THE ISOLATION OF α-DIHYDROTHEELIN FROM HUMAN PREGNANCY URINE

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The quantitative determination of urinary estrogens is being applied widely as a research procedure in the study of normal and pathological conditions. Most investigators have limited their work to the estimation of total estrogenic potency, although it has been known for some time that the urine of pregnant women contains at least two estrogenic compounds, theelin and theelol.

Experiments upon rabbits by Pincus and Zahl (1937) indicated that theelin is converted to theelol by the action of progesterone upon the rabbit uterus. It appeared, therefore, that the separate determination of theelin and theelol in the urine of women might provide a gage of progestin as well as estrogen metabolism, and a study (Smith, Smith, and Pincus, 1938) in which this procedure was carried out during a normal menstrual cycle and a pregnancy gave evidence in support of the supposition. Further studies (Smith and Smith, 1938, 1940) have confirmed this observation and have demonstrated that the separate determination of urinary estrogens yields much more information than may be gained by the determination of total estrogenic potency alone.

In the interpretation of these data it becomes of importance to know whether or not any estrogens other than theelin and theelol are contributing significantly to the estrogenic potency of the separate fractions. Comparison of colorimetric with bioassay

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suggested the presence in the theelin fractions of late pregnancy
urine of some estrogen other than theelin or thee101 and much
more estrogically active in rats than either of them, possibly
\( \alpha \)-dihydrotheelin (Smith, Smith, and Pincus, 1938). It was,
moreover, discovered that semicarbazide treatment of the theelin
fractions of urine from both pregnant and non-pregnant women
failed to inactivate a large part of the estrogentic potency, 30 to
60 per cent in most specimens. Investigation of the quantitative
accuracy of the method (Cohen and Marrian, 1934) used for
separating theelin from thee101 involved recovery experiments in
which crystalline estrogens were added to non-pregnancy urines
of low estrogentic content (Smith, Smith, and Schiller, 1939). It
was found that no appreciable amounts of added thee101 were
taken into the thcelin fractions, indicating that the potency of
theelin fractions after semicarbazide treatment could be very
little if at all attributable to thee101 contamination. Further,
\( \alpha \)-dihydrotheelin added to urine was completely recovered in the
theelin fraction. These results again suggested the presence of
\( \alpha \)-dihydrotheelin in human urine, accounting for the non-ketonic
activity of theelin fractions. Only the ketonic activity of theelin
fractions, therefore, was considered theelin, the rest being design-
nated \( x \) estrogen.

If the so called \( x \) estrogen could be isolated and identified as
\( \alpha \)-dihydrotheelin, the above interpretation of results would be
considerably strengthened. In addition to the work in the Fear-
ing Laboratory both Westerfeld and Huffman had worked on this
problem because of interesting results that Westerfeld and Doisy
obtained in 1937.

Certain reports on the isolation of \( \alpha \)-dihydrotheelin bear at least
superficial evidence on the identity of \( x \) estrogen with \( \alpha \)-dihydro-
theelin. In 1935, MacCorquodale, Thayer, and Doisy isolated
this compound from sow ovaries. Of course, species differences
do occur but in the absence of contradictory evidence it seems
plausible to assume that the same compound is present in human
ovaries. Owing to the difficulty of procuring sufficient human
ovaries for examination, the investigation of this problem has not
been completed but the estrogens of human placenta have been
studied and \( \alpha \)-dihydrotheelin isolated and characterized (Huffman,
Thayer, and Doisy, 1940). Furthermore, the isolation of this
compound from pregnant mare urine by Wintersteiner and co-workers (1935) and the report of the large proportion of non-ketonic estrogen in that source by van Stolk and de Lenchere (1937) indicated that the examination of human urine for dihydrotheelin might be attended with success.

Both laboratories were engaged in experiments designed to separate theelol and theelin from other estrogens of human urine at the time the Fearing group suggested to the St. Louis group that they would supply the partially purified extract for the isolation of \( \alpha \) estrogen.

At the outset of our attempt to isolate \( \alpha \)-dihydrotheelin from human pregnancy urine, it became apparent that rather specialized methods would have to be devised in order to separate this hormone from other non-ketonic estrogenic constituents (such as theelol) which might be present. It was at first tentatively proposed to approach the problem in this general fashion: (1) removal of ketones from the total phenolic extract of urine by means of carboxymethoxylamine; (2) protection of the phenolic hydroxyl in compounds of the remaining non-ketonic fraction by benzoylation; (3) treatment of the benzoates of the non-ketones with lead tetraacetate under such conditions that monocarbinols would not react but under which the 1,2-glycol, theelol, would be oxidized to a dialdehyde; the latter product could then be separated from other carbinols by means of a suitable reagent; (4) saponification of the remaining monobenzoates, recovery of the free phenols, and ultimate isolation of the dihydrotheelin as the di-\( \alpha \)-naphthoate.

It has been possible to realize experimentally all of the above steps with the exception of the quantitative monobenzylation of theelol. We have been unable to perform this step with micro quantities of theelol.

We next tried to adapt to our problem the method used by Whitman, Wintersteiner, and Schwenk (1937) for the separation of \( \alpha \)- and \( \beta \)-dihydrotheelin. According to Wintersteiner (1937, a), theelol and \( \beta \)-dihydrotheelin give no precipitate with digitonin in 80 per cent \( \text{C}_2\text{H}_5\text{OH} \). Our adaptation of this procedure to the digitonin precipitation of micro quantities of dihydrotheelin was actually used at one stage of the isolation process reported in this paper.
Shortly after the experimentation mentioned above was completed, the discovery was made in the St. Louis laboratory by A. Mather that dihydrotheelin could be effectively separated from theelin by partitioning between benzene and 0.3 M Na$_2$CO$_3$. This discovery has, of course, greatly simplified our subsequent work.\(^1\)

It is felt, however, that certain phases of our preliminary experimental work are of sufficient importance to warrant a brief discussion in this report.

**Preliminary Experiments**

*Reaction of Theelin with Carboxymethoxylamine*—Although Westerfeld et al. (1938) had used Girard’s reagent successfully in the separation of theelin from non-ketonic material, Wintersteiner (1937, b) reported that carboxymethoxylamine was superior to this reagent in the removal of ketones from mare pregnancy urine extracts.

The reaction of theelin with carboxymethoxylamine was first carried out according to the method of Anchel and Schoenheimer (1936); 15.2 mg. of theelin, 20 mg. of carboxymethoxylamine hydrochloride, and 54 mg. of NaC$_2$H$_3$O$_7$ $\cdot$ 3H$_2$O were dissolved in 4 cc. of 90 per cent C$_2$H$_5$OH and refluxed for 1 hour. The yield of carboxymethoxime was 78 per cent of the theoretical. Another similar experiment also gave a 78 per cent yield. Other reactions with smaller quantities of theelin were conducted, and bioassay upon the ether fractions (containing theelin which had failed to react) uniformly showed that this method did not give results which approached closely enough the quantitative to be suitable for our purposes. We, therefore, varied the procedure in several ways until an entirely satisfactory method was found.

A solution of 10.0 mg. of theelin, 24 mg. of carboxymethoxylamine hydrochloride, and 37 mg. of KC$_3$H$_5$O$_2$ in 4 cc. of 75 per cent n-propyl alcohol (aldehyde-free) was refluxed for 3 hours. The solution was transferred to a separatory funnel with 100 cc. of 3

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\(^1\) In the recent isolation of α-dihydrotheelin from human placenta (Huffman, Thayer, and Doisy, 1940) the benzene-carbonate distribution was employed successfully. It is likely that better results could have been obtained with the pregnancy urine had this method of separation been used. Actually at the time when we employed digitonin, Mather’s research had not been completed.
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per cent NaHCO₃; the bicarbonate solution (which was water-clear) was extracted twice with 100 cc. portions of ether, and the latter then washed with bicarbonate and with water. Assay of the ether showed that less than 1 per cent of the theelin had failed to react. In Table I, additional data are given which show the degree of conversion of theelin into the bicarbonate-soluble derivative.

Theelin carboxymethoxime crystallizes from aqueous alcohol in beautiful white needles (m.p. 188°, uncorrected) which contain \( \frac{3}{4} \) molecule of C₂H₅OH of crystallization.

**Microcombustion Analysis**

\[ C_{22}H_{20}O_4N_+ C_2H_5OH \]

Calculated, C 68.81, H 7.70; found, C 69.01, H 7.74

**TABLE I**

*Reaction of Theelin with Carboxymethoxylamine*

24 mg. of carboxymethoxylamine hydrochloride and 37 mg. of KC₂H₂O₂ were refluxed in 4 cc. of 75 per cent n-propyl alcohol.

<table>
<thead>
<tr>
<th>Theelin used</th>
<th>Time of refluxing</th>
<th>Theelin in ether phase by assay</th>
<th>Removal of theelin</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ</td>
<td>hrs.</td>
<td>γ</td>
<td>per cent</td>
</tr>
<tr>
<td>50.4</td>
<td>3</td>
<td>0.8</td>
<td>98.4*</td>
</tr>
<tr>
<td>230</td>
<td>3</td>
<td>1.0</td>
<td>99.5</td>
</tr>
<tr>
<td>298</td>
<td>5</td>
<td>0.7</td>
<td>99.8</td>
</tr>
</tbody>
</table>

* The failure to obtain better than 99 per cent removal in this case was probably due to the fact that the n-propyl alcohol had not been freed of aldehydes. Aldehyde-free solvent was used in the other runs.

**Hydrolysis of Theelin Carboxymethoxime**—A solution of 37.0 mg. of theelin carboxymethoxime (with \( \frac{1}{4} \) molecule of C₂H₅OH of crystallization) in 30 cc. of 1 N HCl + 30 cc. of 95 per cent C₂H₅OH was refluxed for 3 hours in a water bath with the thermometer held at 85–90°. Most of the alcohol was then distilled, water added to increase the volume to 100 cc., and the theelin extracted with 300 cc. of ether. The ether was washed once with 3 per cent NaHCO₃ and twice with H₂O. Distillation of ether gave 24.4 mg. (theoretical 23.7 mg.). The crystalline material was treated with a small amount of norit and crystallized from aqueous alcohol; yield 21.8 mg. (92 per cent) of theelin.

Hydrolysis by this procedure of 8 micrograms of theelin carboxy-
methoxime showed a quantitative recovery of theelin, as determined by bioassay.

Monobenzylation of Theelol—As stated before, we were unable quantitatively to obtain monobenzoyl theelol (C₃ hydroxyl only), using micro quantities of theelol. However, theelol monobenzoate can be easily made by the ordinary Schotten-Baumann procedure.

\[
\text{C}_6\text{H}_5\text{C}(-\text{O})\text{R} + \text{Pb(O-C-CH}_3\text{)}_4 \rightarrow \text{C}_6\text{H}_4\text{C}(-\text{O})\text{C}_2\text{H}_4\text{CHO} + 2\text{HO-C-CH}_3
\]

It crystallizes from benzene in white platelets (m.p. 225°, uncorrected).

Microcombustion Analysis—C₂₃H₃₉O₄
Calculated, C 76.50, H 7.19; found, C 76.15, H 7.30

Reaction of Theelol Monobenzoate with Lead Tetraacetate—Lead tetraacetate reacts with theelol monobenzoate as indicated in the equation. Under the conditions of these experiments more than the theoretical amount of lead tetraacetate reacted. However, the aldehydic nature of the product was demonstrated by a positive Schiff's reaction and by the formation of a yellow hydrazone with phenylhydrazine. The reactive aldehyde group or groups
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in the product make possible its complete removal from an ether solution by shaking with an aqueous solution of NaHSO₃-Na₂SO₃, an experiment which we have performed.

Theelol monobenzoate was dissolved in a 0.1 N solution of Pb(Ac)₄ in glacial acetic acid (redistilled from CrO₃). The solution was allowed to remain in a glass-stoppered flask at 20° or 30° for several hours and then the excess Pb(Ac)₄ titrated in the manner described by Criegee (1931). Under these conditions α-dihydrotheelin monobenzoate does not react. The results of typical experiments are shown in Table II.

Precipitation of α-Dihydrotheelin with Digitonin and Decomposition of Resulting Digitonide—The method used follows the general outline of that given by Whitman, Wintersteiner, and Schwenk (1937). A number of experiments were conducted to determine the modifications best suited to the most nearly complete precipitation of α-dihydrotheelin in quantities of the order of a few mg. An example of the most satisfactory procedure is given as follows:

In a centrifuge tube 9.0 mg. of α-dihydrotheelin and 80 mg. of digitonin (Merck) were dissolved with the aid of heat in 2.0 cc. of 80 per cent C₂H₅OH. The tube was stoppered tightly and left at room temperature for 2 days and then centrifuged at 3000 r.p.m. for 30 minutes, after which the alcohol was carefully drawn off with a capillary pipette. A small stirring rod was used to stir up the digitonide thoroughly with 3 to 4 cc. of absolute ether, and then centrifugation and removal of the supernatant liquid were carried out as before. This washing procedure was repeated. Finally the digitonide was dried by being allowed to remain at room temperature overnight.

TABLE II

Reaction of Theelol Monobenzoate with Lead Tetraacetate

<table>
<thead>
<tr>
<th>Hormone used</th>
<th>Temperature °C</th>
<th>Time hrs.</th>
<th>Pb(Ac)₄ used m.eq.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theelol monobenzoate</td>
<td>0.260</td>
<td>0.245</td>
<td>0.105</td>
</tr>
<tr>
<td>&quot;</td>
<td>30</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>Dihydrotheelin monobenzoate</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.462</td>
<td>0.376</td>
<td>0.000</td>
</tr>
</tbody>
</table>
The dried digitonide was dissolved in 0.5 cc. of dry pyridine and 8 to 10 cc. of absolute ether gradually added. The suspended material was packed by centrifugation and the supernatant fluid drawn off. In order to remove the sterol as completely as possible from the digitonin, the process was repeated twice. The combined solutions from the decomposition of the digitonide were added to 300 cc. of ether, and the ether washed once with 100 cc. of HCl (1:10) and five times with 50 cc. portions of water. All of the aqueous layers were combined, washed once with 100 cc. of ether, and the ether then washed three times with 50 cc. volumes of water. Combination of the two ether fractions and distillation to dryness gave a white product which after crystallization at a low temperature from a small volume of aqueous alcohol weighed 7.0 mg. and melted sharply at 172° (uncorrected). Assay of the 80 per cent C2H5OH and ether washings from the digitonide formation showed that between 1.0 and 1.5 mg. of dihydrotheelin had escaped precipitation, indicating therefore that with 9 mg. the completeness of recovery through the digitonide was about 85 per cent.

Experimental Work on Human Pregnancy Urine

Extraction of Estrogens from Urine—Urine from women during spontaneous labor and delivery was chosen for the source, since the highest values for estrogen had been encountered in such specimens (Smith and Smith, 1940). Hydrolysis (by boiling under a reflux for 10 minutes with 15 per cent concentrated hydrochloric acid) and extraction with ether were performed within 12 hours of the time of collection. This precaution was taken in order to avoid the possibility of conversion of urinary estrogens upon standing. Small batches of urine, 200 to 700 cc.,
were handled at a time in order to get maximum recovery and more quantitative separation.

The weakly acidic phenols (theelin and \( x \) estrogen fraction) were separated from the strongly acidic phenols (theelol fraction), according to the method of Cohen and Marrian (1934). They were then partially purified by reextraction from toluene, according to the Cohen and Marrian procedure, and stored in 95 per cent ethyl alcohol. Alcoholic solutions of the weakly acidic phenols from 38 liters of urine were combined and a small portion removed for bioassay before and after semicarbazide treatment. It was determined that the total extract contained 300,000 rat units of estrogenic substance, half of which represented non-ketonic (\( x \) estrogen) material. If the non-ketonic activity were entirely attributable to \( \alpha \)-dihydrotheelin and the ketonic activity to theelin, it was calculated, according to the standardization values of the assay method used, that the extract contained 7.5 mg. of the former and 75 mg. of the latter.

**Preliminary Purification**—The extract of human pregnancy urine from the Fearing Research Laboratory was dissolved in alcohol and filtered through a Jena funnel. Evaporation of the filtrate gave 452 mg. of a dark orange-red material which by assay with mice showed estrogenic activity equivalent to 70 mg. of theelin. It was further purified as indicated in the flow sheet.

**Removal of Ketones**—The 136 mg. of material, which contained all but traces of the estrogenic activity, were dissolved in 20 cc. of 95 per cent \( C_2H_5OH \), 160 mg. of carboxymethoxylamine hydrochloride and 420 mg. of NaC\(_2\)H\(_3\)O\(_2\)-3H\(_2\)O (in solution in 2 to 3 cc. of H\(_2\)O) added, and the whole refluxed for 3 hours. Most of the alcohol was then distilled off and the solution transferred to a separatory funnel with 15 cc. of 2 per cent NaHCO\(_3\) and 50 cc. of ether; the two phases were separated and the bicarbonate extracted twice more with ether. The combined ether solutions were washed once with 5 cc. of 2 per cent Na\(_2\)CO\(_3\), three times with 5 cc. portions of water, and distilled; weight 100 mg. The 100 mg. of non-ketonic phenols were again treated with the ketone reagent but with refluxing for 7.5 hours. After this treatment the final non-ketonic fraction weighed 92 mg. (Fraction 01h). 27 mg. of crude theelin carboxymethoxime (Fraction
α-Dihydrotheelin from Human Urine

452 mg. solids in solution in 135 cc. acidified 70% C₂H₅OH

Extracted 4 times with 40 cc. volumes petroleum ether (b.p. 30-60°); combined extracts washed twice with 6 cc. 60% ethyl alcohol

Aqueous alcohol, distilled to dryness and dissolved in mixture of 12 cc. n-butyl alcohol and 60 cc. petroleum ether (b.p. 30-60°)

Extracted 10 times with 15 cc. portions 0.25 N NaOH

Petroleum ether

Distilled to dryness; 102 mg. >270 to <400 r.u.

Butyl alcohol and petroleum ether, distilled to dryness; 119 mg. >270 to <400 r.u.*

Extracted 10 times with 75 cc. portions ether

Ether

Alkali, acidified with concentrated HCl

Washed with 5% Na₂CO₃, dilute HCl, and H₂O

Extracted 8 times with ether

Ether, distilled to dryness; 160 mg. leached with 25 cc. hot 0.25 N NaOH + NaCl, chilled with ice, and filtered; residue taken up in alcohol, solvent removed by distillation, and leaching repeated; process performed 4 times

Washings (discarded)

Ether, acidified with concentrated HCl; extracted with ether

Alkali

Acidified with HCl; extracted with ether

Ether, washed with water and distilled to dryness; 10.9 mg. >5300 to <8000 r.u.†

Water

Ether, distilled to dryness; 65 mg. >1000 to <1330 r.u.

Washed with water

Ether, acidified with 5% Na₂CO₃, once with dilute HCl, and once with water

Acid (discarded)

Washings (discarded)

Ether, distilled to dryness; 136 mg.

* The bioassays in this flow sheet were conducted at the Fearing Research Laboratory. 1 rat unit = 0.5 to 0.667 microgram of theelin.
† After storage in a dry condition in a refrigerator for 13 months, assay in the St. Louis laboratory showed less than 1000 mouse units.
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01f) were recovered from the aqueous bicarbonate and carbonate fractions.

Isolation of Theelin—Fraction 01f was hydrolyzed by the method given under "Hydrolysis of theelin carboxymethoxime;" yield 19.2 mg. of crude theelin. Treatments with norit and distribution between benzene and 0.3 M Na₂CO₃ yielded 16.0 mg. in the benzene-soluble fraction. This material was naphthoylated with α-naphthoyl chloride and attempts made to purify the theelin through crystallization of the α-naphthoate, but a good product was not obtained. The crude α-naphthoate and mother liquors from crystallizations were united and saponified with alcoholic KOH. The free phenol was then treated with semicarbazide and the semicarbazone recrystallized twice from aqueous ethanol (−5°). Hydrolysis of this apparently pure product was carried out by refluxing in 0.5 N HCl (50 per cent aqueous ethanol). After treatment with norit and one recrystallization of the phenol from aqueous alcohol, 3.79 mg. of white crystals, m.p. 258–258.5° (Anschütz), were obtained. A mixed melting point with authentic theelin (m.p. 258.5–259°) was 258.5–259°.

Microcombustion Analysis—C₁₃H₂₃O₂
Calculated, C 79.95, H 8.21; found, C 79.98, H 8.44

Isolation of α-Dihydrotheelin—Fraction 01h was assayed and found to possess activity equivalent to 50 mg. of theelin. It was dissolved in 95 per cent ethyl alcohol, concentrated to 1 cc., chilled, filtered, and the crystals washed once. This process was performed a total of three times; weight of crystalline fraction (01Hb) 25 mg. From these crystallizations and filtrations the filtrates (Fraction 01Ha) were combined, evaporated, dried, and weighed; weight 69 mg.

Fraction 01Ha was placed in a small Pyrex tube, 1.0 cc. of 80 per cent C₂H₅OH added, and the mixture warmed until solution was complete. Then 0.25 cc. of a solution of digitonin (≈ 10 mg. of digitonin) in 80 per cent ethyl alcohol was added; in a short time a precipitate resembling a digitonide formed. Finally 0.75 cc. of 80 per cent C₂H₅OH and 70 mg. of solid digitonin were added, the mixture being warmed until all solids had dissolved. After the mixture had stood for 2 days, a very large precipitate
was observed, some of which appeared to be digitonide but most of which appeared to be digitonin. From this point on the procedure used is exactly as described under the heading "Precipitation of α-dihydrotheelin with digitonin and decomposition of resulting digitonide." The yield from the decomposition of the digitonide was 5.2 mg. of a faintly yellow crystalline material (Fraction 01HaPD). Assays in mice indicated the presence of 2 to 3 mg. of dihydrotheelin and the assay in rats showed this product to be 3 times as active as pure theelin. A small aliquot of Fraction 01HaPD was distributed between benzene and 70 per cent C₂H₅OH and bioassay then conducted on each fraction. It was found that the activity was equally distributed in this partition. According to Westerfeld, Thayer, MacCorquodale, and Doisy (1938) the partition ratio of α-dihydrotheelin between benzene and 70 per cent C₂H₅OH is 1:1.

The supernatant fluid and ether washings from the digitonide formation were evaporated to dryness and the reddish colored residue again treated with digitonin (20 mg. of digitonin in 2.0 cc. of 80 per cent C₂H₅OH) but no precipitate occurred.

Fraction 01HaPD was acetylated and submitted to fractional distillation in a molecular still, but no substantial concentration of the active material (as determined by bioassay) could be realized. The acetylated material was then saponified, and the phenol recovered and partitioned between 0.3 M Na₂CO₃ and benzene. From the benzene, 2.11 mg. of white crystals were obtained. These crystals were naphthoylated with α-naphthoyl chloride (MacCorquodale, Thayer, and Doisy, 1936) to give 3.31 mg. (≈1.50 mg. of α-dihydrotheelin) of a compound melting at 196.5–197°C (corrected). A mixed melting point taken with an authentic sample of α-dihydrotheelin di-α-naphthoate (m.p. 198–198.5°C, corrected) was found to be 197–198°C (corrected). A mixed melting point taken with an authentic sample of theelin α-naphthoate (210°C, uncorrected) was 180–185°C (uncorrected).

**Microcombustion Analysis** — C₁₅H₁₂O₄  
Calculated, C 82.72, H 6.25; found, C 82.37, H 6.13

**Isolation of Theelin** — Fraction 01Hb was united with Fraction 01HaNPtD (the portion of No. 01Ha remaining after precipitation with digitonin) and dissolved in 74 cc. of 95 per cent C₂H₅OH;
26 cc. of water were added, and the aqueous alcohol was then extracted three times with 25 cc. portions of petroleum ether (b.p. 30-60°). Distillation of the aqueous alcohol gave a nice crystalline product with a brown contaminant. This material was dissolved in 500 cc. of 0.3 M Na₂CO₃ and then extracted once with 500 cc. of benzene. The carbonate fraction was extracted three times with 400 cc. of ether, after which the combined ethers were washed three times with water and distilled to dryness; yield 35 mg. which by assay indicated the presence of approximately 18 mg. of theelol.

The 35 mg. of crystalline material were acetylated with acetic anhydride, and the acetylated product distilled in a molecular still at 110-130° for 5 hours, and then at 180-200° for another period of 5 hours (pressure 0.0001 mm.). Saponification of the distillate gave crystals with very little color. These were crystallized twice from 1 to 2 cc. volumes of absolute acetone (-5°), treated once with norit, and finally recrystallized from aqueous methanol; yield 9.02 mg., melting at 279° (Anschütz). A mixed melting point with authentic theelol (m.p. 282-283°) was 280-282°.

Microcombustion Analysis—C₁₄H₂₂O₃
Calculated, C 74.95, H 8.39; found, C 74.92, H 8.32

SUMMARY

1. A method has been devised by which the ketonic estrogen, theelol, reacts quantitatively with the ketone reagent, carboxymethoxylamine. The resulting methoxime (which has been characterized) is soluble in aqueous bicarbonate and can thus be quantitatively separated from an ether solution of non-ketonic estrogens.

2. Theelol monobenzoate has been prepared and characterized; it reacts with lead tetraacetate in the usual fashion of 1,2-glycols.

3. The method of Wintersteiner et al. for the precipitation of α-dihydrotheelol with digitonin and the decomposition of the resulting digitonide has been adapted for work with micro quantities.

4 The 500 cc. of benzene contained between 1 and 2 mg. of dihydrotheelol by bioassay.
α-Dihydrotheelin has been isolated from human pregnancy urine collected during spontaneous labor and delivery; the α-dihydrotheelin was isolated as the di-α-naphthoate and characterized as such by the melting point, by the mixed melting point with authentic α-dihydrotheelin di-α-naphthoate, and by micro-combustion analysis.

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