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Note

Improved high-performance liquid chromatographic separation of decomposition products of prednisolone by adding sulphite to the mobile phase

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Under anaerobic conditions, decomposition of corticosteroids occurs[1–5], and optimal separation of important anaerobic decomposition products of prednisolone has been obtained by high-performance liquid chromatography (HPLC) using partition chromatography[1].

An important decomposition product of prednisolone is the 17-ketosteroid (11β-hydroxy-1,4-androstadiene-3,17-dione)[5]. Mason[3] earlier reported that under anaerobic alkaline conditions, 50% of the amount of cortisone initially present was converted into the 17-ketosteroid (1,4-androstadiene-3,11,17-trione).

Decomposition of corticosteroids into the 21-dehydro-compound has been reported[6,7] to occur under oxidative conditions.

In our partition chromatographic method, 21-dehydroprednisolone and the 17-ketosteroid (11β-hydroxy-1,4-androstadiene-3,17-dione) have an identical k' value. The separation of these two compounds could be accomplished by adding sulphite to the mobile phase.

In our studies on the anaerobic decomposition of prednisolone, we are now able to detect the presence of 21-dehydroprednisolone, produced by undesirable oxidative decomposition.

MATERIALS AND METHODS

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

21-Dehydroprednisolone was prepared by oxidation of prednisolone with copper (II) acetate in methanol, according to Conbere and Fanwood[8].

The 17-ketosteroid of prednisolone (11β-hydroxy-1,4-androstadiene-3,17-dione) was isolated from the decomposition mixture of prednisolone as described by Dekker and Buijs[5].

A liquid chromatograph (Waters Assoc., Milford, MA, U.S.A.), a stainless-steel column (30 cm × 3.9 mm I.D.), and a variable-wavelength detector (Pye Unicam LC3 UV detector) were used. Detection was performed at 240 nm.

The column packing was porous silica particles, permanently bonded to a monomolecular layer of organosilane (μBondapak C_{18}; Waters Assoc.). The com-

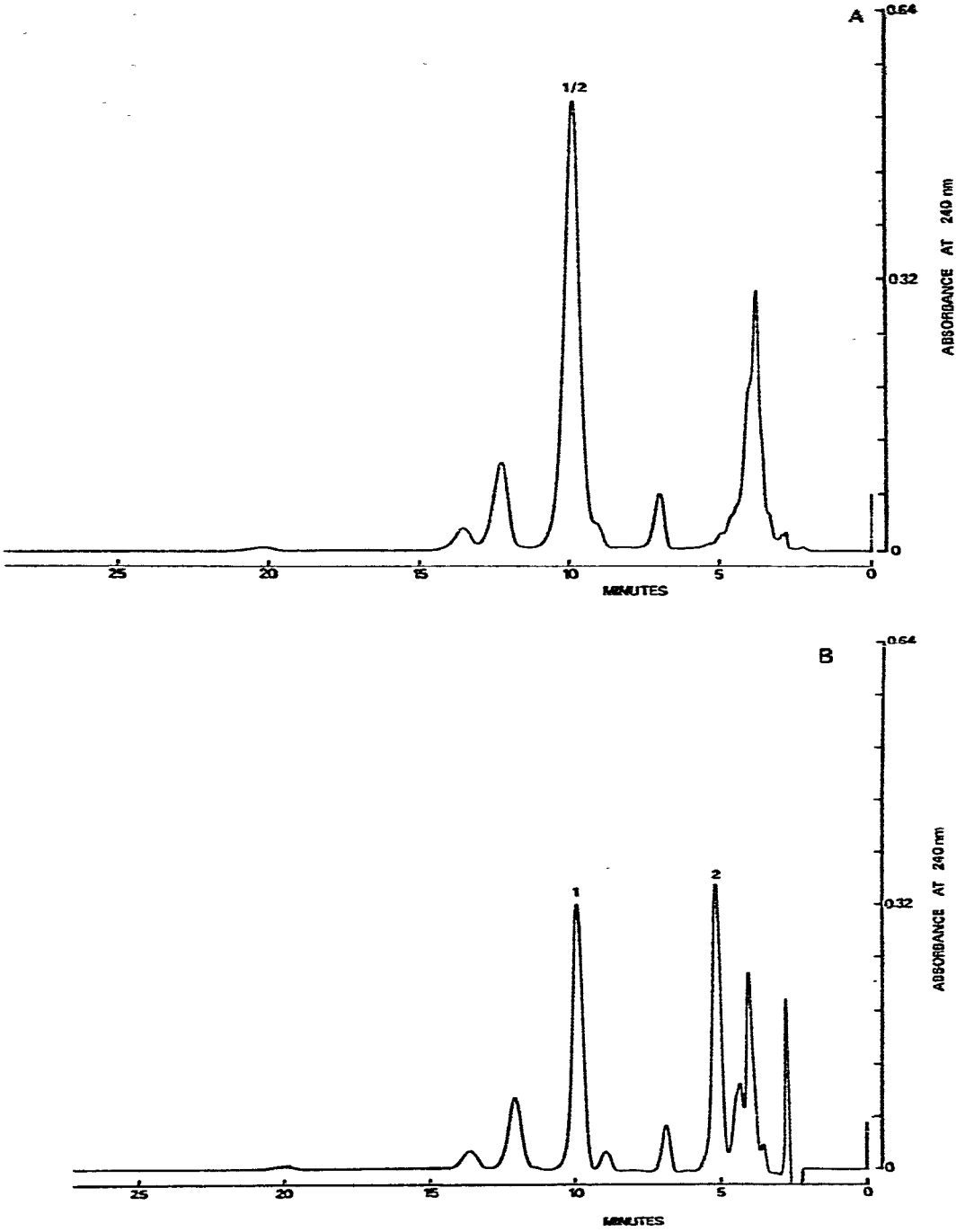

Fig. 1. (A) HPLC of the filtrate of the decomposition mixture of prednisolone with added 21-dehydroprednisolone. Solvent: methanol-water (1:1, w/w), to which 1% (v/w) of a 0.4 *M* sodium phosphate solution of pH 7.0 was added. Column: μBondapak (30 cm × 3.9 mm I.D.); temperature, 25°C; flow-rate, 1.0 ml/min; wavelength, 240 nm; sensitivity, 0.64 a.u.f.s. (B) As (A), except that sodium sulphite (0.1%, w/w) was added to the mobile phase.

position of the mobile phase was methanol (analytical-reagent grade)–water (1:1, w/w). To this solvent, 1% (v/w) of a 0.4 *M* sodium phosphate solution of pH 7.0 was added. Sodium sulphite (analytical-reagent grade) was optionally added to give a concentration of 0.1% (w/w). The flow-rate was 1.0 ml/min and the sensitivity was 0.64 a.u.f.s.

RESULTS AND DISCUSSION

Addition of 21-dehydroprednisolone to the filtrate of a decomposition mixture of prednisolone which was obtained under severe anaerobic alkaline conditions[1] is not detected when the solvent without sodium sulphite is used (Fig. 1A). After the addition of sodium sulphite to the solvent, 21-dehydroprednisolone (peak 2) is separated from the 17-ketosteroid (11β-hydroxy-1,4-androstadiene-3,17-dione) (peak 1) and other products (Fig. 1B). This separation may be due to the formation of an addition product of hydrogen sulphite with the aldehyde group of 21-dehydroprednisolone, resulting in a decrease in lipophilicity.

In this way, we are able to detect the presence of 21-dehydroprednisolone in anaerobic decomposition mixtures of prednisolone. This product, due to oxidative decomposition, should be absent under completely anaerobic conditions. When the formation of the 17-ketosteroid is determined by partition chromatography with a solvent without sodium sulphite, 21-dehydroprednisolone will interfere.

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