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Note

Ascorbic acid as an antioxidant in thin-layer chromatography of corticosteroids

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Autoradiography of ^{14}C- and ^{3}H-labelled corticoids following thin-layer chromatography (TLC) requires exposure of X-ray films for a week or even longer. During exposure, under the influence of oxygen in the air, corticoids become degraded and oxidative products are formed.

This paper describes the use of ascorbic acid as a protective agent during chromatography and autoradiographic detection of corticoids by TLC.

MATERIALS AND METHODS

All the materials and fine chemicals used were of analytical grade: silica gel G, silica gel GF_{254} (Merck, Darmstadt, G.F.R.); ascorbic acid (Riedel de Haen, Seelze, Hannover, G.F.R.); and [4-^{14}C]corticosterone, 61 mCi/mmole (NEN, Boston, Mass., U.S.A.).

Preparation of 4-^{14}C dihydrocorticosterone (4-^{14}C-5-α-pregnan-11β,21-diol-3,20-dione) (DHC)

We obtained [4-^{14}C]dihydrocorticosterone by biological conversion of [4-^{14}C]-corticosterone by incubation with rat kidney tissue slices in Krebs–Ringer hydrogen carbonate buffer at 37° for 3 h. The steroids extracted[1] from the incubation medium were separated on silica gel plates.

Preparation of plates for TLC

Glass plates, 20 × 20 cm, were coated with a 250-μm layer of a mixture containing equal amounts of silica gel G and silica gel GF_{254} in distilled water (30 g per 60 ml) and activated at 100° for 1 h.

Plates that were later treated with ascorbic acid were coated with a 250-μm layer of silica gel G suspended in distilled water (30 g per 60 ml) and activated by heating to 100° for 1 h.

After cooling, the plates were sprayed with a saturated solution of ascorbic acid in absolute ethanol.

Ascorbic acid extinguishes the fluorescence of silica gel GF_{254} in the ultraviolet

region and makes detection with UV light impossible on plates sprayed with ascorbic acid.

After transferring the samples, the plates were first developed in *n*-heptane in order to eliminate impurities and then corticosterone and its metabolites were separated by developing the plates in acetone–benzene (5:9). This mixture was found to be more suitable than chloroform–ethanol (98:2)[1], benzene–acetone (4:1)[2] and other mixtures tested.

Autoradiography

Autoradiography was performed by placing an X-ray film (Sanix, Fotokemika, Zagreb, Yugoslavia) on the plate, exposing it to irradiation for 7 days and developing the film at the end of this period.

Densitometry

Dark spots on the X-ray film were subjected to densitometry using a Kipp and Zonnen DD-2 densitometer and a Kipp and Zonnen BC-1 digital integrator.

RESULTS AND DISCUSSION

Following metabolic degradation of corticosterone, single corticoids were separated on TLC plates. The positions of single fractions were determined by autoradiography. The R_F values of corticosterone and the main metabolites are presented in Table I.

TABLE I

R_F VALUES OF CORTICOSTERONE AND ITS METABOLITES ON SILICA GEL G PLATES
Solvent: acetone–benzene (5:9).

Compound	R_F	*Rel.* R_f *
Corticosterone	0.51	1.00
Dihydrocorticosterone	0.57	1.15
5α-pregnan-3β, 11β, 21-triol-20-one	0.44	0.86

* Relative to corticosterone.

Single fractions were scraped off, eluted with acetone and transferred to the plates again. Re-chromatography using the same solvent system showed that a significant portion of DHC had been converted into its degradation products. At the spot which corresponded to DHC, only a small portion of radioactivity was found, and at the same time the appearance of labelled oxidative products was noted. After re-chromatography, DHC accounted for only 30% of the total activity (Fig. 1). As the remaining activity was found near the start and front, the possibility of incomplete separation during the first chromatography was excluded.

Ascorbic acid was found to be an efficient protective antioxidant for corticosteroids on TLC plates (Fig. 2). DHC accounted for 80% of the total activity transferred to plates sprayed with ascorbic acid, *i.e.*, ascorbic acid increased the DHC recovery 2.5 times.

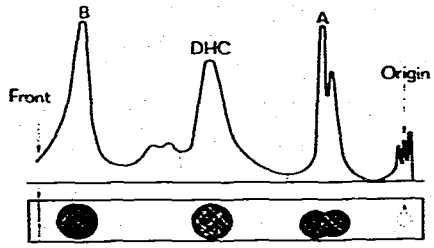

Fig. 1. Autoradiograph of the re-chromatogram of dihydrocorticosterone subjected to densitometry. DHC = dihydrocorticosterone; A and B = degradation products. DHC accounts for 30% of the total radioactivity.

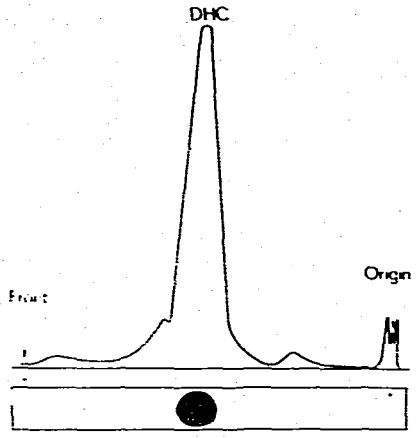

Fig. 2. Autoradiograph of the re-chromatogram of dihydrocorticosterone (DHC) subjected to densiometry on plates sprayed with ascorbic acid. DHC accounts for 80% of the total activity transferred o the plate.

Ascorbic acid, transferred to plates after activation and before the transfer of corticosteroid samples, provides protection against oxidation and keeps corticoids sensitive to oxidation unchanged throughout the long exposure to air during autoradiography. They are then still intact for further use in biological experiments after their elution from thin-layer plates.

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REFERENCES

1 D. W. Frederiksen and J. D. Wilson, *J. Biol. Chem.*, 246 (1971) 2584.
2 Y. Suzuki, *Endocrinology*, 90 (1972) 924.