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Note

Thin-layer chromatographic separation of corticosteroids from their respective 17-oxo-oxidation products

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17-Oxosteroids derived from corticosteroids by oxidation are occasionally found in the dosage forms of these drugs[1], in amounts up to 2%. Some of these oxidation products have a small but measurable hormonal activity and it is important that their presence should be readily detectable at a low level. Since both corticosteroids and oxidation products possess a strong UV chromophore, the α,β-unsaturated ketone moiety, we have developed a thin-layer chromatographic (TLC) system for their separation on fluorescent silica gel. Using the procedure developed by Hall[2] for separating corticosteroids from each other, in which 17-oxosteroid impurities were not considered, we have modified the solvent mixture to suit the particular requirements of a general test to separate corticosteroids from the products of oxidative side-chain cleavage.

EXPERIMENTAL

Glass plates, 20 cm × 20 cm, precoated with silica gel 60, 0.25 mm (Brinkmann, Westbury, N.J., U.S.A.) were used.

The following amounts of steroids were chromatographed: (a) corticosteroid, 200 and 100 μg; (b) oxidation product, 2, 1, 0.8, 0.6, 0.4, and 0.2 μg; (c) mixtures of 100 g of corticosteroids plus 1 μg of its oxidation product, and 200 μg of corticosteroid with 2 μg of oxidation product. The corticosteroids and their oxidation products used are listed in Table I.

The following corticosteroids, whose oxidation products were not available, were included in the study: triamcinolone, fludrocortisone acetate, and paramethasone acetate, each being applied at the 200-μg level.

The solvent used was methylene chloride–dioxane–water (100:50:50) (ref. 2) and (120:30:50). The mixture was shaken together and allowed to separate. The lower layer was run through filter paper into the tank.

Ultraviolet light, 254 nm, was used for the detection of spots after chromatography.

TABLE I

CORTICOSTEROIDS AND THEIR OXIDATION PRODUCTS

Corticosteroid	*Oxidation product*
Prednisone + 21-acetate	1,4-Androstadiene-3,11,17-trione
Prednisolone + 21-acetate	11β-Hydroxy-1,4-androstadiene-3,17-dione
Dexamethasone + 21-acetate	9α-Fluoro-11β-hydroxy-16-methyl-1,4-androstadiene-3,17-dione
Hydrocortisone + 21-acetate	11β-Hydroxy-4-androstene-3,17-dione
Cortisone acetate	4-Androstene-3-11,17-trione
Betamethasone + 21-acetate	9α-Fluoro-11β-hydroxy-16β-methyl-1,4-androstadiene-3,17-dione
Methylprednisolone + 21-acetate	11β-Hydroxy-6α-methyl-1,4-androstadiene-3,17-dione
Fluprednisolone	6α-Fluoro-11β-hydroxy-1,4-androstadiene-3,17-dione

TABLE II

hR_F VALUES OF CORTICOSTEROIDS AND THEIR OXIDATION PRODUCTS

Solvent systems: methylene chloride–dioxane–water (100:50:50) (A) and (120:30:50) (B).

Compound	*Solvent system*	
	A	*B*
Prednisone	54	27
Oxidation product	92	79
Acetate	79	58
Prednisolone	34	13
Oxidation product	77	55
Acetate	71	45
Dexamethasone	42	17
Oxidation product	78	60
Acetate	79	52
Hydrocortisone	43	17
Oxidation product	84	63
Acetate	80	56
Cortisone acetate	83	67
Oxidation product	91	87
Betamethasone	43	17
Oxidation product	78	59
Acetate	78	52
Methylprednisolone	36	12
Oxidation product	94	88
Acetate	77	50
Fluprednisolone	37	14
Oxidation product	79	61
Fludrocortisone acetate	78	56
Paramethasone acetate	78	55
Triamcinolone	22	07

RESULTS

The results obtained are summarized in Table II. In all cases, oxidation products could be detected down to a level of 0.4 μg.

DISCUSSION

The modified mixture (120:30:50) gives a greater range of R_F values and separates the oxidation products from their respective corticosteroids by wider margins than the previously reported mixture (100:50:50). The 21-acetates were included in this study to assist in the prediction of the behaviour of the oxidation products of fludrocortisone acetate and paramethasone acetate, for which the respective 17-oxosteroids were not available. Since it is our experience that 21-acetates may be found at low levels as impurities in formulations containing the respective parent corticosteroids, it is important that when present they do not mask the presence of any 17-oxo-oxidation product. It was found that the modified mixture would effect this separation for betamethasone and dexamethasone and their respective derivatives but that the mixture (100:50:50) would not. By comparing the R_F values of fludrocortisone acetate and paramethasone acetate with those of the compounds to which they are most related structurally, it may reasonably be extrapolated that each would be separated from its respective oxidation product. The remaining compound, triamcinolone, with its 16α-hydroxy group, is more difficult to compare with any of the other compounds on a structural basis, although it might be expected that the 16α-hydroxy-17-ketosteroid derived from triamcinolone would be easily separated from the corticosteroid.

Since the 17-oxosteroids are detectable at a level of 0.4 μg, by applying 100 μg of corticosteroid, the oxidation products can be detected at the 0.4% level, and their presence is easily demonstrable at the 1% level.

REFERENCES

1 B. A. Lodge, H. Watanabe and L. Watts, *Can. J. Pharm. Sci.*, 10 (1975) 107.
2 A. Hall, *J. Pharm. Pharmacol.*, 16 (1964) 9T.