THE ISOLATION OF THE PRINCIPAL ESTROGENIC SUBSTANCE OF LIQUOR FOLLICULI

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Since the introduction of the vaginal smear method of detecting and assaying the follicular hormone (Allen and Doisy, 1923) many investigators have studied the occurrence and distribution of estrogenic substances. Chemists interested in the sex hormones have isolated at least six different pure compounds from the urine of pregnant women and mares but until recently the nature of the active principle of the ovary was unknown.

In their early work, Allen and Doisy used liquor folliculi aspirated from sow ovaries as the source of the follicular hormone. Extensive work on the purification of the estrus-producing component had given rather potent non-crystalline products which, however, were less active than theelin. Therefore, it was assumed by many that the activity of these preparations was due to theelin. However, in October, 1934, when we obtained a preparation having a potency exceeding that of theelin, it became clear that at least a part of the activity of the follicular fluid was due to some other substance. Promptly, we tested the effect of semicarbazide on this preparation but contrary to the effect on the potency of theelin no significant alteration in the activity was observed. These observations stimulated our interest to such a degree that we have processed almost 4 tons of sow ovaries and have worked out a procedure which permits the recovery of approximately 50 per cent of the activity of liquor folliculi extracts as the pure crystalline di-α-naphthoate of dihydrotheelin.

Several important factors have contributed materially to our success in isolating one of the active principles of the ovary. Our
earlier experience in the preparation and purification of extracts and the isolation of pure crystalline estrogenic compounds has been valuable. Furthermore, a supply of theelin for experimental work has been useful. The preparation by Schwenk and Hildebrandt (1933) and others of the reduction product of the carbonyl of theelin has been of assistance. Finally, it should be stated that we have been fortunate, for if we had encountered a new and unknown compound, it would have been necessary to extract many more tons of ovaries. Complete recovery of all of the estrogenic substance as dihydrotheelin from the 4 tons of ovaries that we have processed would have given no more than 25 mg. Since our overall recovery was less than 50 per cent, the quantity of crystalline hormone would have been entirely inadequate for the identification of a new and unknown compound.

Actually we have used about 400 liters of liquor folliculi in our work. Since unselected ovaries from the packing plants yield about 50 cc. of liquor folliculi per pound, 4 tons would be required to obtain 400 liters. However, owing to the cooperation of some of the packing plants which permitted selection of ovaries, we have not aspirated the full 4 tons.

We attempted to use hashed ovaries but owing to the increased difficulties of extraction and purification decided against this procedure. Although the aspiration of the liquor is laborious, it is really an important step in purification and, furthermore, the discarding of the residual ovary is probably not an important source of loss of the follicular hormone (see Doisy, Ralls, Allen, and Johnston, 1924).

In a preliminary paper (MacCorquodale, Thayer, and Doisy, 1935) we reported the isolation of an active principle of sow ovaries. The compound was obtained as the crystalline \( m \)-bromobenzoate which had the same melting point as an authentic specimen of dihydrotheelin \( m \)-bromobenzoate. The derivative was hydrolyzed and the hormone itself recovered in a crystalline condition. The melting point agreed with that of dihydrotheelin and a mixed melting point showed no depression. Furthermore, the assays of the hormone and of dihydrotheelin gave identical values.

Although this evidence seemed satisfactory to us, it was realized that more convincing proof was desirable. Since our yields had
been rather disappointing at times, it seemed that we might econo-
mize by studying the derivatives of dihydrotheelin. Accordingly,
a number of derivatives were made and their solubilities studied. 
The most suitable compound for our work seemed to be the di-
α-naphthoate and, as can be seen from a later section, this deriva-
tive was used rather advantageously.

The next attempts at purification were conducted on synthetic
dihydrotheelin. The inactive oil recovered from earlier fractiona-
tions was added to small quantities of dihydrotheelin and the
stability of the latter studied under various conditions. It was
found that a quantitative recovery could be obtained by sublima-
tion in vacuo of the acetylated mixture. Furthermore, dihydro-
theelin was readily isolated in good yield from the hydrolyzed
sublimate through the di-α-naphthoate.

The last phase of our isolation experiments was effected on the
extract of 107 liters of liquor folliculi plus 56,000 rat units of the
partially purified hormone left from our previous work. The total
amount of hormone in the 107 liters was about 128,000 rat units, of
which about 15 per cent was lost in the preliminary purification,
leaving about 109,000 units in a form similar in purity to the
56,000 units saved from earlier work. This total of 165,000 rat
units is equivalent to approximately 10.0 mg. of dihydrotheelin.

EXPERIMENTAL

Preparation of Derivatives Used in Our Experiments on Isolation;
Dihydrotheelin.—The reduction of the carbonyl group of theelin to
a secondary alcohol was reported by Schwenk and Hildebrandt
(1933) and by Girard, Sandulesco, and Fridensen (1933). * The
former investigators do not state the method used but they suc-
cceeded in obtaining both of the theoretically possible stereoisomers.
The latter investigators carried out the reduction by means of
sodium in alcohol, and also catalytically by means of nickel in the
cold. By both methods they obtained only the isomer melting at
174° (corrected). Laqueur, David, and de Jongh (1935) have also
prepared this isomer by means of sodium and alcohol and Danielli,
Marrian, and Haslewood (1933) obtained it by a catalytic reduc-
tion with platinum. For our work we used the platinum oxide-
platinum black catalyst of Adams, Voorhees, and Shriner (1932)
in alcohol and obtained practically a quantitative yield of the
lower melting isomer which after crystallization from dilute alcohol melted at 173°.\(^1\)Dirscherl (1936), using the same catalyst, has recently reported results in agreement with ours.

Preparation of \(m\)-Bromobenzoates of Dihydrotheelin and Theelin—Dihydrotheelin (21.9 mg.) was dissolved in 10.0 cc. of 10 per cent sodium hydroxide and 4.0 cc. of water. To the solution 0.25 cc. of \(m\)-bromobenzoyl bromide was added and the flask was shaken vigorously until the reaction was complete. The ester was filtered off, washed with water, and then dissolved through the filter with acetone. It was crystallized once from 70 per cent alcohol, once from dilute acetone, and twice from 95 per cent alcohol. The yield was 27.3 mg. of pure white crystals melting at 155–156°. The analysis showed it to be the mono-\(m\)-bromobenzoate. The yield was 74.6 per cent of the theoretical.

\[
\begin{align*}
C_{26}H_{27}O_3Br. & \quad \text{Calculated. } C \ 65.91, \ H \ 5.98, \ Br \ 17.56 \\
& \quad \text{Found. } \" \ 65.93, \ " \ 5.77, \ " \ 17.75, \ 17.60
\end{align*}
\]

From 30.0 mg. of theelin by the same procedure there were obtained 45.6 mg. of \(m\)-bromobenzoate (90.6 per cent of theoretical) after two recrystallizations from alcohol. Theelin \(m\)-bromobenzoate crystallizes in stout, well formed needles melting at 221.5–223°.

\[
\begin{align*}
C_{25}H_{25}O_3Br. & \quad \text{Calculated. } C \ 66.21, \ H \ 5.56, \ Br \ 17.64 \\
& \quad \text{Found. } \" \ 66.13, 66.38, \ " \ 5.47, 5.61, \ " \ 17.55
\end{align*}
\]

Preparation of \(\alpha\)-Naphthoates of Dihydrotheelin and Theelin—Dihydrotheelin (10.1 mg.) was heated for 1 hour with an excess of \(\alpha\)-naphthoyl chloride in 2.0 cc. of dry pyridine. In order to facilitate the removal of the excess acid chloride which reacts extremely slowly with water or dilute alkali at room temperature, an excess (0.5 gm.) of glycine was added and the mixture heated for 1 hour. In this way the water-insoluble \(\alpha\)-naphthoylglycine which readily dissolved when the reaction mixture was diluted with a saturated solution of sodium bicarbonate. The insoluble dihydrotheelin dinaphthoate was removed by extraction with ether, the ether was distilled, and the residue dissolved in acetone and treated with norit. The filtrate

\(^1\) All melting points are uncorrected and were taken with a Bureau of Standards calibrated, long stem thermometer.
was diluted with 95 per cent alcohol and heated on the water bath to remove the acetone. It was concentrated until crystals began to separate and was then allowed to stand at 5°. Two recrystallizations gave 15.2 mg. (71 per cent of the theoretical) of white crystals melting at 195–196°.

\[
\text{C}_{10}\text{H}_{14}\text{O}_4. \quad \text{Calculated. C 82.72, H 6.25}
\]

\[
\text{Found. " 82.71, 82.81, " 6.02, 6.16}
\]

By the same procedure theelin \(\alpha\)-napthoate was obtained as pure white crystals melting at 200.5–202°.

\[
\text{C}_{12}\text{H}_{16}\text{O}_4. \quad \text{Calculated. C 82.03, H 6.65}
\]

\[
\text{Found. " 82.02, 82.04, " 6.58, 6.41}
\]

Attempts to prepare the di-\(m\)-bromobenzoate of dihydrotheelin by this procedure resulted only in the formation of a mono-\(m\)-bromobenzoate identical with the one obtained by the Schotten-Baumann reaction.

**Preparation of Extracts of Liquor Folliculi**—Liquor folliculi was aspirated from sow ovaries and preserved with 2 volumes of 95 per cent ethyl alcohol. This mixture was heated to boiling, filtered, and the material remaining on the filter transferred to a beaker and extracted three times with boiling alcohol. The filtrates and extracts were concentrated to dryness by distillation in vacuo and the residue emulsified in a little hot dilute sodium hydroxide. This emulsion was extracted with ethyl ether (peroxide-free); the extract was washed with a solution of sodium carbonate and water and distilled. The residue, composed largely of cholesterol and its esters, was partitioned between 70 per cent ethyl alcohol and petroleum ether, as described in an earlier publication (Ralls, Jordan, and Doisy, 1926). The alcoholic solution containing most of the hormone was distilled to dryness, the residue dissolved in a small volume of butyl alcohol (20 cc. for the extract of 8 to 16 liters of liquor folliculi), 5 volumes of petroleum ether were added, and the solution was repeatedly extracted with small volumes of 0.25 N NaOH (see Veler, Thayer, and Doisy, 1930). The alkaline solution was passed through a sintered glass filter and then enough hydrochloric acid added to produce a faint haziness. Exhaustive ether extraction removed the hormone. The ether was distilled, the residue dissolved in toluene, and the
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hormone removed from toluene by shaking repeatedly with 0.2 N NaOH containing enough salt to prevent emulsions from becoming troublesome. The alkaline solution was filtered, partially neutralized, and extracted with ether. The ethereal solution was distilled, the residue leached with benzene, and the leachings filtered. The weight of the hormone in this fraction is approximately one-fourth of the total solids.

Isolation of Hormone As m-Bromobenzoate—It was found that upon subjection of an active extract to distillation at 0.02 mm. pressure very little activity was obtained in the distillate collected below 95° but that sublimation of the active material took place rapidly at 130–140°. Three preparations, containing a total of 32,000 rat units, were separately fractionated at 0.02 mm., each being divided into a volatile portion collected below 95° and containing little activity, a middle fraction distilling mainly at 130–140° and containing most of the active substance, and an inactive fraction which did not distil below 150°. The three middle fractions which had a combined weight of 4.23 mg. were dissolved in 100 cc. of 65 per cent alcohol (by volume) and shaken with 20 cc. of benzene. The alcoholic solution was evaporated to dryness and the residue (3.38 mg.) was dissolved in 1.0 cc. of benzene and filtered from a small amount of insoluble material. The solution was evaporated to a volume of 0.3 cc. and 1 cc. of petroleum ether (b.p. 30–60°) was added. The solution was cooled to 5° and the white precipitate filtered off and washed with petroleum ether. It was dissolved in 95 per cent alcohol, concentrated to 1.5 cc., and a few drops of water were added. On standing overnight at 5° the material crystallized from the solution in fine white needles which were filtered off and washed with water. The weight of the crystals was 1.05 mg. and the bioassay showed the rat unit to be 0.165 microgram (Preparation 2-M-16).

Another extract (weight 2.26 mg.) containing 8000 rat units was carefully and repeatedly fractionated at 0.02 mm. pressure and a white crystalline sublimate obtained which weighed 1.73 mg. The bioassay showed it to be of approximately the same degree of purity as Preparation 2-M-16 (0.165 microgram) and so the two were combined for further treatment. The precipitation from benzene by means of petroleum ether was repeated and the material then was combined with Preparation 2-M-26 (weight 2.05
mg.) which contained 26,000 rat units and had been purified in the same manner as Preparation 2-M-16. The total weight of these combined preparations obtained from 70 liters of liquor folliculi was 4.83 mg., with an activity of 43,000 rat units. It was sublimed carefully at a pressure of 0.02 mm. by heating at 110–115° for 48 hours. The sublimate was nicely crystalline and white with a fringe of clear, glassy material. The melting point of the crystalline portion was 162–163.5°. The weight was 3.48 mg. and the rat unit 0.08 microgram. The material was dissolved in 4 cc. of 10 per cent sodium hydroxide solution and 2 cc. of water, and shaken vigorously with 0.1 cc. of m-bromobenzoyl bromide. When the reaction was complete, the precipitate was filtered off and washed with water. It was dissolved in acetone and alcohol and filtered. The filtrate was concentrated to 0.25 cc. and allowed to stand overnight at 5°. The crystals which separated were filtered off, washed with methyl alcohol, and dried at 110°; weight 2.6 mg. The product was recrystallized by dissolving in acetone, diluting with 95 per cent ethyl alcohol, concentrating to 0.25 cc., and allowing to stand at 5°. As the material thus obtained was still slightly yellow, it was dissolved in acetone and treated with norit. Crystallization from 95 per cent alcohol then gave 1.3 mg. of pure white fluffy crystals identical in appearance with the m-bromobenzoate of dihydrotheelin. The melting point was 154–155°.

The ester was hydrolyzed by heating for 1 hour in a platinum vessel with 2 cc. of 5 per cent alcoholic potassium hydroxide. The solution was diluted with water, heated to remove the alcohol, acidified to Congo red with hydrochloric acid, and allowed to stand at 5° overnight. The precipitate was filtered off, washed with sodium bicarbonate solution and then with water, and dried at 110°; weight 0.45 mg. It was dissolved in 0.4 cc. of 95 per cent alcohol, treated with a few grains of norit, and filtered. A few drops of water were added to the filtrate, which was then concentrated to 0.2 cc. on the water bath and allowed to stand at 5°. The hormone crystallized in small white needles (see Fig. 1) identical in appearance with those of dihydrotheelin obtained from theelin. The crystals were filtered off, washed with water, and dried at 110°; weight 0.35 mg. The melting point was 170–171°. A mixed melting point with dihydrotheelin showed no depression. The
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bioassay of the hormone crystals showed the rat unit to be 0.06 microgram.

Isolation of the Hormone As the Di-α-Naphthoate—Preparation 177-d (76,000 rat units) was refluxed for 1 hour with 1 cc. of acetic anhydride. The excess acetic anhydride was removed by distillation and the brown glassy residue subjected to distillation at 0.02 mm. pressure. Two fractions were obtained, one distilling mainly at 95–105° and the other at 135–150°. The latter was entirely oily but the former contained a considerable quantity of well formed white crystals. This fraction was carefully refractionated and a pure crystalline sublimate obtained at 65–70°, which, however, proved to have no estrogenic activity. The amount was small and it has not yet been investigated. As the fractionation was continued, the temperature was raised to 130–135° and a clear, colorless, oily distillate of the hormone acetate was obtained. This fraction, together with the first one ob-

Fig. 1. X 290. Dihydrotheelin from the ovary. The hormone crystallizes in needles and in platelets, both of which appear in the photomicrograph.
tained at 135–150°, was hydrolyzed with alcoholic potassium hydroxide, and the solution acidified with hydrochloric acid and extracted thoroughly with ether. Distillation of the ether left a pale yellow oil (8.60 mg.; the rat unit was 0.130 microgram). The oil was dissolved in alcohol and heated with semicarbazide acetate for 1½ hours. The solution was evaporated to dryness and leached alternately with benzene and water. The insoluble fraction thus obtained weighed 1.33 mg. but it contained practically no theelin semicarbazone for after hydrolysis with hydrochloric acid the bioassay showed no significant increase. By control experiments it had been found that 0.5 mg. of theelin could be easily separated in this way. From our investigations it appears that if there is any theelin in the follicular fluid of sow ovaries, it accounts for only an insignificant fraction of the estrogenic activity.

The benzene-soluble fraction was dissolved in alcohol and the solvent allowed to evaporate spontaneously. A residue remained which was partly crystalline and partly oily. The oily droplets were washed from the crystalline material by means of 1.0 cc. of benzene. The crystals remaining in the tube were sublimed at 0.02 mm. and 160°; weight 1.875 mg.

Preparation 185-c-11 (12.5 mg.; 54,000 rat units) was purified by fractional distillation of the acetate, followed by hydrolysis and sublimation in vacuo. In this way 5.15 mg. of crystals were obtained. This material was combined with the 1.875 mg. of crystals of Preparation 177-d and dissolved in 2.0 cc. of dry pyridine. To this solution 0.10 cc. of α-naphthoyl chloride was added and the solution heated for 1 hour on the water bath. Then 0.5 gm. of glycine was added and the mixture heated on the water bath for 1 hour, after which the reaction mixture was diluted with an excess of saturated sodium bicarbonate solution. The precipitate which remained was taken up by shaking with ether. Distillation of the ether extract produced a residue of yellow oil containing fine white crystals. This was dissolved in acetone, treated with norit, and filtered. The filtrate was diluted with alcohol and the acetone removed by boiling. The alcoholic solution was concentrated to 1.5 cc. and allowed to stand at 5°. Crystals of the very sparingly soluble dihydrotheelin dinaphthoate separated, leaving practically all of the contaminating substances in the
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The crystals were filtered off, washed with methyl alcohol, and recrystallized by the same procedure. After drying at 110° the crystals, which had a faint yellow color, weighed 7.8 mg. They were dissolved in acetone, treated with norit, and recrystallized from 95 per cent alcohol, producing 7.0 mg. The fourth recrystallization gave 6.4 mg. of pure white crystals which melted at 191–193°. A specimen of pure dinaphthoate of dihydrotheelin which had been made from theelin melted at 194–195° (taken simultaneously in the same bath) and the mixed melting point was 192–194°. The melting point of a mixture of the naphthoates of dihydrotheelin and theelin was 175–180°.

Preparation 189-c-2 (12.5 mg.; 35,000 rat units) was combined with the benzene washings of the impure crystals obtained from the preceding extract (Preparation 177-d) and purified by the process used for Preparation 185-c-11. After four recrystallizations from 95 per cent alcohol there were obtained 4.6 mg. of pure dinaphthoate of the hormone with a melting point of 191 192.5°. On mixing with pure dihydrotheelin dinaphthoate the melting point was not depressed. The total amount of pure hormone dinaphthoate obtained from 165,000 rat units (≈ 10.0 mg. of dihydrotheelin) was thus 11.0 mg., which corresponds to a recovery of 52 per cent. Of this, 1.243 mg. were used for a molecular weight determination (Rast) and combustion analysis (Pregl).

C_{40}H_{36}O_4. Calculated. C 82.72, H 6.25, mol. wt. 580

The remaining hormone dinaphthoate (9.8 mg.) was saponified by refluxing with alcoholic potassium hydroxide and the solution diluted with water and acidified with hydrochloric acid. An excess of sodium bicarbonate was added and the cold solution extracted five times with ether. The extract was washed with water and the ether distilled, leaving a white crystalline residue which was dissolved in methyl alcohol, treated with norit, and crystallized from 1.5 cc. of dilute methyl alcohol. The weight of the pure white needles was 3.5 mg. and the melting point 171–172°. A specimen of dihydrotheelin beside it on the thermometer melted at 172–173°. The melting point of a mixture of the two was 171.5–173°. The melting point of a 3:1 mixture of dihydrotheelin and theelin was 164–168°. Of the 3.5 mg. of hormone 2.030 mg. were
used for a molecular weight determination and two combustion analyses.

$$C_{15}H_{20}O_2.$$ Calculated. C 79.36, H 8.88, mol. wt. 272  
Found. " 79.12, 79.27, " 8.91, 8.79, " " 267

**Bioassay**—Since the establishment in 1932 of the "international standard" for the assay of the follicular hormone, we have conducted many experiments in a study of factors affecting the accuracy of assays. This work, which is now being prepared for publication, has indicated some of the precautions to be observed to obtain accurate results. We are convinced that in order to secure consistent results, uniformity of past history of animals is essential. Furthermore, for the greatest precision we find it necessary to conduct a parallel assay of the standard preparation on the same day with a group of comparable animals. In assaying this crystalline material from the liquor folliculi we have carried out on the same day parallel assays of dihydrotheelin prepared from thecin.

**Modified Marrian-Parkes Procedure**—Ovariectomized mice were given subcutaneous injections of four equal quantities of an aqueous solution at intervals of 12 hours.

**Assay**—Dihydrotheelin 200,000 units per mg.  
Crystals from ovary 200,000 " " "

**Modified Butenandt Procedure**—A single subcutaneous injection of ovariectomized mice with the substance dissolved in oleum sesami was made.

**Assay**—Dihydrotheelin 67,000 units per mg.  
Crystals from ovary 67,000 " " "

**Modified Allen-Doisy Procedure**—Ovariectomized rats were given subcutaneous injections of three equal quantities of an aqueous solution at intervals of 4 1/2 hours.

**Assay**—Dihydrotheelin 16,600 units per mg.  
Crystals from ovary 16,600 " " "

**Curtis-Doisy Procedure**—Immature female rats were given subcutaneous injections of an aqueous solution in the morning and evening of 3 successive days beginning at 18 days of age.

**Assay**—Dihydrotheelin 5000 units per mg.  
Crystals from ovary 5000 " " "
The identity of the active principle isolated from the Graafian follicle with dihydrotheelin prepared in the laboratory from theelin of pregnancy urine is thus established.

**DISCUSSION**

Our first isolation of the hormone as the m-bromobenzoate was preceded by a series of careful and time-consuming fractional sublimations in vacuo. Although the pure crystalline hormone sublimes unchanged under the conditions of our experiments, its separation from the other substances present in the extracts cannot be accomplished by this method without considerable loss of activity. It was subsequently found that acetylation of the extract before fractionation greatly increased the stability of the hormone and permitted an easy separation of the diacetate by one or two fractionations.

The later work in which the naphthoate was employed gives an over-all yield of approximately 50 per cent which is much more satisfactory than was obtained with the bromobenzoate. While the m-bromobenzoyl bromide reacts with crystalline dihydrotheelin to give a good yield it cannot be advantageously employed to separate the hormone from the impurities present in the extract. The solubilities of the ester and the impurities are such that the separation is unsatisfactory. On the other hand, the slight solubility of the dinaphthoate in ethyl alcohol permits a ready separation of the hormone from contaminating substances.

Shortly after our announcement of the isolation of dihydrotheelin from sow ovaries, Wintersteiner, Schwenk, and Whitman (1935) reported the isolation of the same substance and its isomeric form from the urine of pregnant mares. These two investigations are the only reports on the occurrence of natural dihydroxy estrogenic substances.

Though we have obtained a large proportion of the estrogenic substances of sow ovaries as crystalline dihydrotheelin, this finding does not exclude the presence of other estrogenic compounds. However, measured in physiological potency the amount of such compounds is small. One would expect the presence of other active compounds, since it is highly probable that some of the compounds of the intermediate steps in the formation by the ovary would possess some activity.
The isolation of dihydrotheelin from sow ovaries does not permit us to say that the ovaries of other species contain this hormone. One needs only to recall the difference between the estrogenic substances of human and mare urine to realize the danger of exceeding the data in drawing conclusions in this field. However, in spite of the limitation mentioned the isolation of the principal estrogenic compound of sow ovaries is a source of satisfaction, since it represents the conclusion of work begun by one of us in 1923.

Assay by the vaginal smear method shows that dihydrotheelin is more potent than any other known substance in producing cornification in the vagina of the ovariectomized rat or mouse. Again, it would be unwise to exceed our data. This compound may be less active if assayed by other types of response produced by estrogenic substances.

Since the statements of the large number of rat units used may convey an erroneous impression to those not active in this field it should be stated that the total amount of estrogenic substance, calculated as dihydrotheelin, in the liquor folliculi of a ton of sow ovaries is about 6 mg. The concentration in the liquor folliculi is about 1 part in 15,000,000 and in the ovary of the sow 1 part in 150,000,000.

**SUMMARY**

1. Dihydrotheelin has been isolated from the liquor folliculi of sow ovaries as the m-bromobenzoate and also as the di-α-naphthoate. Both derivatives were obtained as pure crystalline compounds agreeing in melting points with the corresponding derivatives prepared from dihydrotheelin obtained from theelin. A mixed melting point determination on the naphthoates showed no depression. The derivatives were hydrolyzed and the hormone obtained in the pure crystalline condition. In each case the melting point was in agreement with that of dihydrotheelin prepared from theelin, and the mixed melting point showed no depression.

2. Complete analytical data, as well as molecular weight determinations, have been obtained for the naphthoate and for the hormone obtained from it by hydrolysis.

3. Bioassay of the hormone by four standardized procedures shows it to have the same estrogenic activity as dihydrotheelin prepared in the laboratory.
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