Table V. Compound Strainability

No.	Ingredient	Strainability *Gal./sq. ft.*	Amount Added *Gal.*
A	Neoprene latex (Type 571)	77.0	12.00
B	Stabilizer solution	∞	0.80
C	Zinc oxide dispersion	16.8	1.00
D	Filler dispersion	17.5	2.00
E	Antioxidant dispersion	2.8	0.25
F	Pigment dispersion	8.3	1.00
	Total compound	35.0 (experimental)	17.05

For example, mix a gallons of A with a strainability of S_1 requiring a/S_1 square feet of felt with b gallons of B with a strainability of S_2 requiring b/S_2 square feet of felt. Then $a + b$ gallons of mixture with a strainability of S_T requiring $(a+b)/S_T$ square feet of felt will result.

Thus $\frac{a}{S_1} + \frac{b}{S_2} = \frac{a+b}{S_T}$, or for any number of noninteracting ingredients A, B, C, D, . . . of a, b, c, d, . . . gallons each $\frac{a}{S_1} + \frac{b}{S_2} + \frac{c}{S_3} + \frac{d}{S_4} + \ldots = \frac{a+b+c+d+\ldots}{S_T}$ where S_T is the strainability of the mixture.

By comparing the last equation with strainability results obtained during latex compounding, interactions between ingredients can be detected.

The data in Table V indicate a compound produced without interaction.

By the noninteraction formula

$$\frac{12.00}{77.0} + \frac{0.80}{\infty} + \frac{1.00}{16.8} + \frac{2.00}{17.5} + \frac{0.25}{2.8} + \frac{1.00}{8.3} = 0.5393 = \frac{17.05}{S_T}$$

$$S_T = \frac{17.05}{0.5393} = 31.6 \text{ gallons per square foot,}$$

which agrees fairly closely with the experimental result and indicates little or no interaction.

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Identification of Crystalline Progesterone with 2,4-Dinitrophenylhydrazine

Quantitative Determination of Progesterone in Oil

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A procedure is described for identification of crystalline progesterone by quantitative precipitation of a derivative with 2,4-dinitrophenylhydrazine. Evidence is presented that the reaction product of progesterone with 2 molecules of 2,4-dinitrophenylhydrazine is a monopyrazoline-monohydrazone. The method developed is suitable for the gravimetric determination of pure progesterone in oil. Results of analyses show excellent recoveries of crystalline progesterone by itself and when added to oil. Analyses of commercial samples of pure progesterone in oil yielded values close to the labeled amounts.

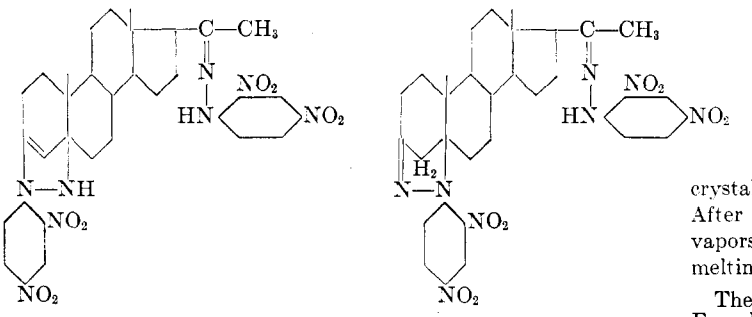
IN THE course of preparing crystalline progesterone in a vegetable oil for parenteral use, it was necessary to determine the progesterone content of the oil preparations for analytical control purposes. A recent review (*3*) shows the inadequacies of present chemical methods. Any specific chemical methods which could be developed would have obvious advantages over bioassay procedures (*1*). With the ketonic reagents available, a chemical assay method seemed possible. The Hughes procedure (*6*) with Girard reagent T was first tried but quantitative recoveries from oil could not be obtained. The determination of estrone with 2,4-dinitrophenylhydrazine (*8*) then suggested this reagent for the estimation of crystalline progesterone. No information regarding previous use of this hydrazine for the identification and determination of the hormone of the corpus luteum could be found.

Progesterone with two carbonyl groups reacts as expected with two molecules of 2,4-dinitrophenylhydrazine. Evidence is adduced that the compound formed is a monopyrazoline-monohydrazone, isomeric with the expected progesterone, bis(2,4-dinitrophenylhydrazone). This derivative may be either 3,5-{3′-[2′-(2″,4″-dinitrophenyl)]pyrazolyl}pregnene-3-one-20,2‴,4‴-dinitrophenylhydrazone (Ia), or 3,5-{2′-[1′-(2″,4″-dinitrophenyl)]pyrazolyl}pregnane-one-20,2‴,4‴-dinitrophenylhydrazone (Ib).

In attempting to demonstrate the chemical relationship of the derivative to progesterone by acid hydrolysis and recovery of the parent compound, no progesterone was isolated under a variety of conditions employed. In fact, the extreme insolubility of the derivative necessitated prolonged periods of heating to dissolve the compound, apparently by reaction. The reaction product was a lower melting compound (decomposition point, 150° C.) corresponding to a mono-2,4-dinitrophenylhydrazone. Treatment of this mono-compound with 2,4-dinitrophenylhydrazine regenerated the original derivative described above (decomposition point, 282–283° C.), thus demonstrating that the second, difficultly hydrolyzable, hydrazine residue has the same structure in both compounds. The great resistance to hydrolytic cleavage of the second hydrazine residue suggests a cyclic structure involving the conjugated system of the 3-keto-Δ-4,5 structure of progesterone. This cyclization reaction appears to be general in simple conjugated systems (*4*, *7*), and its occurrence can be deduced from the facts presented. No evidence is available for a choice between the two alternate structures, Ia and Ib. The hydrolytic cleavage product may also have alternate structures.

Regardless of the structure of the derivative, the conversion factor for the weighed precipitate to progesterone is the same: 0.466.

The derivative is insoluble in water, ethanol, and hexane. Vegetable oils are immiscible with 90% ethanol, whereas progesterone is soluble in this concentration of alcohol. These properties permit gravimetric determination of progesterone in oil solution.



Ia Ib

PROGESTERONE METHOD

The following analytical procedure was adopted for the determination of progesterone in oil.

Apparatus. Separatory funnels of 125-ml. capacity, glass-stoppered. Erlenmeyer flasks of 125-ml. capacity, standard taper, with pourout lip. Reflux condensers, standard taper. Filter crucibles of 20- or 30-ml. capacity, 10 porosity Selas or equivalent.

Reagents. Mixed hexanes, approximate boiling point range 55° to 70° C. Ethyl alcohol, specially denatured absolute or 95%, containing 0.5% benzene. Ethyl alcohol, 90%. 2,4-Dinitrophenylhydrazine. Hydrochloric acid, concentrated. Hydrochloric acid, approximately 0.5 *N*.

Extraction of Progesterone from Oil Solution. Measure a portion of the oil solution, containing about 20 mg. of progesterone, into a separatory funnel. Depending on the volume of the oil taken, usually between 2 and 20 ml., add 20 to 40 ml. of hexane. Extract the hexane solution five times with 10- to 20-ml. portions of 90% alcohol, combine the alcohol extracts in an Erlenmeyer flask, and evaporate to dryness on a steam bath with the aid of a current of air.

When progesterone is determined in pharmaceutical preparations, no difficulty from interfering substances is met in those containing only progesterone. In pharmaceutical preparations containing estrone and progesterone, advantage may be taken of the extractability of the estrone from hexane solution by 2 *N* sodium hydroxide as in the method of Carol and Rotondaro (*2*). Progesterone may then be determined on the estrone-free hexane solution.

Preparation of 2,4-Dinitrophenylhydrazine Derivative of Progesterone. To the residue or to a weighed sample of pure progesterone, add approximately three times its weight of 2,4-dinitrophenylhydrazine, followed by 30 ml. of ethanol, absolute or 95%. Connect the condenser to the flask and reflux on a steam bath for 15 minutes. Add 1 ml. of concentrated hydrochloric acid, and continue refluxing for 15 minutes more. The hydrazone precipitates either upon the addition of the acid or during the second refluxing period. Cool the mixture to room temperature and filter off the precipitate in a previously tared filter crucible. Wash the precipitate carefully with two 10-ml. portions of ethanol, followed by six 10-ml. portions of hexane. Since this solvent removes any oil trapped in the precipitate, it is omitted when the extraction from oil solution is not used. To remove any unreacted hydrazine wash with 5-ml. portions of alcohol, then with 0.5 *N* hydrochloric acid, until the filtrate comes through colorless. Remove the residual acid by washing with distilled water. Finally transfer the filter and contents to a desiccator and dry in vacuo to constant weight over a drying agent—e.g., Drierite. Weigh on an ordinary analytical balance.

The precipitate formed is deep orange-red in color. The weight of the precipitate × 0.466 = mg. of progesterone in the sample taken.

RECOVERIES AND IDENTIFICATION OF PROGESTERONE DERIVATIVE OF 2,4-DINITROPHENYLHYDRAZINE

A series of samples of crystalline progesterone (melting point 128.5–129.5° C.) was precipitated by the method outlined. The results are summarized in Table I. Recoveries obtained (99.9 ± 0.7%) show that progesterone reacts with 2 molecules of dinitrophenylhydrazine to yield a weight of precipitate that agrees with the theoretical values.

This derivative gave melting point values with decomposition, ranging from 271° to 278° C. (Table I). [Melting points, corrected, were taken with the Hershberg apparatus (*5*) using completely immersed, uncalibrated, Anschütz thermometers.] The combined precipitates were twice recrystallized by solution in toluene and dilution with ethyl alcohol. Both first and second recrystallized products decomposed without melting at 282–283° C. After the purified product was dried in vacuo over xylene vapors in an Abderhalden drying tube, it still had the same melting point.

Theory ($C_{32}H_{38}N_8O_8$): C, 58.75%; H, 5.68%; N, 16.60%. Found: C, 58.3%; H, 5.4%; N, 16.55%. Analysis by William Saschek, Columbia University, College of Physicians and Surgeons, New York, N. Y.

STRUCTURE OF 2,4-DINITROPHENYLHYDRAZINE DERIVATIVE OF PROGESTERONE

Preliminary experiments were carried out to determine the least drastic conditions for carrying out the acid hydrolysis. Mixtures of hydrochloric acid with the following organic solvents were employed: glacial acetic acid, *n*-butyl alcohol, isobutyl alcohol, and ethyl alcohol. In all cases deep orange residues were isolated, insoluble in 2 *N* hydrochloric acid and soluble in ethyl ether and ethyl alcohol. The lowest refluxing temperature could be obtained with acid–ethyl alcohol mixture.

Approximately 150 mg. of the dihydrazine derivative of progesterone were refluxed for 66 hours with 600 cc. of ethyl alcohol–6 *N* hydrochloric acid mixture (3 to 1). The long heating period was required to dissolve the compound. After evaporation to dryness in vacuo the residue was taken up with 350 cc. of ethyl ether. The ether solution was extracted with 2 *N* hydrochloric acid, washed with distilled water, and dried with anhydrous sodium sulfate. After removal of the solvent, the residue was recrystallized three times by solution in ethyl alcohol and dilution with water. The product had a deep orange-red color.

The products obtained after the second and third recrystallizations exhibited similar melting point properties. Decomposition with softening started at about 150° C. and decomposition was not complete until about 170° C. After drying in vacuo over

Table I. Analysis of Progesterone with 2,4-Dinitrophenylhydrazine

Progesterone Taken	Dinitrophenylhydrazine Derivative Recovered		Progesterone Recovered	
Mg.	*Mg.*	*M.P., ° C.*	*Mg.*	%
25.7	54.7	275–6	25.5	99.2
44.0	94.5	271–2	44.0	100.0
37.7	80.1	275–6	37.3	99.8
61.1	131.4	277–8	61.2	100.1
35.6	76.6	276–7	35.7	100.2
30.6	65.6	275–7	30.6	100.0

Table II. Analysis of Progesterone in Oil[a]

Identification of Sample		Dinitrophenylhydrazine Derivative Recovered		Progesterone Recovered	
Progesterone taken	Sesame oil taken				
Mg.	*Cc.*	*Mg.*	*M.P., ° C.*	*Mg.*	%
18.9	2	40.3	276–7	18.8	99.4
20.1	5	45.0	273–4	21.0	104.4
28.3	5	60.9	273–4	28.4	100.3
18.9	10	40.7	273–4	19.0	100.5
30.1	15	63.8	274–5	29.7	98.8
18.9	20	40.3	270–1	18.8	99.4
20.1	20	43.2	270–1	20.1	100.0
Progesterone in Oil (Commercial)					
Labeled Amount of Progesterone					% of labeled amount
Mg./cc.					
5	5	56.1	273–4	26.1	104.5
5	5	54.6	271–3	25.4	101.8
5	5	55.8	272–3	26.0	104.0
5	5	54.8	276–8	25.5	102.0
5	5	54.8	274–6	25.5	102.0
10	2	43.5	272–3	20.2	101.0
10	2	44.0	271–2	20.4	102.0
10	2	42.1	278–9	19.6	98.0
10	2	41.8	276–7	19.5	97.5

[a] Some determinations done by A. Albamonte.

water in an Abderhalden dryer, a nitrogen determination was carried out. As postulated this compound contained one dinitrophenylhydrazine residue with an empirical formula of $C_{27}H_{34}N_4O_5$. Nitrogen found 11.97%, theory 11.32%.

Approximately 10 mg. of the hydrolytic cleavage product and an equal quantity of 2,4-dinitrophenylhydrazine in 5 cc. of ethyl alcohol were made to react as in the progesterone determination. The resulting orange-red precipitate, without being recrystallized, melted with decomposition at 276.5° C. Mixed with the pure dihydrazine derivative of progesterone, melting point with decomposition was 279–281° C.

DETERMINATION OF PROGESTERONE IN OIL

Progesterone was added to sesame oil, and recovery determinations were carried out. Results shown in Table II are similiar to the recoveries and melting point values of the derivative obtained with pure crystalline progesterone (Table I). Application of the method to oil solutions for control purposes yielded values in agreement with the labeled amounts (Table II).

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Boron Microdetermination in Fresh Plant Tissue

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Boron is extracted from 5 sq. cm. of leaf at room temperature, with a mild chemical extractant, in the same dish in which the color is to be developed. This prevents possibility of contamination from boron in filter papers and loss of boron at elevated temperatures. A sampling device is described for cutting four foliage strips 2.5 × 25.0 mm. at one time; eight such strips constitute the sample for extraction. Representative sampling is effected by using a single strip from each one of eight leaves. Where advisable, foliage can be gathered, prepared, and extracted, and the color developed and read within one working day. The normal range is from 0.2 to 1.2 microgram of boron per determination.

IN THE analysis of soils and plants for boron, where submicrogram levels are routinely encountered, great care must be exercised against contamination. In sample preparation, especially of plants, equal attention must be given to retention in the sample of the small but very essential amount of boron initially present. A discussion of the major causes of contamination and loss is presented, preliminary to an analytical procedure designed to eliminate these difficulties.

In an early attempt to study plant uptake of boron as related to soil applications, valid differences were obscured in analysis by a heavy and variable background contamination. High quality double-acid-washed filter paper was found responsible for this contamination and its use in boron microanalysis was discontinued in January 1943. Twelve widely used filter papers have been analyzed for boron content (Table I). A composite sample of each kind consisted of four 90° segments obtained one each from 4 disks selected at random through the pack. These were torn roughly into centimeter squares and extracted as indicated in Table I, with occasional stirring. Duplicate determinations were made, using 1-ml. aliquots from these solutions.

The data in Table II were obtained by placing a Whatman No. 40 paper in a Kimble 58° funnel, using the conventional method of folding. The successive 10-ml. portions of solution were delivered from a pipet, the tip being directed in a circle just below the rim of the paper. Each portion was caught in a separate size 00 Coors porcelain dish, and boron was determined in the usual manner. The high amount of boron in filter paper, as shown in Table I, and the ease with which it is carried into the filtrate (Table II) constitute sufficient reason for avoiding its use. On the other hand, the residual value of 1.95 micrograms after seven washings (Table II) suggests that prior washing of the paper before filtering an unknown may not give adequate protection against contamination from that source.

The apparatus shown in Figure 1 was used to study the amount of boron volatilized from pecan foliage during normal, carefully regulated drying and ashing.

A size 00 Coors porcelain evaporating dish was employed for the lower half of the retort, *R*, while a 65-mm. Kimble 58° funnel with stem bent as shown was used for the upper half. Concentric-style ring clamps attached to a short piece of iron rod were used to hold these together as a rigid unit. Volatilized materials were conducted away through a 9-mm. soft-glass tube, *T*, into soft-glass 32 × 160 mm. centrifuge tubes, used as condensing tubes, each containing 12 ml. of a 0.1 *N* suspension of calcium hydroxide.

One gram of fresh leaf tissue was placed in the lower half of the retort, and, after the two condensing tubes, *A* and *B*, were connected slow aspiration was applied. A third tube was used in the series experimentally, but was found unnecessary, as most of the boron was stopped in *A*, the remainder in *B*. In this manner foliage from the borax-treated tree (Figure 2) was raised through successive temperatures in such a way as to allow the

Table I. Easily Extractable Boron in Standard Filter Papers[a]

No.	Make	Maker's No.	Quality (Acid Wash)	Size, Cm.	Ash Weight, Gram	Micrograms of Boron per Paper
1	Whatman	4	None	9	0.00052	1.80
2	Whatman	1	None	9	0.00039	5.80
3	Whatman	2	None	11	0.00880	65.60
4	Whatman	31	Single	11	0.00033	7.00
5	Whatman	30	Single	9	0.00021	4.80
6	Whatman	32	Single	11	0.00031	8.20
7	Whatman	41	Double	11	0.00015	7.20
8	Whatman	40	Double	11	0.00013	7.80
9	Whatman	42	Double	9	0.00006	3.60
10	Schleicher and Schüll	595	None	11	Not stated	8.80
11	Schleicher and Schüll	597	None	11	0.00340	14.40
12	Schleicher and Schüll	5893	Double	9	0.00005	17.60

[a] Extracted 2.5 hours at room temperature in 250-ml. boron-free beakers, using 20 ml. of a solution 0.125 *N* in respect to HCl and 0.0125 *M* in respect to KCl.