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STABILITY OF CORTICOSTEROIDS UNDER ANAEROBIC CONDITIONS. C6 AND C9 FLUORINE-CONTAINING CORTICOSTEROIDS

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SUMMARY

The decomposition of corticosteroids due to a fluorine atom at C6 and/or C9 is investigated. Chromatographic properties, the isolation and the structure elucidation of decomposition products are given.

INTRODUCTION

Recently, the decomposition of the dihydroxyacetone side-chain of prednisolone under anaerobic conditions was investigated. One of the decomposition products of prednisolone was 17-deoxyprednisolone (Dekker, 1979a). This compound was found to be stable under the decomposition conditions.

To obtain information about the stability of fluorine atoms at C6 and C9, it is important to use corticosteroids with this stable side-chain, in order to avoid decomposition products which are not due to the C6 or C9 fluorine atoms.

In order to achieve that goal, desoximetasone (Fig. 1A) with a fluorine atom at C9, fluocortolone (Fig. 1B) with a fluorine atom at C6, and diflucortolone (Fig. 1C) with fluorine atoms at C6 and C9 were used. These corticosteroids only differ in the position of the fluorine atoms.

Desoximetasone and diflucortolone are not known to be used parenterally. They are investigated only in order to obtain information about the stability of the fluorine atoms at C6 and C9 under anaerobic conditions. This information can then be used for analogous corticosteroids with a dihydroxyacetone side-chain, which are used parenterally, e.g. dexamethasone (Fig. 1D).

In this publication the structure elucidation of decomposition products due to the presence of fluorine is given. Chromatographic properties are given to detect the compounds in a decomposition mixture and the isolations are described.

It will also be shown that the decomposition due to a fluorine atom at C6 can be neglected under the chosen decomposition conditions and at C9 it can be neglected under

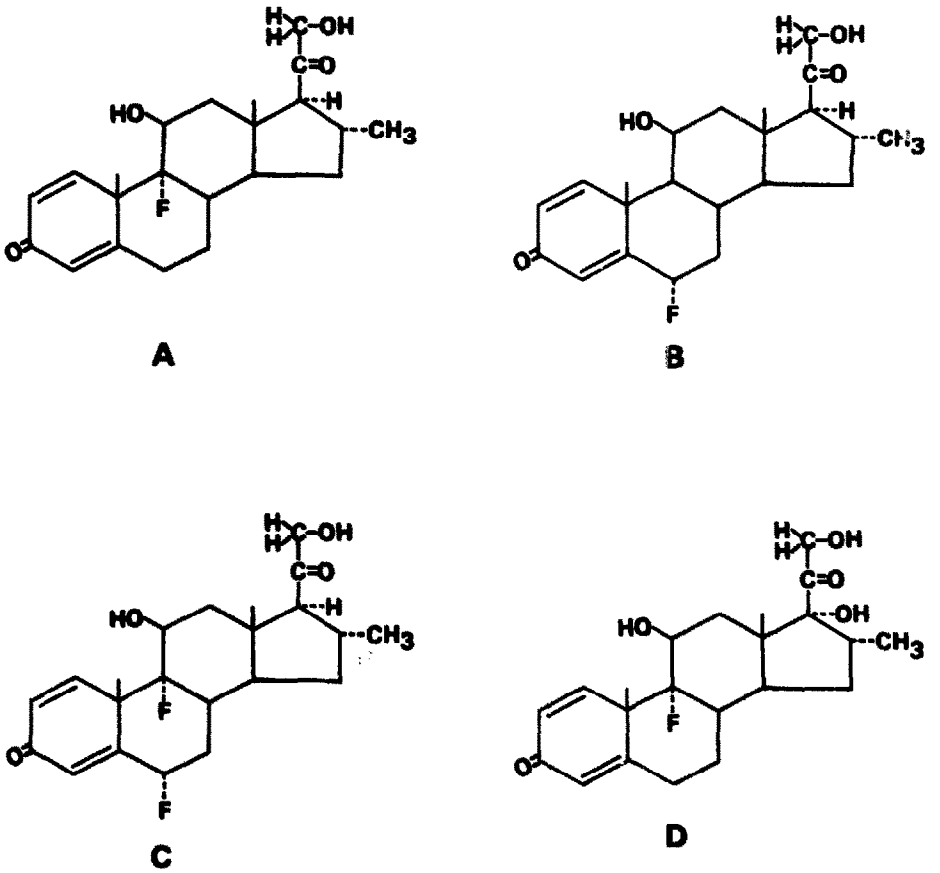

Fig. 1. Structural formulae of desoximetasone (A), fluocortolone (B), diflucortolone (C) and dexamethasone (D).

normal conditions (20 min at 120°C), especially when this decomposition is compared with the decomposition of the dihydroxyacetone side-chain. To achieve this goal, the decomposition of dexamethasone (Fig. 1D) is compared with desoximetasone (Fig. 1A).

MATERIALS AND METHODS

The chemicals used were of European Pharmacopoeia quality unless otherwise mentioned.

Fluocortolone and diflucortolone were gifts from Schering AG, desoximetasone was a gift from Roussel and dexamethasone is commercially available.

Chromatography

A. Thin-layer chromatography. Precoated silica gel plates (60 F254 Merck, thickness 0.25 mm) were used. 2 µl of the filtrate of the decomposition mixture as well as 2 µl of a chloroform extract of the alkalized filtrate (1 part of chloroform + 1 part of alkalized filtrate) were applied. For preparative purposes, the chloroform extract was applied as a

narrow band on plates of 20 X 20 cm, which were cleaned twice with methanol (analytical grade) before use. The chromatograms were developed with the solvent mixture: chloroform–methanol (9 : 1). Spots were visualised under UV light (254 nm) for non-destructive purposes. Destructive methods of visualisation were: (a) spraying with a sulphuric acid–ethanol (20 : 80) mixture, heating at 120°C for about 10 min, and viewing under daylight or UV light (365 nm); (b) spraying with a freshly prepared mixture of 1 part of volume of 0.2% (w/v) tetrazolium blue in methanol and 3 parts of volume 12% (w/v) sodium hydroxide in methanol.

B. High pressure liquid chromatography. A liquid chromatograph (model Waters, Waters Assoc., Milford, Mass., U.S.A.) with a UV absorption detector (254 nm) and a stainless steel column (30 cm X 3.9 mm internal diameter) was used. The column packing was porous silica particles permanently bonded to a monomolecular layer of organosilane (μ-Bondapak C18, Waters Assoc.). The composition of the mobile phase was methanol (analytical grade)–water (50 : 50) (w/w). To this mixture 1% (v/w) acetic acid was added. The flow was 1.0 ml/min and the sensitivity 0.64 AUFS.

Generally 10 μl filtrate of the decomposition mixture was injected. The chloroform of the chloroform extract of the alkalized filtrate (1 part of chloroform + 1 part of alkalized filtrate) was removed with nitrogen. The residue was dissolved in an equal part of the mobile phase – 10 μl of this solution was injected. Methanol and water were added to the suspension (thus including the filtrate) until a concentration of 0.1–1 mg corticosteroid in 1 ml methanol (analytical grade)–water (1 : 1) (w/w) was obtained, 50–100 μl and 5–10 μl, respectively, of these solutions were injected.

Mass spectrometry

The mass spectrometer was combined with a computer (Finnegan 6000). CI-mass spectra were obtained with a Finnegan 2300 mass spectrometer at temperatures ranging from 150 to 250°C; the gas used was CH_4. The emission current was 0.20 mA, the muitiplier voltage, 1.8 kv and the electron energy, 120 eV.

Decomposition conditions

A 3% sodium biphosphate solution, adjusted to pH 8.0, was used to investigate the decomposition at pH 8. For the decomposition studies at pH 4–8, Britton buffer was adjusted to the desired pH-values.

The corticosteroids were suspended (10 mg/ml) in the buffer solutions. The suspensions were saturated with nitrogen and were heated for 16 h at 120°C. Under these conditions the dihydroxyacetone side-chain of prednisolone is nearly completely decomposed (Dekker, 1979a).

The influence of cations, present as impurities which can give catalytic decomposition, was investigated by adding disodium edetate to the buffer to give a concentration of 0.5 mg/ml.

Isolation of the decomposition products

Microgram quantities were isolated with methanol (analytical grade) from thin-layers using the apparatus described by Dekker (1979b). With this apparatus a particle-free eluate is obtained, which can be used directly for high performance liquid chromatography and mass spectrometry.

RESULTS AND DISCUSSION

I Desoximetasone

1. Chromatographic properties

Fig. 2A–D illustrates the thin-layer chromatogram of the filtrates of the decomposition mixtures obtained at pH 4, pH 6 and pH 8 and the thin-layer chromatogram of the chloroform extract of the filtrate of the decomposition mixture obtained at pH 8. After spraying with the tetrazolium blue reagent, two blue-coloured spots appeared in the thin-layer chromatogram of the decomposition mixture obtained at pH 8. Spot a is desoximetasone and spot b is a decomposition product, which still has an α-ketol group (Dekker, 1979a).

After spraying with the sulphuric acid–ethanol reagent a third spot (c, Fig. 2) appeared mainly at pH 6. This spot is not present in the chloroform extract of the decomposition mixture obtained at pH 8, but is present in the filtrate of the decomposition mixture obtained at pH 8. Consequently, this compound has acidic properties.

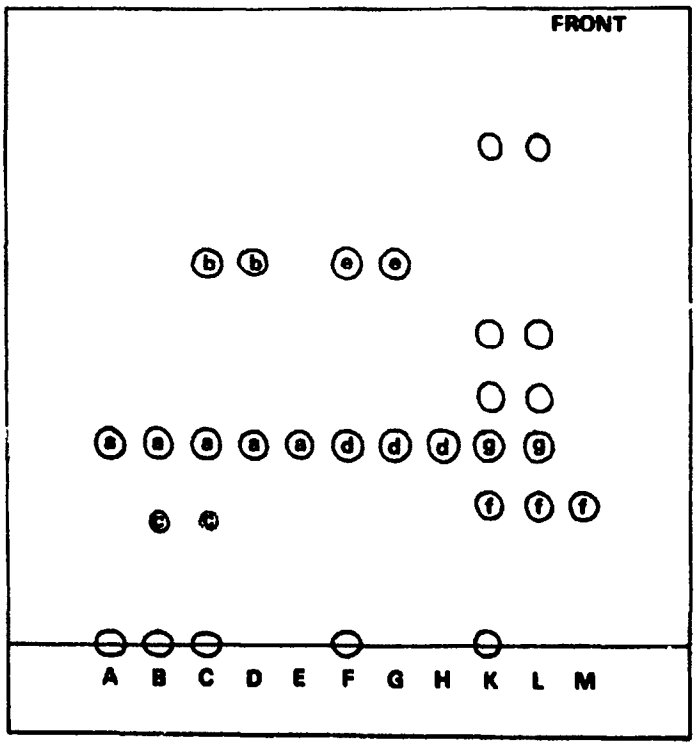

Fig. 2. A: the filtrate of the decomposition mixture of desoximetasone (at pH 4). B: ibid. at pH 6. C: ibid. at pH 8. D: the chloroform extract of the filtrate of the decomposition mixture of desoximetasone (at pH 8). E: desoximetasone. F: the filtrate of the decomposition mixture of diflucortolone (at pH 8). G: the chloroform extract of the filtrate of the decomposition mixture of diflucortolone (at pH 8). H: diflucortolone. K: the filtrate of the decomposition mixture of dexamethasone (at pH 8). L: the chloroform extract of the filtrate of the decomposition mixture of dexamethasone (at pH 8). M: dexamethasone. Spots a to f see text.

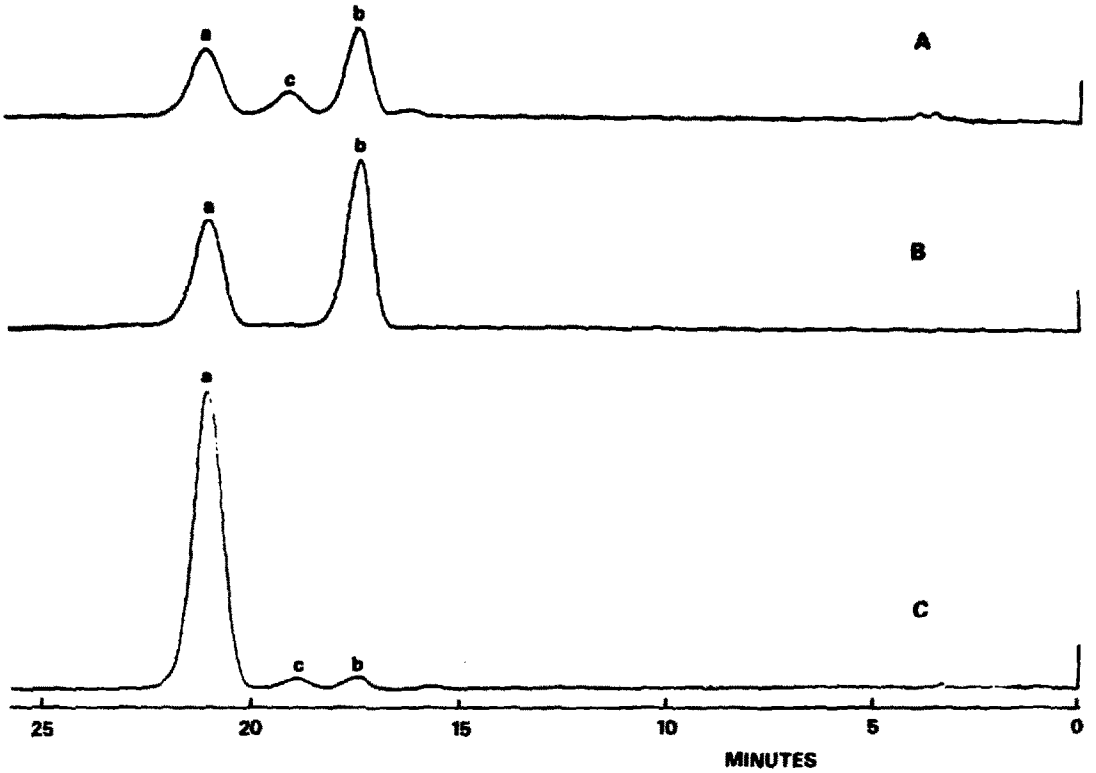

Fig. 3. A: the HPLC chromatogram of the filtrate of the decomposition mixture of desoximetasone. B: the chloroform extract of this filtrate. C: the HPLC chromatogram of the total suspension of desoximetasone.

The partition chromatogram of the filtrate of the decomposition mixture (pH 8) obtained with high pressure liquid chromatography is illustrated in Fig. 3A. In Fig. 3B is illustrated the partition chromatogram of the chloroform extract of this filtrate. The peaks a, b and c correspond with the spots a, b and c. The absence of peak c in Fig. 3B also shows that this compound has acidic properties.

2. Structure elucidation

2.1. Compound b

Thin-layer chromatography. Compound b gives a positive reaction with the tetrazolium blue spray reagent. Consequently the 20-keto-21-hydroxy side-chain of desoximetasone is still present in compound b.

Mass spectrometry. In Fig. 4 the CI-mass spectrum of desoximetasone is given. Peaks derived from the parent peak ($M^+ = 376$; CI 377) of desoximetasone are: 357 (M^+–HF); 339 (M^+–H_2O, HF); and 279 (M^+–H_2O, HF, $\dot{C}O$–CH_2OH). In Fig. 5 the CI-mass spectrum of the decomposition product b is illustrated. The parent peak of this compound is 356 (CI 357). The difference with the parent peak of desoximetasone of 20 mass units is due to the disappearance of one molecule of HF, resulting in a compound with a double bond between C11 and C9 or between C9 and C8 (Fig. 6).

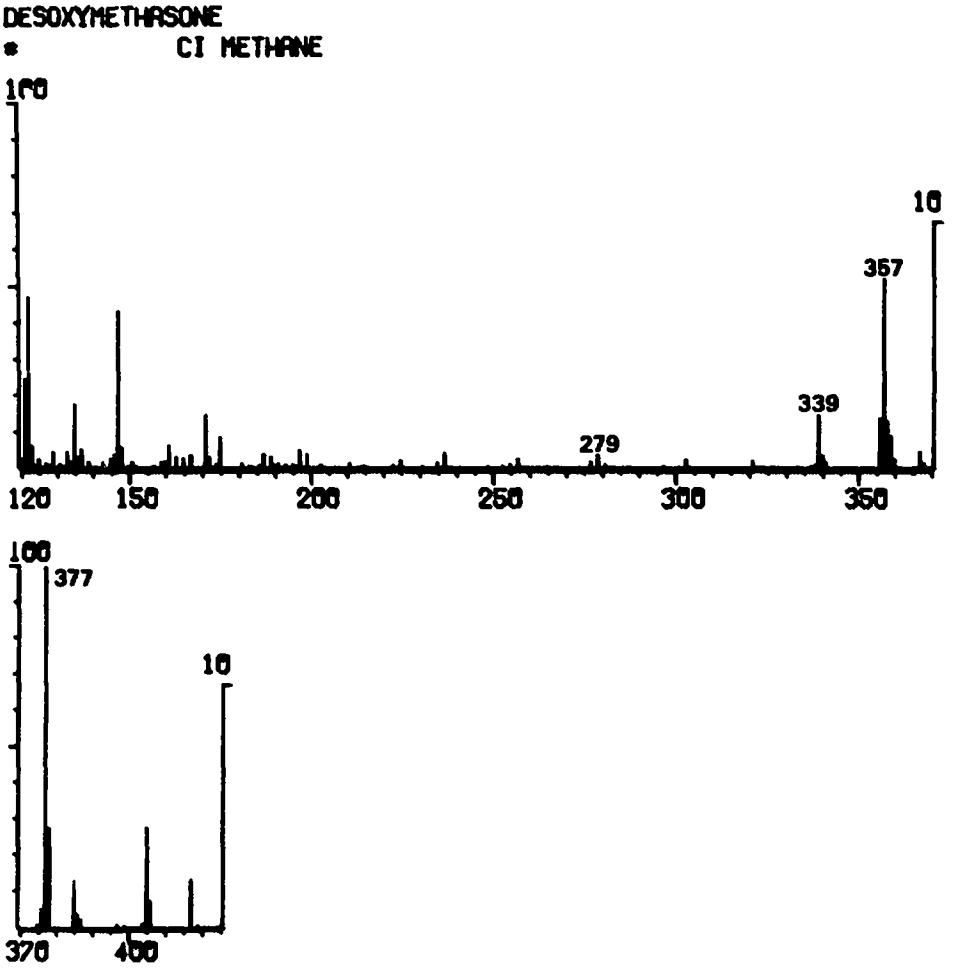

Fig. 4. The CI-mass spectrum of desoximetasone.

Peaks derived from the parent ($M^+ = 356$, CI 357) of this compound are: 339 ($M^+ - H_2O$); 297 ($M^+ - \dot{C}O\text{-}CH_2OH$).

2.2. Compound c

Mass spectrometry. The CI-mass spectrum of compound c is given in Fig. 7. The parent peak of this compound is 362 (CI 363). The parent peak of desoximetasone is 376 (CI 377), consequently compound c has a parent peak of 14 mass units less. The fluorine atom is still present, which can be derived from peak m/z 343 due to the parent peak minus HF and from peak m/z 325 due to the parent peak minus HF and minus H_2O from a hydroxyl group or a carboxyl group.

The A-ring system has not been changed, which can be derived from peak m/z 121–122 (Dekker 1979a). As compound c has acidic properties (see chromatographic properties) the structure of this decomposition product is the 17-deoxy-17-carboxylic acid derivative of desoximetasone.

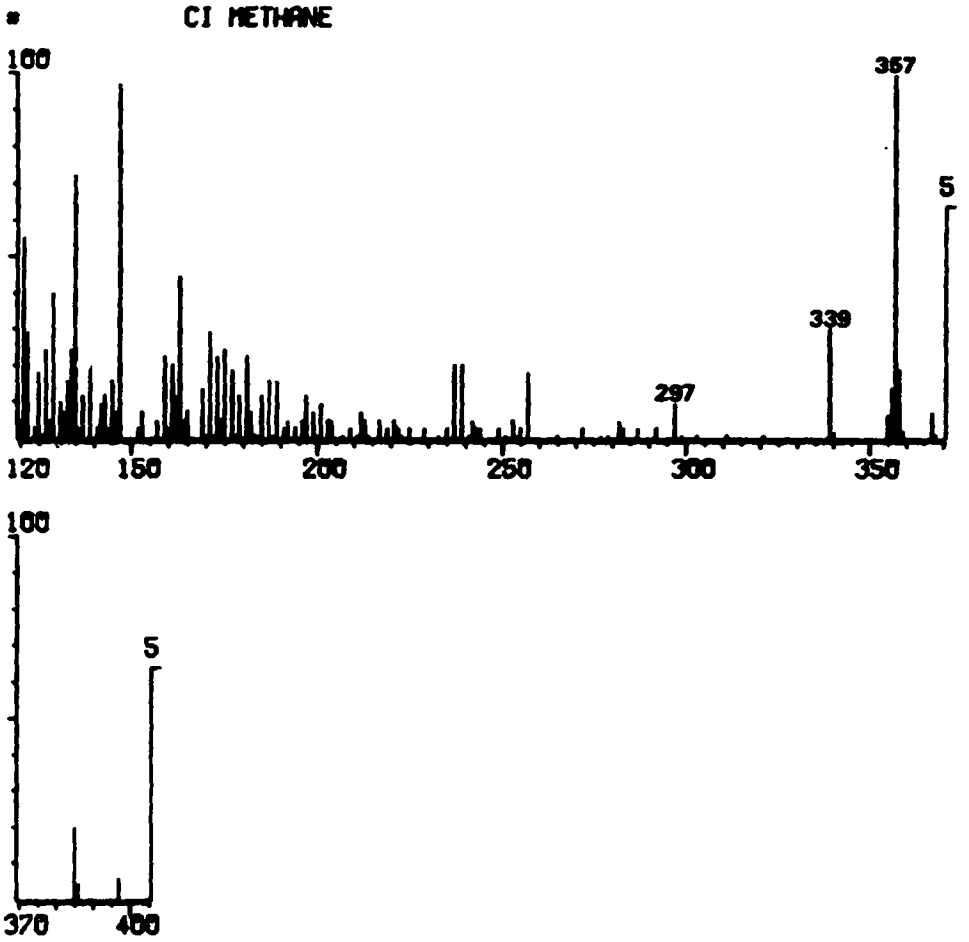

Fig. 5. The CI-mass spectrum of compound b.

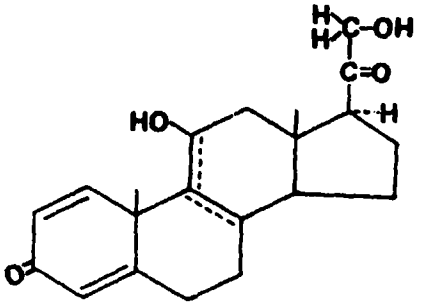

Fig. 6. Possible structure of compound b. The dotted lines indicate the possible presence of the double bonds.

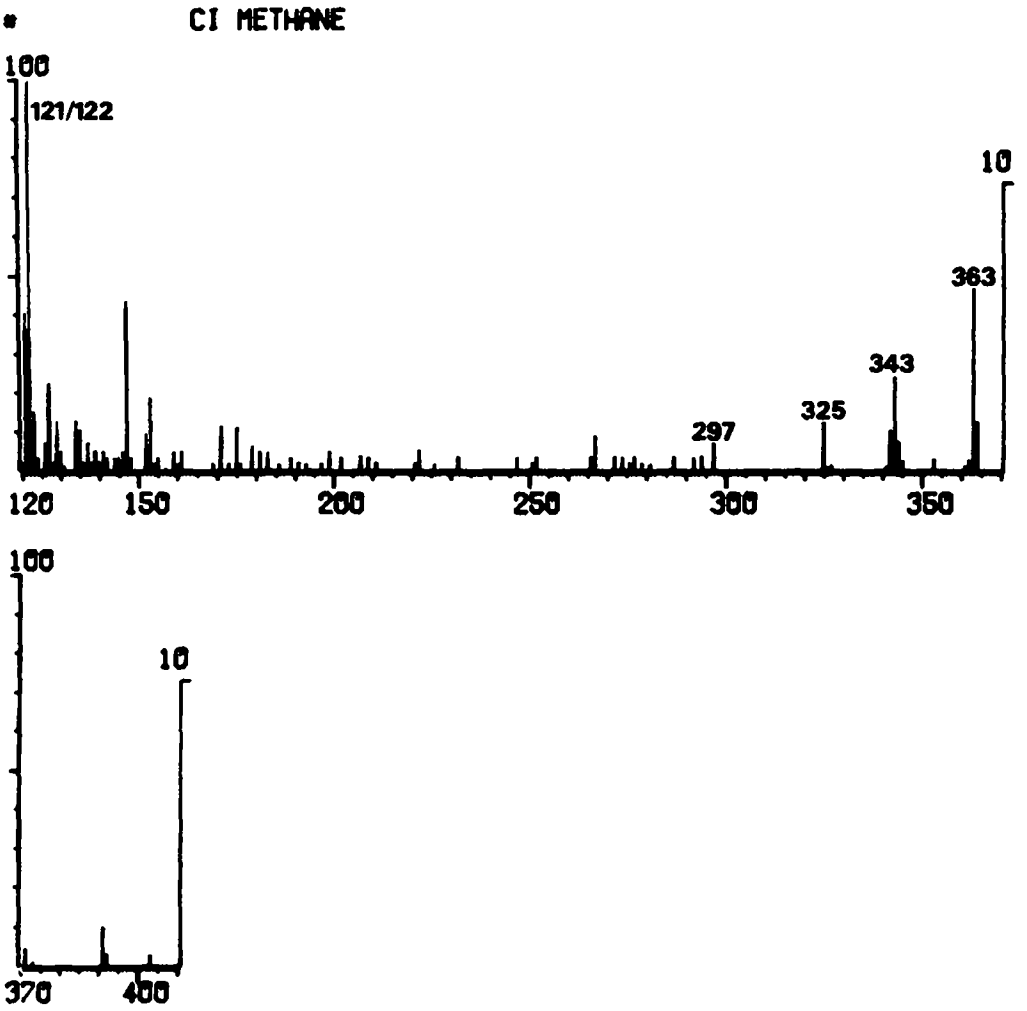

Fig. 7. The CI-mass spectrum of compound c.

3. Discussion

The 17-deoxy-17-carboxylic acid may be due to some cations, because after addition of disodium edetate to the buffer pH 6 and pH 8, this compound was not formed. It should be mentioned that compound b (Fig. 6) is still formed after addition of disodium edetate. In Fig. 3C is illustrated the partition chromatogram of the total suspension (at pH 8) obtained with high pressure liquid chromatography. It can be seen, that under these extreme conditions (16 h at 120°C) only a little of compound b was formed in the total suspension. Therefore, it can be concluded that decomposition under anaerobic conditions due to a fluorine atom at C9 can be neglected under normal sterilization conditions (e.g. 20 min at 120°C).

II Fluocortolone

No decomposition product due to the fluorine atom at C6 was observed.

III Diflucortolone

1. Chromatographic properties

The thin-layer chromatogram of the filtrate of the decomposition mixture obtained at pH 8 and the thin-layer chromatogram of the chloroform extract of this filtrate is illustrated in Fig. 2F and G. Spot d (diflucortolone) and spot e (a decomposition product) gave a positive reaction with tetrazolium blue reagent. After spraying with the sulphuric acid–ethanol reagent, a third spot appeared at the start. This spot is present in the chromatogram of the filtrate and absent in the chromatogram of the chloroform extract. Consequently, at the start acidic compounds are present. These acidic compounds are likely to be due to some oxidation of the side-chain. With partition chromatography using the high pressure liquid chromatograph, diflucortolone has a k′ value of 5.2 and compound e has a k′ value of 3.6.

2. Structure elucidation

2.1. Compound e

A. Thin-layer chromatography. Compound e gives a positive reaction with the tetrazolium blue spray reagent. Consequently the 20-keto-21-hydroxy side-chain of diflucortolone is still present in compound e.

B. Mass spectrometry. In Fig. 9 the CI-mass spectrum of diflucortolone is given. Peaks derived from the parent peak ($M^+ = 394$; CI 395) of diflucortolone are: 375 (M^+–HF); 357 (M^+–HF, H_2O); 315 (M^+–HF, $\dot{C}O$–CH_2OH); 355 (M^+–2x HF); 337 (M^+–2x HF,

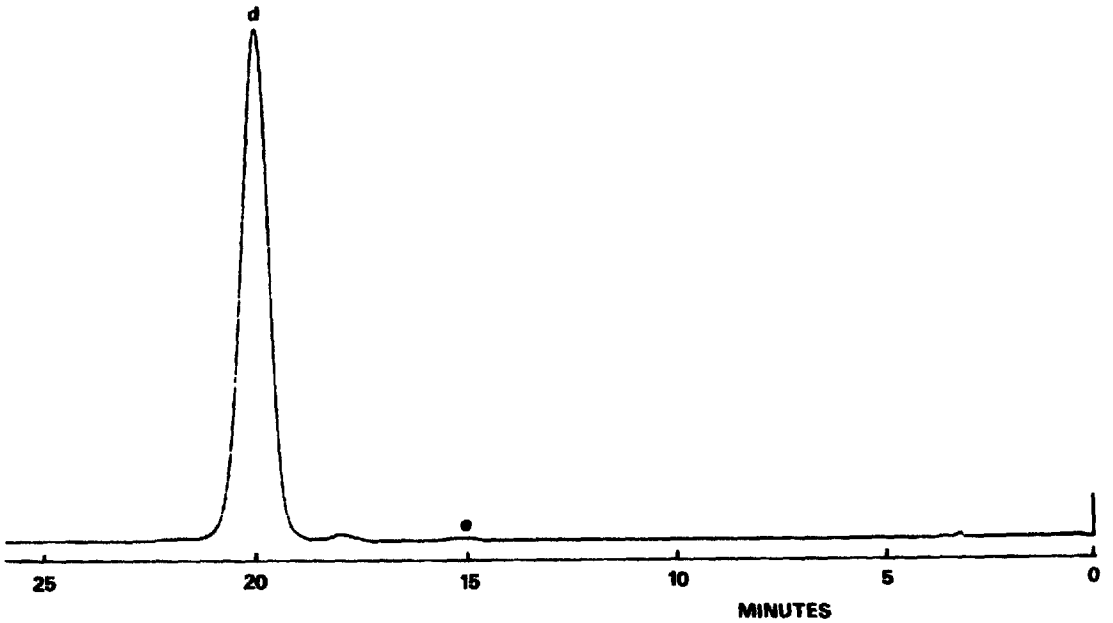

Fig. 8. The HPLC chromatogram of the total suspension of diflucortolone.

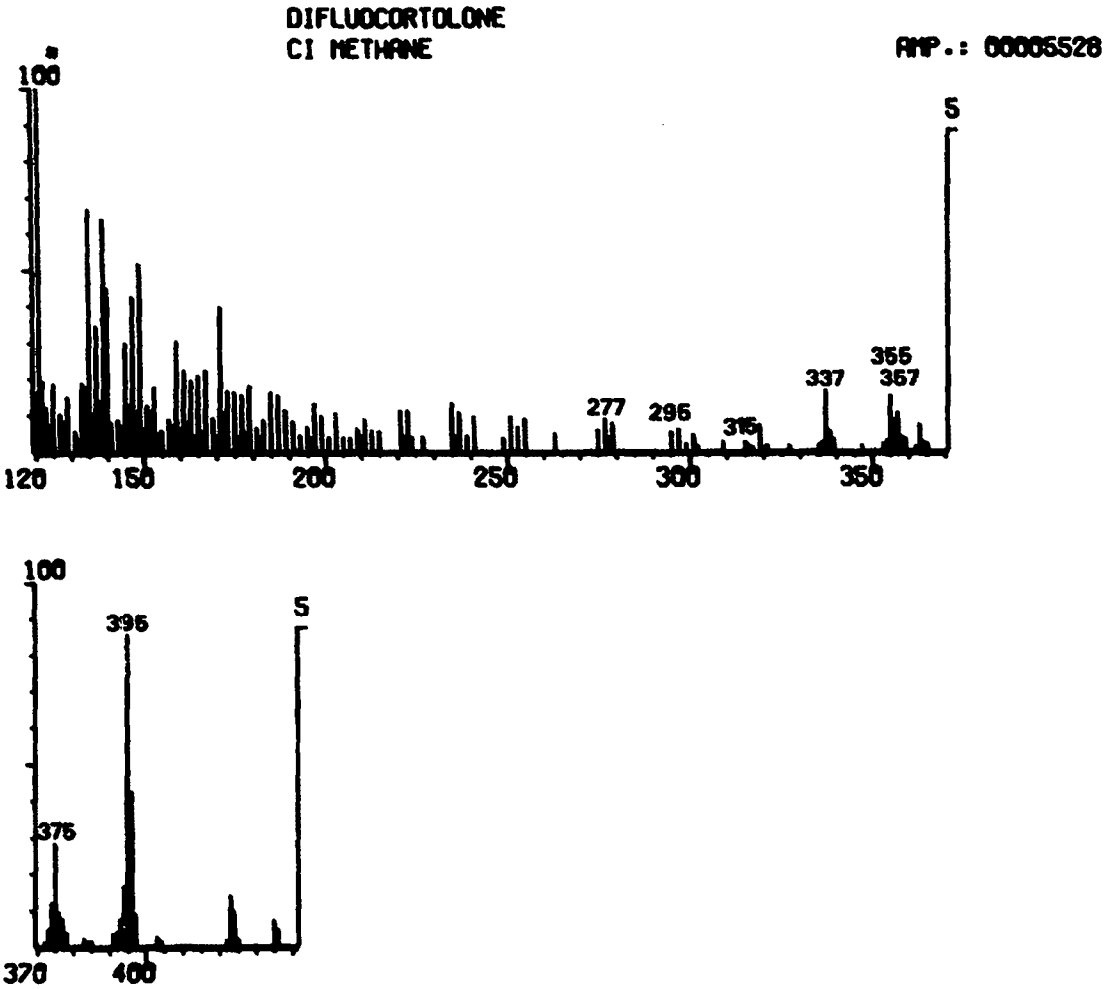

Fig. 9. The CI-mass spectrum of diflucortolone.

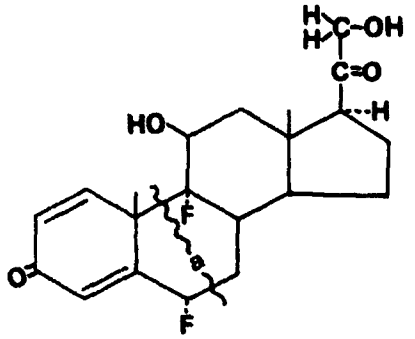

Fig. 10. The fragmentation of diflucortolone leading to m/z 139–140.

H_2O); 295 (M^+–2x HF, CO–CH_2OH); 227 (M^+–2x HF, H_2O, $\dot{C}O$–CH_2OH).

In Fig. 10 is illustrated fragmentation a which has been discussed previously (Dekker, 1979a).

With prednisolone this fragmentation leads to m/z 121–122 and with diflucortolone the presence of a fluorine atom leads to analogous peaks with 18 mass units more (an H atom at C6 has been changed for a fluorine atom), the peaks 139–140. In Fig. 11 the CI-mass spectrum of compound e is given. The parent peak of this compound is 374 (CI 375). The difference with the parent peak of diflucortolone is 20 mass units. Consequently one molecule of HF has disappeared from diflucortolone. The presence of the peaks 139–140, as discussed in the mass spectra of diflucortolone, shows that the fluorine atom at C6 is still present. Thus only the fluorine atom at C9 has disappeared, resulting in a compound with a double bond between C11 and C9 or between C9 and C8, as illustrated in Fig. 6 for the decomposition product of desoximetasone. Peaks derived from the parent peak (M^+ = 374; CI 375) of this compound are: 355 (M^+–HF); 357 (M^+–H_2O); 337 (M^+–HF, H_2O); 297 (M^+–H_2O, $\dot{C}O$–CH_2OH); 295 (M^+–HF, $\dot{C}O$–CH_2OH).

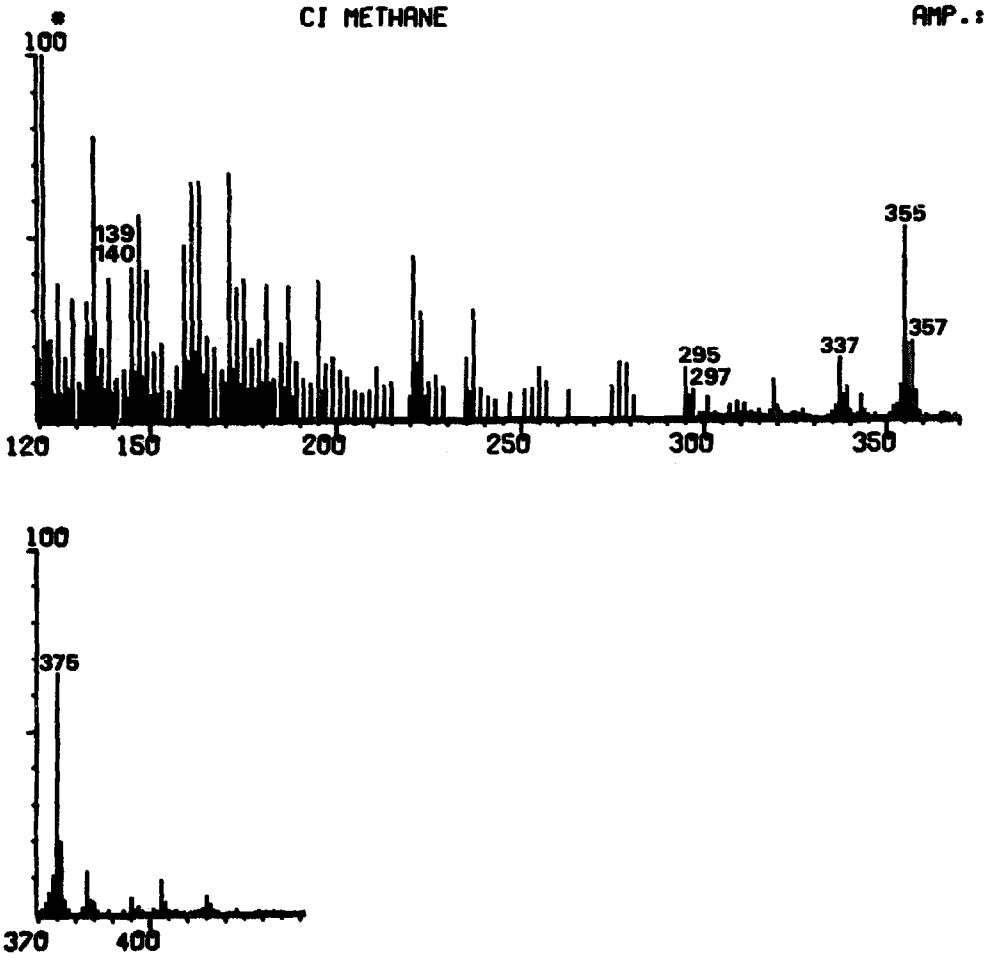

Fig. 11. The CI-mass spectrum of compound e.

3. Discussion

In Fig. 8 is illustrated the partition chromatogram of the total suspension (at pH 8) obtained with high pressure liquid chromatography. It can be seen, that under these extreme conditions (16 h at 120°C) only a little of compound e was formed in the total suspension (peak d is diflucortolone). Therefore, it can be concluded, analogous to desoximetasone, that decomposition under anaerobic conditions due to a fluorine atom at C9 can be neglected under normal sterilization conditions (e.g. 20 min at 120°C).

It is also obvious that the fluorine atom at C6 is stable under the chosen decomposition conditions. This is in agreement with the fact that no decomposition due to the fluorine atom at C6 was observed with fluocortolone.

IV Dexamethasone

Fig. 2K and L illustrates the thin-layer chromatogram of the filtrate of the decomposition mixture obtained at pH 8 and the thin-layer chromatogram of the chloroform extract of this filtrate. Spot f (dexamethasone) and spot g (a decomposition product) gave a positive reaction with tetrazolium blue reagent. Spot g has the same R_f value as desoximetasone, and the same k′ value was obtained with the used partition chromatography (k′ = 3.6). The decomposition of a 17-hydroxy-corticosteroid to a 17-deoxy-corticosteroid has been described for prednisolone (Dekker, 1979a). In that publication it was shown that prednisolone was decomposed to a complex mixture of decomposition products. It is likely that dexamethasone decomposed to an analogous mixture of decomposition products. In this mixture of decomposition products the compound b (as illustrated in Fig. 6) could not be detected, either with thin-layer chromatography or with the high pressure liquid chromatography used. As desoximetasone could be detected as an important decomposition product, it is obvious that the decomposition due to a fluorine atom at C9 can be neglected in comparison with the decomposition of the side-chain under the chosen conditions.

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