THE BEHAVIOR OF DEHYDROISOANDROSTERONE
AND ANDROSTERONE IN THE m-DINITRO-
BENZENE REACTION

BY G. O. LANGSTROTH AND N. B. TALBOT

(From the George Eastman Laboratories of Physics, Massachusetts Institute
of Technology, and from the Biological Laboratories, Harvard University,
Cambridge, and the Department of Pediatrics, Harvard Medical
School, Boston)

(Received for publication, June 9, 1939)

The data presented in this paper extend work previously re-
ported (1) on the behavior of synthetic androgens in the m-dinitro-
benzene reaction. The investigations have been undertaken in
the attempt to provide a basis for the development of assay meth-
ods which will permit a detailed quantitative determination of the
amounts of various androgens in extracts of biological material.
The present data, obtained in a comparative study of the absorp-
tion spectra of the compounds formed by dehydroisoandrosterone
and androsterone, have an additional interest in view of the numer-
ous recent applications of the reaction to the specific problem of
the colorimetric assay of urinary extracts.

There is some disagreement in the literature concerning the
comparative behavior of dehydroisoandrosterone and androsterone
in the m-dinitrobenzene reaction. Neustadt (2) states that the
two hormones give practically identical color reactions, while
Friedgood and Whidden (3) have reported that, weight for weight,
the former gives rise to a greater extinction in the 5200 Å. region.
Callow, Callow, and Emmens (4), on the other hand, have pub-
lished data which suggest that dehydroisoandrosterone gives rise
to slightly less extinction. These results were obtained with the
aid of filters, and the various investigators used different modifi-
cations of the reaction conditions. Aside from a rough curve
published by the last named authors, there appear to be no data
in the literature on the absorption spectrum of dehydroisoandro-
sterone reaction mixtures. Since an interpretation of the colori-
metric or spectrochemical assay value for an extract containing these hormones in comparable amounts (e.g. normal male urines (5–7)) must involve some consideration of the extinction produced by each, it appeared desirable to obtain more definite information concerning their comparative behavior in the reaction.

In the present investigation extinction-wave-length curves were studied over the range 3100 to 6500 Å, under reaction conditions similar to those previously employed (1). The curve shapes and the absolute values of the extinction were found to be identical for the two hormones within the limit of experimental error. Moreover, marked changes in the reaction conditions affected the curves for the two hormones in the same way. Without exception the data indicate that dehydroisoandrosterone and androsterone behave identically in the reaction, and that it is impossible to distinguish between them without the aid of additional procedures. This finding is not surprising in view of the structural similarity of the two hormones in the neighborhood of the chromogenic group.

On the basis of these data, the assay value obtained from examination of the extinction of a reaction mixture containing both hormones represents the total content of dehydroisoandrosterone and androsterone. The procedure previously described (1) may therefore be applied without modification in the analysis of synthetic mixtures for dehydroisoandrosterone plus androsterone, and testosterone. A convenient procedure for the separate determination of androsterone and dehydroisoandrosterone is being developed by Mr. A. Fineman in this laboratory (Massachusetts Institute of Technology), and will be described in a later publication.

**EXPERIMENTAL**

The m-dinitrobenzene reaction conditions, the standardization of reagents, and the method of determining the extinction-wave-length curves were identical with those previously described (1). Samples of crystalline dehydroisoandrosterone and androsterone were obtained through the kindness of Dr. Erwin Schwenck of the Schering Corporation. They were made up in known solutions with absolute alcohol, without further purification.
Results

The extinction, $K$, for any particular wave-length is defined by the equation $KL = \log(i'/i)$, where $i'$ and $i$ represent respectively

![Diagram showing extinction wave-length curves for reaction mixtures containing various amounts of dehydroisoandrosterone, and comparison with that for mixtures containing androsterone. The reaction mixtures were made up with an 11.6 mg. per cc. stock dinitrobenzene solution, and were developed for 80 minutes at 25.1°. The solid line represents the curve shape for androsterone reaction mixtures (cf. (1)). The plotted points represent observations on the curve shape for reaction mixtures containing various amounts of dehydroisoandrosterone, as follows: $\bullet$ 0.10 mg., + 0.20 mg., $\bigcirc$ 0.40 mg., and $\bullet$ 0.50 mg.]

the intensities of the light transmitted through a thickness, $l$, of the blank and test reaction mixtures. The $K-\lambda$ curves therefore
Androsterones correspond to the absorption spectra of the compounds associated with the presence of the hormones. All $K$ values have been reduced to the basis of a 4.0 cc. dilution after development by an application of Beer's law (cf. (1)). The error in the spectroscopic

![Graph](https://via.placeholder.com/150)

**Fig. 2.** Comparison of the shape of the extinction-wave-length curves for corresponding dehydroisoandrosterone and androsterone reaction mixtures (a) with decreased time of development, and (b) with decreased m-dinitrobenzene addition. (a) The reaction mixtures were made up with an 11.6 mg. per cc. stock dinitrobenzene solution, and were developed for 20 minutes at 25.1°. ○ and ● denote observations made on reaction mixtures containing respectively 0.20 mg. of dehydroisoandrosterone and 0.20 mg. of androsterone. (b) The reaction mixtures were made up with a 2.0 mg. per cc. stock dinitrobenzene solution, and were developed for 73 minutes at 25.1°. △ and ▲ denote observations made on reaction mixtures containing respectively 0.20 mg. of dehydroisoandrosterone and 0.20 mg. of androsterone.
determination of the $K$ values was less than 0.03 for values between 0.0 and 1.0, less than 0.06 for values between 1.0 and 2.0, and less than 0.09 for values greater than 2.0. In the following,

![Graph showing extinction-wave-length curves for reaction mixtures containing both dehydroisoandrosterone and androsterone, and comparison with that for mixtures containing only one of the hormones. The reaction mixtures were made up with an 11.6 mg. per cc. stock dinitrobenzene solution, and were developed for 80 minutes at 25.1°. The dashed line represents the curve shape for mixtures containing only one of the hormones. The plotted points represent observations on mixtures containing both hormones, as follows: ○ 0.30 mg. of dehydroisoandrosterone plus 0.10 mg. of androsterone, and ● 0.10 mg. of dehydroisoandrosterone plus 0.30 mg. of androsterone.](http://www.jbc.org/)

the designation standard reaction conditions means that the reaction mixtures were made up with an 11.6 mg. per cc. stock dinitrobenzene solution (0.2 cc.), and were developed for 80
minutes at 25.1°; these conditions were found to be suitable for simple assay work.

![Graph](attachment:image.png)

**FIG. 4.** Comparison of the shape of the extinction-wave-length curves for reaction mixtures containing dehydroisoandrosterone plus testosterone, with those for mixtures containing androsterone plus testosterone. The reaction mixtures were made up with an 11.6 mg. per cc. stock dinitrobenzene solution and were developed for 80 minutes at 25.1°. O and ● represent observations on reaction mixtures containing respectively 0.30 mg. of dehydroisoandrosterone plus 0.30 mg. of testosterone, and 0.30 mg. of androsterone plus 0.30 mg. of testosterone. As previously described (1) the curve shape does not correspond to that obtained by superimposing the curves for equivalent amounts of androsterone and testosterone developed separately.

The typical data on the shape of the extinction curves given in Figs. 1 to 4, and on the absolute value of the extinction at 5200 Å, given in Table I, are sufficient to fix the absolute extinction-
wave-length curves. This mode of presentation has been adopted since it serves to show most clearly the closely identical behavior

### Table I

**Absolute Extinction Values at 5200 Å for Dehydroisoandrosterone Reaction Mixtures, and Comparison with Those for Corresponding Androsterone Mixtures**

<table>
<thead>
<tr>
<th>Reaction conditions</th>
<th>Composition of sample</th>
<th>$K$ (5200 Å)</th>
<th>Fig. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dehydroisoandrosterone</td>
<td>Androsterone</td>
<td>Testosterone</td>
</tr>
<tr>
<td>Standard</td>
<td>mg.</td>
<td>mg.</td>
<td>mg.</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.10</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>0.20</td>
<td>1.86</td>
</tr>
<tr>
<td></td>
<td>0.40</td>
<td>0.40</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>0.50</td>
<td>4.2</td>
</tr>
<tr>
<td>20 min. development</td>
<td>0.20</td>
<td>0.20</td>
<td>1.7</td>
</tr>
<tr>
<td>2.0 mg. per cc. stock dinitro-benzene solution</td>
<td>0.20</td>
<td>0.20</td>
<td>0.93</td>
</tr>
<tr>
<td>Standard</td>
<td>0.30</td>
<td>0.10</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.30</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>0.30</td>
<td>3.1</td>
</tr>
</tbody>
</table>

*Owing to errors in making up the stock hormone solutions used in the work on which the previous article (1) was based, the unit of hormone content expressed as mg. in that article differs somewhat from actual mg.; this circumstance has no other effect on the results and hence, for the purpose of that investigation, has no importance.

of dehydroisoandrosterone and androsterone in the m-dinitro-benzene reaction.

The following observations concerning the results may be made:

(a) Under standard reaction conditions, the shape of the extinction-wave-length curves is independent of the amount of de-
hydroisandrosterones present; this characteristic has also been found with androsterone reaction mixtures (1). The curve shape throughout the wave-length range studied is identical with that for androsterone reaction mixtures, as are the absolute values of the extinction for equal amounts of the two hormones (Fig. 1 and Table I). (b) A decrease in development time (from 80 to 20 minutes) results in an identical decrease in the absolute extinction values for both hormones; if there is any modification in the shape of the extinction-wave-length curves, it is the same for both hormones (Fig. 2 and Table I). (c) A decrease in the amount of dinitrobenzene added (from 2.3 to 0.4 mg.) results in an identical decrease in the absolute extinction values for both hormones; any modification of curve shape which may occur is the same for both hormones (Fig. 2 and Table I). (d) Reaction mixtures containing both hormones have the same curve shape and the same absolute extinction values as those containing an equivalent amount of either of the hormones alone. This feature is especially noteworthy, since it is known (1, 4) that the androsterone calibration curve is not linear; i.e., that the “efficiency” of the reaction in producing the androsterone compound decreases with increasing androsterone content. The extinction-wave-length curve for a reaction mixture containing androsterone does not correspond exactly, therefore, with that obtained by superimposing the curves for two reaction mixtures each containing, say, half the amount of androsterone. In spite of this characteristic, the addition of dehydroisandrosterone to a mixture containing androsterone is just equivalent to adding a similar amount of androsterone (Fig. 3 and Table I). (e) It has been shown that the presence of testosterone appreciably influences the formation of the androsterone compound in the reaction (1). The data of this article show that it has the same effect on the formation of the dehydroisandrosterone compound (Fig. 4 and Table I).

DISCUSSION

The data indicate that dehydroisandrosterone and androsterone behave identically in the m-dinitrobenzene reaction, and that marked variations in the reaction conditions affect the extinction-wave-length curves for both hormones in the same way. This is not surprising, since the two hormones have identical
structures in the neighborhood of the chromogenic group. A single set of data obtained by Callow et al. (4) under similar reaction conditions, suggested a slightly lower (7 per cent) extinction in the 5200 Å. region for the dehydroisoandrosterone reaction mixtures; this difference could well be due to experimental error and in view of the results of the present investigation cannot be regarded as significant. Neustadt (2) states, without giving supporting evidence, that the two hormones give practically identical color reactions in the procedure used by him; this agrees with the present findings, since it appears likely that use of aqueous alcohol and somewhat modified reaction conditions will affect the behavior of both hormones in the same way. On the other hand, Friedgood and Whidden (3) state, also without giving supporting data, that dehydroisoandrosterone reaction mixtures have a greater extinction in the 5200 Å. region than do the corresponding androsterone mixtures; it is difficult to reconcile this statement with the results of the present investigation.

On the basis of the present evidence, the assay value obtained from examination of the extinction of mixtures containing both dehydroisoandrosterone and androsterone, represents the total content of both hormones. Analysis of synthetic mixtures for dehydroisoandrosterone plus androsterone, and testosterone, may therefore be made by the procedure already described (1), when all three hormones are present. This fact furnishes the basis for a convenient method of determining androsterone and dehydroisoandrosterone separately. The procedure, now being developed by Mr. A. Fineman in this laboratory, involves (a) a determination of the total content of both hormones in the original sample, (b) precipitation of dehydroisoandrosterone with digitonin, and (c) a determination of the androsterone left in the supernatant. The details of this procedure will be published later.

We are indebted to Mr. H. J. Rugo for valuable assistance in the experimental work. The investigation has been carried out under a grant from the Rockefeller Foundation to Professor G. R. Harrison for the application of spectrophotometry to medical problems; part of the chemical equipment and supplies was obtained with the aid of funds from the Rockefeller Foundation administered by Professor F. L. Hisaw.
A comparative study has been made of the absorption spectra of the compounds formed by synthetic dehydroisoandrosterone and androsterone in the m-dinitrobenzene reaction. The wavelength range investigated extended from 3100 to 6500 Å. The results furnish strong evidence that the two hormones behave identically in the reaction. This fact has some interest with respect to commonly used colorimetric assay methods, since interpretation of the assay value obtained for samples containing both hormones must involve some consideration of the extinction due to each. Furthermore, it provides the basis for a convenient method, now being developed, of determining dehydroisoandrosterone and androsterone separately.

BIBLIOGRAPHY
THE BEHAVIOR OF DEHYDROISOANDROSTERONE AND ANDROSTERONE IN THE \textit{m}-DINITROBENZENE REACTION

G. O. Langstroth and N. B. Talbot


Access the most updated version of this article at http://www.jbc.org/content/129/2/759.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/129/2/759.citation.full.html#ref-list-1