatus

For the high-performance liquid chromatographic measurements a Hewlett-Packard 1090 liquid chromatograph equipped with a diode-array detector and ChemStation software were used. The HPLC-MS systems were, (a) Waters 600 M liquid chromatograph equipped with a Waters 490 UV detector attached to a Finnigan MAT 95 SQ hybrid-tandem mass spectrometer and (b) Hewlett-Packard 1100 Series LC/MSD system and ChemStation software.

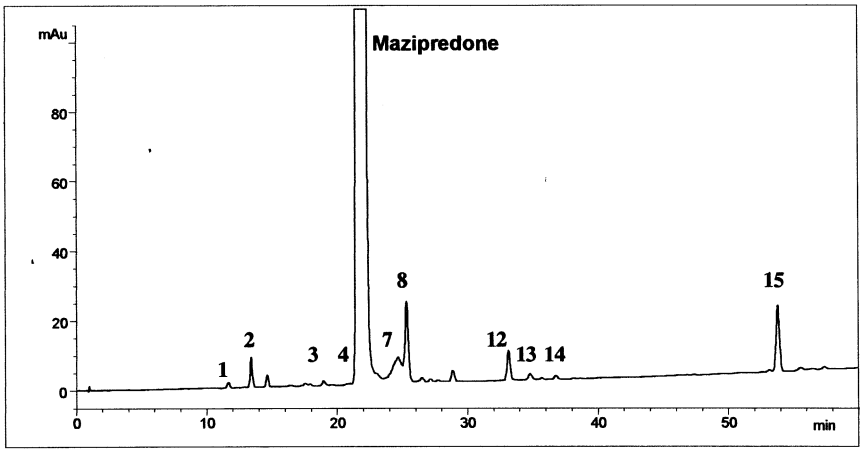

Fig. 2. Chromatogram of a production batch of mazipredone. For the HPLC system and the numbering of the peaks see Section 2.3.1 and Table 1, respectively.

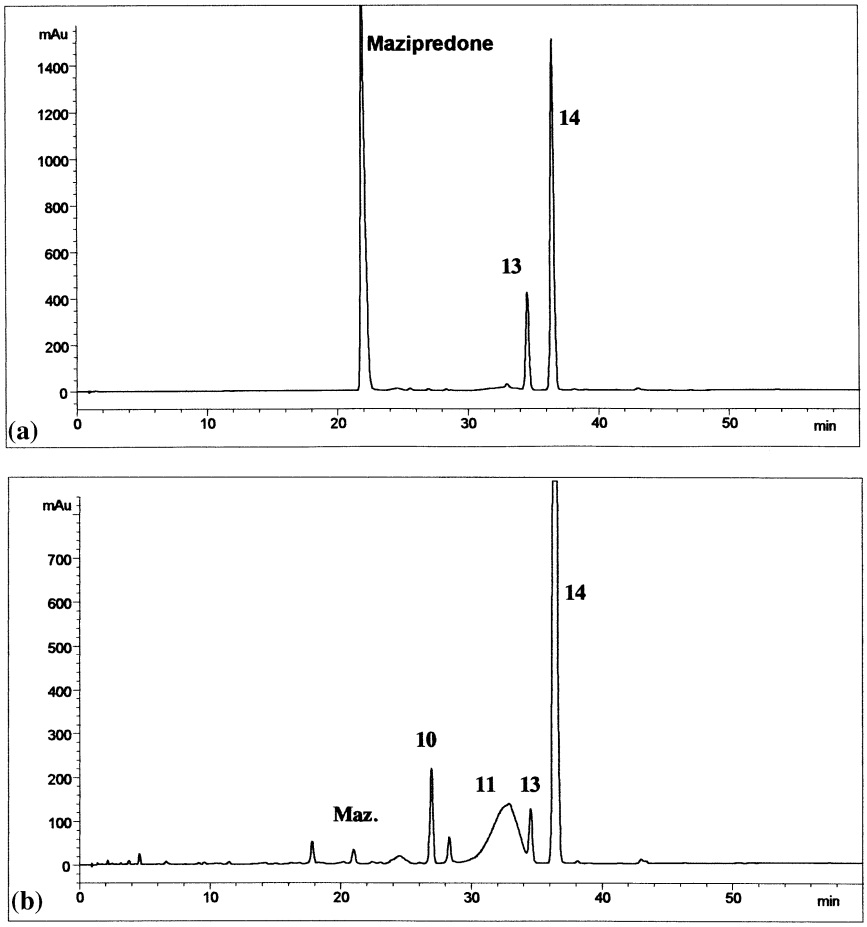

Fig. 3. Chromatograms of mazipredone after treatment with 0.1 M hydrochloric acid at 80°C. (a) Chromatogram after 30 min reaction time. (b) Chromatogram after 24 h reaction time. For the HPLC system and the numbering of the peaks see Section 2.3.1 and Fig. 4, respectively.

2.2. Materials

The mazipredone samples and the synthesized potential and real impurities were products of the Chemical Works of Gedeon Richter, Budapest. Methanol and acetonitrile were Merck LiChrosolv® gradient grade products. Water was purified using a Millipore Milli-Q 185 Plus system. Ammonium acetate, of p.a. quality was purchased from Merck.

2.3. Methods

2.3.1. HPLC and LC/MSD method

Column: Purospher® RP-18e, 5 μm, 125 × 4 mm I.D. (Merck). Eluent: A: water-acetonitrile-methanol (85:5:5, v/v/v), B: water-acetonitrile-methanol (20:40:40, v/v/v) both containing 50 mM ammonium acetate. Gradient profile: linear gradient from 10% B at 0 min to 90% B at 70 min, 90% B at 80 min, 10% B at 82 min, postrun 15

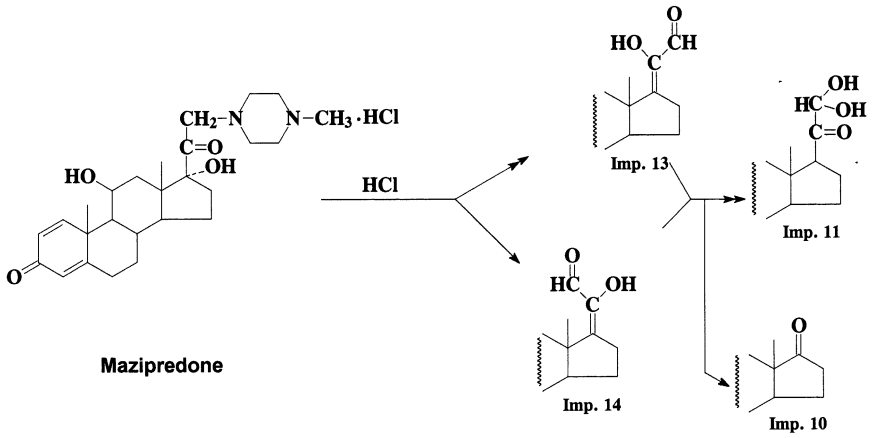

Fig. 4. Reaction scheme of the degradation of mazipredone in 0.1 M hydrochloric acid at 80°C.

min. Flow rate 1 ml min^{-1}. Temperature: 40°C. UV detector set to 240 nm or used in the spectrum scanning mode. MSD parameters: ionization mode: APCI positive; drying gas (nitrogen) flow: 10 l min^{-1}, nebulizer pressure: 40 psig, drying gas temperature: 325°C, vaporizer temperature: 450°C, capillary voltage: 4 kV, corona current: 4 μA.

2.3.2. HPLC-MS method

Column: Hypersil BDS C8, 3 μm, 100 × 4.6 mm I.D. (Shandon). Eluent: system 1: ammonium acetate (25 mM)-methanol (1:1, v/v); system 2: A: ammonium acetate (25 mM), B: methanol. Gradient profile: linear gradient to 80% B at 80 min, 80% B at 85 min, 35% B at 90 min, postrun 15 min. Flow rate 1 ml min^{-1}. Temperature: ambient. UV detector set to 240 nm. Mass spectrometric parameters: interface: Finnigan MAT API; accelerating voltage: 5 kV; resolution: 1450; ionization mode: APCI positive; vaporizer temperature: 300°C; capillary temperature: 200°C; corona current: 5 μA; sheath gas: nitrogen, 70 psi.

3. Results and discussion

3.1. The impurity profile of mazipredone

The structures for the impurities were suggested primarily on the basis of the molecular masses obtained from the HPLC-(APCI)-MS investigations using the HPLC-MS systems described in Section 2.3.2 In some problematic cases the information obtained from the diode-array UV spectra and from the chemical background of the synthesis was also helpful. The suggested structures were

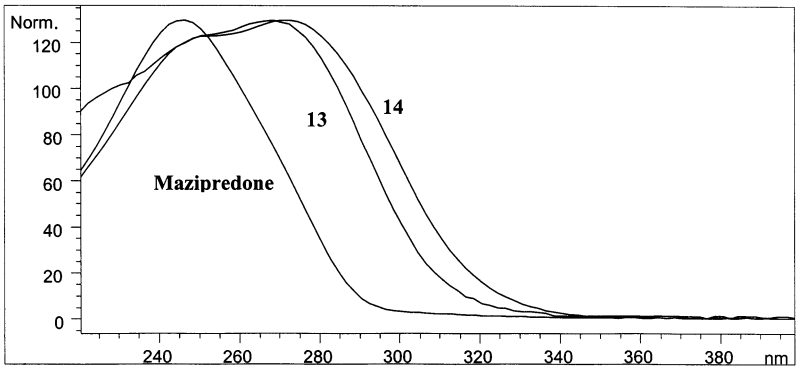

Fig. 5. Diode-array UV spectra of mazipredone and degradation products **13** and **14** (see Table 1).

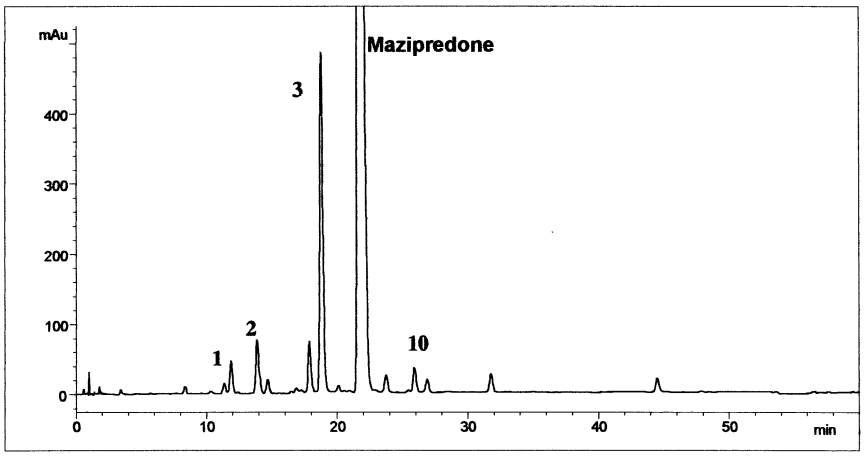

Fig. 6. Chromatogram of mazipredone after treatment with 0.1 M sodium hydroxide at 80°C for 4 h in the presence of atmospheric oxygen. For the HPLC system and the numbering of the peaks see Section 2.3.2 (system 2) and Fig. 7, respectively.

then synthesized and on the basis of these impurity standards and some potential impurities the HPLC system for the purity control optimized. All impurities being present above 0.05% in production batches of mazipredone were identified. Table 1 summarizes the retention data of 15 potential and real impurities and degradation products.

As described in Section 1, some of the derivatives in Table 1 originate from the (potential) impurities of prednisolone (**6**): 1,2-dihydromazipredone (**5**), 11-deoxymazipredone (**9**), 11-

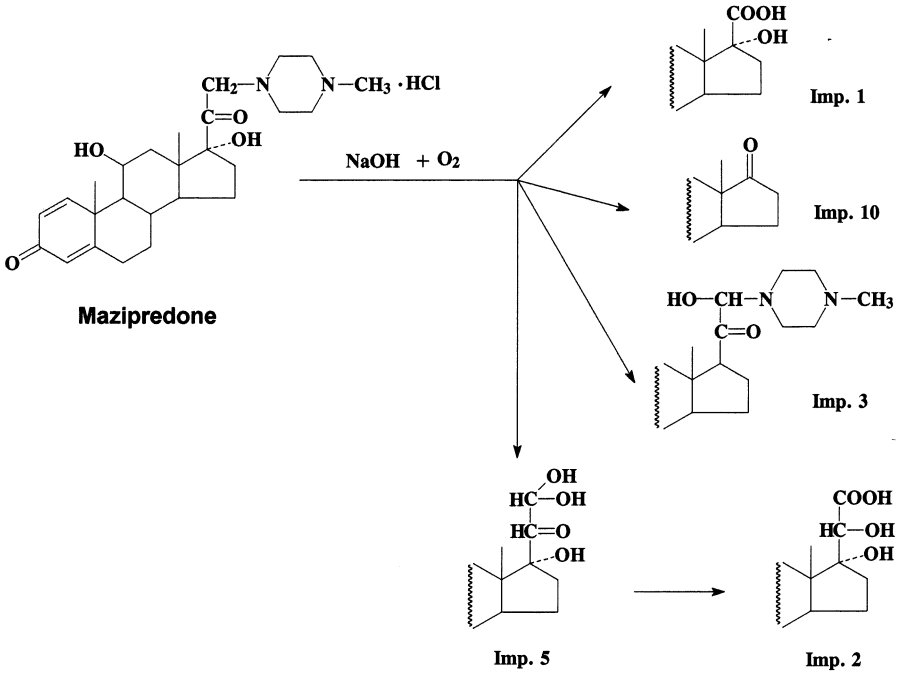

Fig. 7. Reaction scheme of the degradation of mazipredone in 0.1 M sodium hydroxide at 80°C in the presence of atmospheric oxygen.

keto-mazipredone (**4**). It is to be noted that **5** is poorly resolved from mazipredone using the HPLC method described in Section 2.3.1 For the separation and quantitation of this impurity HPLC system 2 described in Section 2.3.2 was used (relative retention time 0.97). Two other impurities originate from the synthetic steps. Prednisolone-21-mesylate (**12**) is the last intermediate of the synthesis (see Fig. 1). The chemical basis for the formation of the 11-deoxy-$\Delta^{9(11)}$-derivative (**8**) is the not entirely regioselective mesylation of prednisolone leading to the 11,21-dimesylate which loses one mole of methanesulphonic acid in the course of the subsequent step of the synthesis. The reason for the formation of the dimeric derivative (**15**) is the presence of traces of piperazine as an impurity in methylpiperazine. The two side reactions are also included in the reaction scheme in Fig. 1. It is worth mentioning that in the case of **5** the lack of one of the double bonds and in the case of **8** the presence of the $\Delta^{9(11)}$-double bond result in the distortion of the shape of the UV-band of the 1,4-diene-3-keto group and this information was successfully used when the structures of the two impurities were selected from among other possible isomeric structures with molecular masses of 444 and 424, respectively [1].

All other impurities (**1**, **2**, **3**, **7**, **10**, **13**, **14**) are degradation products and will be discussed in Section 3.2 and Section 3.3.

On the basis of the investigation of several production batches of mazipredone using the above described HPLC methods with area normalization it was found that impurities **1**, **4**, **6**, **9**, **10**, **13** and **14** cannot be detected or their quantity was below 0.1%. Impurities **2**, **5**, **7**, **8**, **11** and **15** were found to be present in the range 0.1–0.4%.

Fig. 2 is the chromatogram of a typical production batch of mazipredone.

3.2. Identification of the products of acid-catalyzed degradation

As described in Section 1 it was predictable that the α-hydroxy-α'-aminoacetone type 17-side chain of mazipredone is likely to undergo acid-catalyzed transformations. The degradation in acidic media of the analogous corticosteroids bearing a dihydroxyacetone side chain at position 17 is of intramolecular oxidoreduction type and leads to 17-deoxy-20-keto-21-aldehyde derivatives [4,5,6,7 and several references therein]. In the course of the acidic treatment of mazipredone (a 6 mg ml^{-1} solution of the test substance in 0.1 M hydrochloric acid at 80°C for 30 min) two isomeric reaction products were identified with the aid of the HPLC-MS and HPLC-UV analysis (see chromatogram (a) in Fig. 3). As seen in the reaction scheme in Fig. 4 these are the *E* and *Z* isomers of the enolaldehyde type degradation product, 11β,20-dihydroxypregna-1,4,17(20)-triene-21-al-3-one which are identical with the minor impurities **13** and **14** in Table 1 and Fig. 2 and with those found by Lewbart et al. [7] in the course of the investigation of the acidic degradation products of prednisolone. In this case diode-array UV spectra of **13** and **14** (Fig. 5) were of diagnostic value in establishing the structures of the degradation products. In the spectra the band of the 1,4-diene-3-keto group around 248 nm is partially overlapped by the band of the *E* and *Z* isomers of the 17(20)-ene-20-ol-21-al group at 269 and 276 nm. The difference between the absorption maxima of the long wavelength band is certainly due to steric hindrance in the case of the *E* isomer (minor component).

If the heating of the acidic reaction mixture is prolonged for 24 h chromatogram (b) in Fig. 3 is obtained. This shows that **13** and **14** are only intermediates of the acidic degradation. As shown in the reaction scheme in Fig. 4 the main transformation reaction of the enolaldehydes is the acid catalyzed rearrangement of the 17(20)-ene-20-hydroxy system followed by the addition of one mole of water to the aldehyde group leading to **11** which is a 17-deoxy-20-keto-21-aldehyde hydrate derivative but a small amount of a breakdown product (17-keto derivative, **10**) was also detected. The probable reason for the large peakwidth of **11** is that in the aqueous eluent an equilibrium exists between the 21-aldehyde group and its hydrated form. Similarly broad peaks were found for **7**, too, which is also a 20-keto-21-aldehyde derivative (see the reaction scheme in Fig. 7). It is to be

noted that new peaks with longer retention time than that of **15** appear in the chromatograms if the reaction mixture of the acidic treatment contains alcohols. These are peaks of the hemiacetal derivatives of the 21-aldehyde group.

3.3. Identification of the degradation products formed in alkaline media

Mazipredone is more stable in alkaline than in acidic media: 80% of mazipredone was found unchanged when 6 mg ml^{-1} mazipredone was heated in 0.1 M sodium hydroxide at 80°C for 4 h. In addition to this, the degradation products identified in the reaction mixture were found to be oxidation products due to the presence of atmospheric oxygen rather than products of base-catalyzed degradation.

The chromatogram of the reaction mixture and the scheme of the reactions taking place in alkaline solution in the presence of atmospheric oxygen are shown in Figs. 6 and 7, respectively. Of the oxidative degradation products the 17-keto derivative (**10**) and the two carboxylic acids (**1** and **2**) have already been mentioned in Section 3.1 as minor impurities in bulk mazipredone. The 17α-hydroxy-20-keto-21-aldehyde hydrate (**7**) which is a major oxidation product cannot be detected in the alkaline reaction mixture since under these reaction conditions it is quantitatively rearranged to the 17α,20-dihydroxy-21-carboxylic acid derivative (**2**).

Unlike with all the other impurities and degradation products there is no preparative evidence for the structure of the degradation product **3** (Fig. 7). On the basis of its molecular mass which is identical to that of mazipredone and its higher polarity an isomeric 17-deoxy-20-keto-21-hydroxy-21-(4-methyl-1-piperazinyl) structure has been suggested where the unusual geminal oxyamine structure is believed to be stabilized by the electron attracting 20-keto group. Work is underway to furnish further evidence for this structure.

4. Conclusions

On the basis of HPLC-MS investigations, supplemented by HPLC-UV data it was possible to describe the structures of all impurities in bulk mazipredone above 0.1% and many of the minor impurities below 0.1% (altogether 13 compounds). These achievements and the elucidation of the mechanism of the decomposition reactions taking place in acidic and alkaline media may serve as the basis for validated purity and stability tests for bulk mazipredone and its liquid formulations. The similarities found between the degradation pathways of mazipredone and prednisolone are or special interest with respect to the 21-aldehyde-type degradation products (**7**, **11**, **13**, **14**), which have been described as being capable to bind to the arginine residues of proteins and are therefore considered to be responsible as pro-antigens for the allergic reactions caused by corticosteroids [14].

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