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SHORT COMMUNICATION

Determination of 21-acyloxy corticosteroids and other steroid esters*

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Corticosteroids in which the 21-hydroxyl group is acylated form an important group of steroid drugs. The usual methods for their determination include ring A spectrophotometry or colorimetry as well as tetrazolium methods, neither of them being specific to the ester group.

Methods based on the ester group, such as the ferric hydroxamate method (Forist & Theal, 1958) and the differential kinetic variant of the tetrazolium method (Guttman, 1966), are seldom used in pharmaceutical analysis.

Although the classical method of alkaline hydrolysis followed by the back-titration of excess base can be used successfully for the determination of some steroid esters, it cannot be applied directly to 21-acyloxy corticosteroids. This is because the α-ketol side-chain produced on hydrolysis is oxidized by atmospheric oxygen under alkaline conditions, the main oxidation products being the corresponding etianic acids and formic acid (Velluz, Petit & others, 1947). Even under anaerobic conditions products such as α-hydroxy acids are formed (Guttman & Meister, 1958).

The difficulties caused by such side-effects may be overcome by reducing the 20-keto-group with sodium borohydride when the resulting glycol side-chain is insensitive to further change either by alkali or atmospheric oxygen.

Experimental

Reagents. All chemicals were of analytical reagent grade.

Reagent for hydrolysis: an aqueous solution of sodium hydroxide (0·2N) containing 0·1 mol/litre of sodium borohydride. Stored at room temperature, this solution is usable up to 2 months. 0·1N Hydrochloric acid. Phenol red indicator, 0·1% aqueous solution containing 20% v/v ethanol. 0·5M aqueous solution of sodium acetate.

Apparatus. A Radelkis OP–204 pH-titrimeter was used with the usual glass and saturated calomel electrodes.

Procedure. Dissolve an accurately weighed sample of the steroid ester (about 0·5 m-equiv) in ethanol (15 ml, 96% v/v). Add reagent for hydrolysis (5·0 ml) and reflux (1 h) at 100°. Add methyl ethyl ketone (0·5 ml) and reflux a further 5 min. After cooling add water (20 ml) and phenol red indicator (5 drops). Titrate the solution with 0·1N hydrochloric acid to a salmon-pink colour.

Make a blank titration treating the solutions in a manner identical to the actual estimation but substituting 0·5M sodium acetate solution (1 ml) for the steroid ester. The percentage of the steroid ester can be calculated from the difference between blank and sample titrations.

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Results

Table 1 summarizes the results obtained using the recommended procedure on different types of steroid esters. The steroids used for this work were of the highest quality available. This was controlled by thin-layer chromatography and measuring their physical constants.

As it can be seen from the data of Table 1, the standard deviation of the method does not exceed $\pm 0{\cdot}7\%$. This precision is better than that of the colorimetric methods.

Discussion

By reducing the 20-keto-group of steroids, side reactions are avoided; unreacted sodium borohydride is decomposed before back-titration of excess alkali by boiling with methyl ethyl ketone.

Before the titration, solutions thus contain sodium hydroxide, sodium borate and the sodium salt of the esterifying acid. Fig. 1 shows the titration curves both of sample and blank solutions.

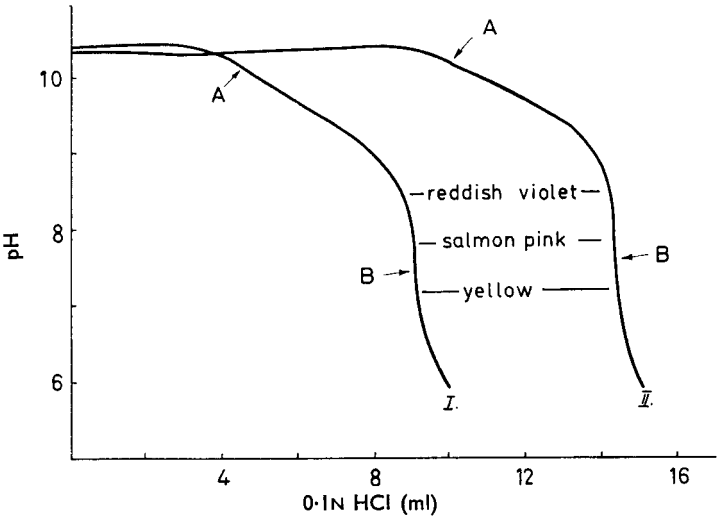

FIG. 1. I. Titration curve taken after hydrolysing 0·2276 g of pregn-5-ene-3β,17α,21-triol-21-acetate as described in the Experimental. II. Titration curve of the blank.

The point A on the curves indicates the indistinct neutralization point of sodium hydroxide while B shows the sharp end point of the displacement titration of sodium borate. The first end point has no importance while the difference between the second end points of the blank and sample solutions is characteristic of the amount of the steroid ester. 0·5 m-equiv of sodium acetate was added to the blank to keep the conditions for the titration similar to those of the sample titration.

It was found that phenol red exhibited a sharp colour change at a point corresponding exactly with the potentiometric end point and therefore this indicator has been used routinely. Dilution with water before the titration gave a sharper end point. Occasionally, as a result of the dilution (or during the titration), precipitation of the steroid occurred. This, however, did not affect the titration.

Although some groups of the compounds mentioned in Table 1 may be determined by the classical method of saponification with boiling alcoholic alkali followed by back-titration using phenolphthalein as indicator, the application of the proposed

Table 1. *Determination of some steroid esters by the proposed method*

	Number of determinations	Purity %	Standard deviation ±%
21-*Acyloxy derivatives*			
Cortisone acetate	3	100·6	—
Hydrocortisone acetate	5	100·2	0·7
Prednisone acetate	3	99·0	—
Prednisolone acetate	3	99·5	—
Prednisolone *p*-toluenesulphonate*	3	99·3	—
Desoxycorticosterone acetate	3	99·7	—
Pregn-5-ene-3β,17α,21-triol 21-acetate	8	99·7	0·5
Pregn-4-ene-17α,21-diol-3-one 21-acetate	5	100·3	0·3
17-*Acyloxy derivatives*			
Testosterone propionate	3	100·1	—
Testosterone phenylpropionate	5	99·2	0·5
19-Nortestosterone phenylpropionate	3	98·9	—
Norethisterone acetate	6	100·0	0·4
3-*Acyloxy derivatives*			
Oestrone acetate	3	99·0	—
Oestrone caproate	3	100·4	—
Oestradiol benzoate	5	99·6	0·6
Dehydroepiandrosterone acetate	5	98·5	0·5
Pregna-5,16-diene-3β-ol-20-one acetate	3	98·9	—
3,17-*Diacyloxy derivatives*			
Oestradiol dipropionate	3	100·5	—
Ethynodiol diacetate	6	99·8	0·4

* Sodium acetate in the blank omitted.

method offers the following advantages: 1. Ketosteroids as well as carbonyl impurities of ethanol may undergo polymerization, when boiled with a base. This can give rise to coloured solutions and the formation of acidic by-products. The former makes it difficult to observe the colour change of the indicator while the latter causes high results. The use of sodium borohydride obviates these sources of error. 2. Phenol red is less sensitive to atmospheric carbon dioxide than phenolphthalein. 3. The reagent for hydrolysis described above is more stable than alcoholic alkali.

From rate curves for some characteristic steroid esters, using the recommended conditions, all but one of the compounds (17α-hydroxyprogesterone caproate) examined could be determined.

The recommended method seems to be useful for the determination of steroid esters especially in conjunction with other methods such as spectrophotometry, colorimetry and thin-layer chromatography.

Any substances consuming alkali interfere with the determination. The interference of acid type substances can be overcome by preliminary titration with standard sodium hydroxide. Ester type solvents have to be removed by careful drying. Halogen-containing steroids suffer partial or complete hydrolysis under the reaction conditions described and therefore interfere with the determination.

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