A SPECTROPHOTOMETRIC DETERMINATION OF TRACES OF PHENOLIC STEROIDS IN 3-KETOSTEROIDS

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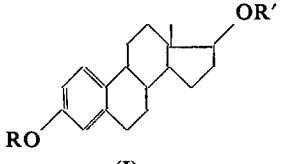
Received July 10, 1958

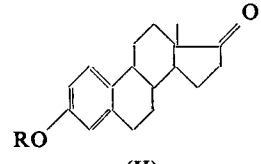
As little as 0·02 per cent of phenolic steroid may be determined in the presence of an excess of 3-ketosteroid. The interfering ketonic absorption is eliminated by selective reduction of ketone by potassium borohydride. The slight absorption of the reduction products may be corrected graphically.

In the presence of large amounts of ketosteroids the determination of oestradiol type steroids (I, II) is not possible by direct ultra-violet spectrometry since the phenolic band about 280 mμ is completely masked by the weak ketonic absorption (maximum about 300 mμ) (Fig. 1).



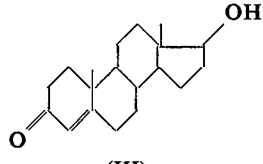
(I)

(a, R=R'=H; b, R=Me, R'=H; c, R=H, R'=MeCO

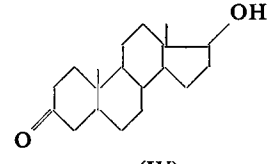


(II)

(a, R=H; b, R=Me)

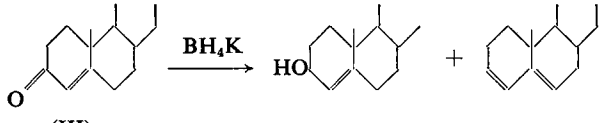


(III)



(IV)

Working with testosterone (III) or androstanolone (IV) the ketonic absorption can be eliminated by selective reduction using potassium borohydride in alkaline methanolic medium as follows.



(III)

Satisfactory results can be obtained with 4 moles BH_4K per mole of ketosteroid, the reaction being allowed to continue for 6½ hours at room temperature. When the reduction is complete and the solution re-acidified, the phenolic band becomes apparent. It is necessary to correct for the weak absorption arising from the reduction products of the ketosteroid. This can be most easily done by a graphical construction. The

residual absorption is nearly linear in the range 272 mμ–300 mμ. In the spectrum of the pure phenol, four wavelengths are selected within this range such that, $\epsilon_{\lambda 1} = \epsilon_{\lambda 3}$, $\epsilon_{\lambda 4}$ equals or is nearly 0, and $\frac{\epsilon_{\lambda 2}}{\epsilon_{\lambda 1}}$ is as large as possible, where λ_2 is the wavelength of the maximum of the phenol.

In the spectrum of a mixture after reduction, the difference in optical density at wavelengths λ_1 and λ_3 is due solely to parasitic absorption and this difference determines the slope s of a straight line △ which represents this absorption to the first approximation. The line △ intercepts the spectrum of the mixture at wavelength λ_4 since by hypothesis the optical density at this point is due solely to the parasitic absorption. The phenol concentration is obtained from the absorbance $DD' = d$ using an apparent E (1 per cent, 1 cm.) for compensating for the weak phenolic absorption at wavelength λ_4.

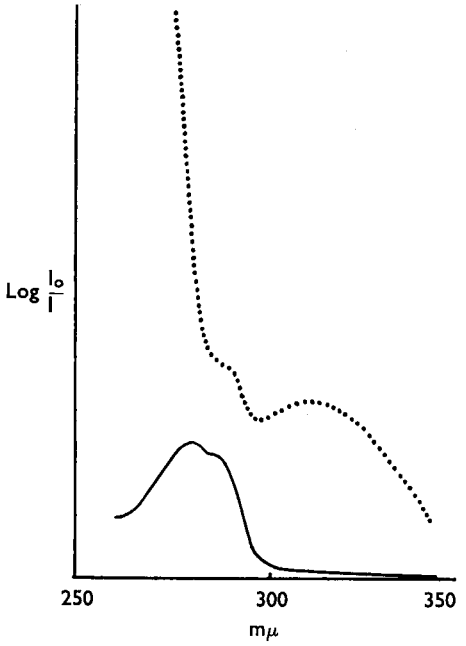


FIG. 1. Mixtures of testosterone and oestradiol.

. . . . . Mixture containing 2 per cent oestradiol before reduction.

——— Mixture containing 1 per cent oestradiol once reduced.

For the smallest concentrations in phenol one can no longer neglect the slight curvature of the true background and it is necessary to add to d an empirical correction $\delta = -a(s + b)$ where a and b are empirical factors, and s is the slope of △ expressed in absorbance variation for an increase in wavelength of 10 mμ.

EXPERIMENTAL METHOD

The steroids are pharmaceutical grade products. Methanol is refluxed with potassium borohydride for 4 hours: methanol 5000 ml., BH_4K 8g., NaOH N 40 ml. and then distilled. The potassium borohydride is a commercial product containing about 90 per cent pure BH_4K. The spectra have been recorded on a model 11 or 14 Cary spectrophotometer.

Recommended Procedure

In a 50 ml. calibrated flask dissolve P mg. (see Table I) of mixture in about 35 ml. of methanol. Add $\frac{4}{5}$ P mg. of potassium borohydride previously dissolved in 4 ml. of 0·1N aqueous sodium hydroxide. Prepare simultaneously a blank containing the same amounts of methanol, borohydride and sodium hydroxide. Allow the reaction to proceed for $6\frac{1}{2}$ hours at room temperature (20–25°) then add to both solutions 4 ml. of

normal aqueous hydrochloric acid. Eliminate dissolved gases by shaking and make up to 50 ml. with methanol. Record the spectrum of the steroid

TABLE I

QUANTITY OF SAMPLE AND CELL LENGTH USED

Phenol content of the sample (per cent)	P mg.	l cm.
About 1	200	5
0·5–0·05	200	10
0·1–0·02	500	10

solution with the blank solution in the reference cell. The weight p′ in mg. of the phenolic steroid per gram of mixture is

$$p' = \frac{5 \cdot 10^5 \, (d + \delta)}{l.\, E\,(1 \text{ per cent, } 1 \text{ cm.}).\, P}$$

where l is the cell-length in cm.

Determination of the Numerical Values used in the Graph Correction

Reduce, as previously, known amounts of pure phenolic steroid. Select correct values for λ_1, λ_2, λ_3, λ_4; draw $\triangle$ and determine with respect to $\triangle$

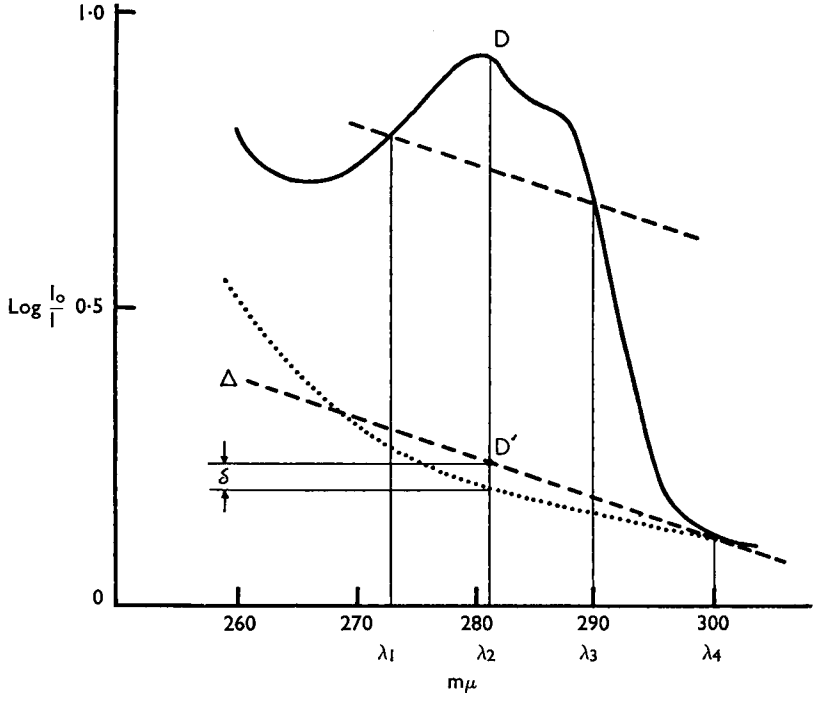


FIG. 2. Graphical correction.

. . . . . Pure testosterone after reduction by BH_4K.
——— Testosterone + 0·1 per cent oestradiol mixture after reduction by BH_4K.

taken as background the apparent E (1 per cent, 1 cm.) for the pure phenol. The values of a and b are determined under similar conditions on several reductions of pure ketosteroid. The slope s usually ranges from zero to $-0{\cdot}1$. The selected numerical values are collected in Table II. They are the same for mixtures containing either testosterone or androstanolone except that in the last case $\delta = 0$.

TABLE II

FIGURES USED FOR THE GRAPHICAL CORRECTION

Phenol determined	λ_1	λ_2 max.	λ_3	λ_4	Apparent E (1 per cent, 1 cm.)	a	b	Standard deviation on a
Ia	273	281	290	300	71·4	1·15	0·01	0·43
Ib	273	279	288·5	300	67·8	0·97	0·01	0·35
Ic	272	281	290	300	62·0	1·04	0·01	0·26
IIa	273	281	290	300	73·2	1·15	0·01	0·43
IIb	272	278·5	289	300	68·0	1·01	0·01	0·27

TABLE III

RESULTS ON KNOWN MIXTURES

Phenol content	Number of measurements (n)	$\sqrt{\frac{\Sigma e^2}{n}}$
Testosterone mixtures		
0·5 per cent	14	2 per cent
0·2	12	2·5
0·1	18	5
0·05	13	6
0·02	9	12·5
Androstanolone mixtures		
0·5 per cent	4	2·5 per cent
0·2	4	3·5
0·1	2	7·5
0·05	2	10
0·02	2	18

RESULTS

The results obtained with synthetic mixtures are tabulated (Table III) where e is the relative error expressed in per cent. For very small amounts of phenol the determining error is that calculated with reference to δ, i.e., $\Delta\delta = (s + b)\,\Delta a + a\Delta\,(s + b) \simeq (s + b)\,\Delta a + a\Delta s$.

Δa is indicated in Table II and Δs can be evaluated from the graph. The method is considered to be applicable to mixtures containing other types of borohydride reducible ketones so long as the reduction products present no chromophore absorbing below 300 mμ.