2.95 g. of the acrylic acid[15] was mixed with 2.25 g. of red phosphorus and 14 cc. of acetic anhydride and 14 cc. of hydriodic acid (57%) was added dropwise with shaking. The containing flask was connected to a reflux condenser and heated for three hours in a bath at 120°. After cooling, the phosphorus was filtered off and washed with acetic acid. The filtrate was evaporated to dryness, taken up in water and then extracted with ether. Concentration of the aqueous phase gave a sirup, to which excess ammonium hydroxide was added and the resulting clear solution again evaporated to dryness. The residue was extracted with hot absolute alcohol and the alcohol insoluble portion crystallized from hot water yielding 1.40 g. (70% of the calcd.) of the amino acid, stout white prisms, containing one-half molecule of water which was not lost on drying at room temperature.

*Anal.* Calcd. for $C_7H_{10}N_2SO_2 \cdot {}^1/_2H_2O$: C, 43.08; H, 5.69; N, 14.36. Found: C, 43.02; H, 5.76; N, 14.17.

The amino acid is readily soluble in hot water; practically insoluble in absolute alcohol. When heated in a capillary, it did not melt but decomposed with evolution of gas at about 237°; an almost colorless liquid remained in the tube. By heating an aqueous solution of the acid with cupric carbonate it was found possible to prepare the blue copper salt which crystallized from the concentrated solution and from which, on treatment with hydrogen sulfide, the original material could be regenerated. A determination[16] of the percentage of sulfur labilized[11] by plumbite, by bromine and by fuming nitric acid gave respectively the figures 51, 75 and 70.

(15) An attempt was made to accomplish the reduction of the acid using Adams platinum catalyst. Although the recrystallized thiazole did not "poison" the catalyst, as evinced by undiminished subsequent activity of the same catalyst using benzaldehyde as a test object, only unchanged starting material could be recovered.

**Acknowledgment.**—Grateful acknowledgment is made to Dr. R. T. Major of Merck and Co., Inc., for providing chemicals and microanalyses and to the Research Corporation for a grant of funds, defraying the costs of the investigation.

## Summary

There has been described the synthesis of an amino acid which is a theoretically possible precursor of the thiazole half of vitamin $B_1$.

(16) We are indebted to Mr. Harold W. Strickler for these analyses.

PASADENA, CALIF. RECEIVED FEBRUARY 2, 1939

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND THE DEPARTMENT OF ZOÖLOGY OF THE UNIVERSITY OF WISCONSIN]

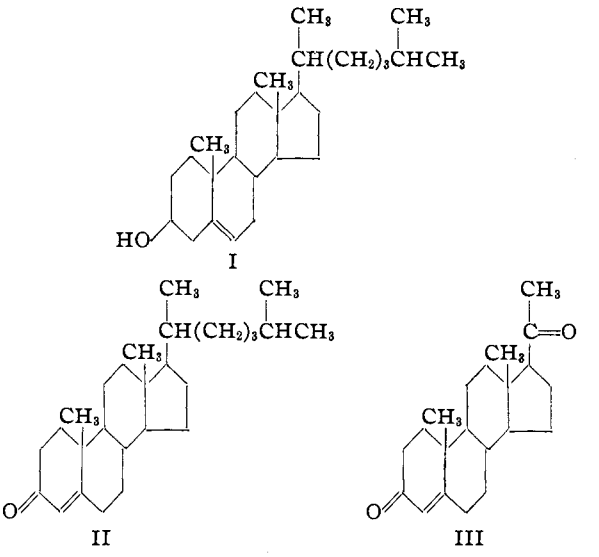
# The Preparation of Progesterone from Cholesterol

BY M. A. SPIELMAN AND R. K. MEYER

Although the corpus luteum hormone, progesterone (III), is very much in demand, there is no really satisfactory way of obtaining it. Corpus luteum tissue is expensive and the extraction laborious, while stigmasterol, from which it is made artificially,[1] is virtually unavailable in America.

Cholesterol (I) is a cheap sterol and therefore a desirable starting material. However, it is obvious that a very small yield would be expected in the direct removal of most of the cholesterol side chain by oxidation, and to offset this, the preparative method would have to be both simple and inexpensive. This paper reports the details of such a method.

Two ways of preparing progesterone from cholesterol have been published, and in each, cholestenone (II) is an intermediate. Tavastsherna[2] converted cholestenone to the dibromide and oxidized it in benzene solution with aqueous sulfuric acid and potassium permanganate. The yield claimed is 40–60 mg. from 10 g. of cholestenone dibromide,[3] but several attempts made in this Laboratory have produced no hormone what-




(1) Butenandt, Westphal and Cobler, *Ber.*, **67**, 1611 (1934); Butenandt and Westphal, *ibid.*, **67**, 2085 (1934).

(2) Tavastsherna, *Arch. sci. biol. (U. S. S. R.)*, **40**, 141 (1936); *C. A.*, **31**, 6670 (1937).

(3) The 15% yield given in the German summary and quoted in *Chemical Abstracts* is not in keeping with the Russian text.

ever. Dirscherl and Hanusch[4] made progesterone in unspecified yield by direct oxidation of cholestenone with chromic acid in acetic acid solution.

The essential feature of the method here described is that the preparation of cholestenone is avoided entirely except as a by-product. Cholesterol is brominated in benzene, and oxidized in the same solvent by shaking with aqueous acid permanganate; the product is debrominated with zinc and finally fractionated by suitable partition between solvents. During the past year we have made more than 3000 Corner–Allen rabbit units of crude progesterone with an average yield of 1.8 units per gram of cholesterol. Since the rabbit unit is 1 mg., the yield is 0.2%—a figure which seems infinitesimal; nevertheless, the method has given us a constant supply of hormone at a fraction of the cost from any other source.

The crude progesterone prepared as above has proved to be entirely satisfactory for physiological work. Its biological activity has been established by the usual sensitization of rabbit uteri,[5] by elicitation of the copulatory reflex in castrate female guinea pigs,[6] and by the stimulation of placentomata formation in castrate pseudo-pregnant rats.[7] The method is not suited to the elaboration of crystalline progesterone because of difficulties in final purification. The crystalline hormone has been isolated only for characterization. Androgenic material in the crude preparation may be removed to any desired extent as described in the Experimental Part.

The method yields as the principal by-product 50% of the theoretical amount of cholestenone which may be converted to more progesterone by the Dirscherl and Hanusch technique. In our hands this latter procedure yields 2 rabbit units per gram of cholestenone.

This work was supported in part by the Wisconsin Alumni Research Foundation. We are indebted to Mr. Clyde Biddulph for hundreds of animal assays.

## Experimental Part

**Preparation of Crude Progesterone.**—A suitable vessel for the oxidation reaction is a 12-liter bottle, the neck of which has been ground to fit a standard taper joint. The latter is sealed at right angles to a 50-cm. section of 16-mm. tubing so that when the bottle is placed in a horizontal position on the shaking machine there is formed a trap for the escape of carbon dioxide.

In the oxidation bottle are placed 3 liters of benzene and 50 g. of cholesterol. Twenty grams of bromine is added, and after five minutes a test is made with moist starch–iodide paper to ensure complete absorption. An excess of bromine must be avoided, by the addition of a little more cholesterol, if necessary. There is added a cold solution of 250 cc. of concd. sulfuric acid in 4 liters of water and then 25 g. of potassium permanganate. The bottle is placed on the machine and shaken until the purple color has faded. Three more 25-g. portions of permanganate are added at appropriate intervals. Completion of the oxidation requires eight to twelve hours.

A liter of shaved ice and 90 g. of sodium sulfite are introduced into the mixture and shaking is continued until the sludge of manganese dioxide has disappeared. The aqueous layer is siphoned out and the upper layer is washed once by shaking with 3 liters of water. The benzene is distilled under diminished pressure during which the internal temperature must not rise above 40°.[8]

The residual sirup is taken up in 150 cc. of glacial acetic acid, and zinc dust is added in 10-g. portions with shaking until further additions cause no heat of reaction. Any separation of zinc acetate is cleared up by the addition of a few drops of water. The liquid is decanted into a separatory funnel, diluted with 500 cc. of water and extracted with 2 portions of petroleum ether (60–68°), the first of which is used to wash the zinc dust. The petroleum ether is extracted with five 100-cc. portions of concd. hydrochloric acid and set aside to be worked up for cholestenone. The hydrochloric acid is diluted with 2 volumes of water and extracted twice with petroleum ether. Evaporation of the petroleum ether leaves the crude product as a brown oil which is best preserved in alcohol. The yield as determined by assay is 50–100 Corner–Allen rabbit units.

The factor most likely to give trouble in the process is the quality of the cholesterol. Impurities, generally revealed by too rapid consumption of potassium permanganate, frequently lead to intractable emulsions and little or no hormone. In those cases it is best to begin with cholesterol dibromide[9] which has been thoroughly washed with glacial acetic acid and air-dried. Addition of bromine to the benzene solution is, of course, omitted.

**Characteristics of the Product.**—The crude progesterone weighs 2–10 mg. per rabbit unit and hence the purity ranges from 10–50%. The amount of androgenic material in a typical sample was determined by assay, using albino rats which were castrated at forty days of age and given injections of the product in corn oil beginning the next day and continuing once a day for five days. The seminal vesicles were dissected out and weighed on the morning of the sixth day. Controls (7 rats) averaged 16 mg. At a dose level (total) of 1 rabbit unit there was no significant response (13 mg., 3 rats), but at 10 units the vesicles averaged in weight 149 mg. (3 rats).

(4) Dirscherl and Hanusch, *Z. physiol. Chem.*, **252**, 49 (1938). Since this manuscript was submitted, Butenandt and Schmidt-Thomé, *Ber.*, **72**, 182 (1939), have announced their partial synthesis of progesterone from cholesterol via dehydroisoandrosterone cyanohydrin.

(5) Allen, *Am. J. Physiol.*, **92**, 174 (1930).

(6) Hertz, Meyer and Spielman, *Endocrinology*, **21**, 533 (1937); Collins, Boling, Young and Dempsey, *ibid.*, **23**, 188 (1938).

(7) Meyer and Rothschild, unpublished results.

(8) The recovered benzene contains hydrogen bromide and may not be used in further oxidations until washed with alkali.

(9) Windaus, *Ber.*, **39**, 518 (1906).

The androgenic material present in the product is undoubtedly androstenedione,[4] and the method for separating it from progesterone is based upon a difference in partition coefficients which the two compounds show when divided between petroleum ether (35–40°) and 35% alcohol. Calculations made from the data of Wintersteiner and Allen[10] show that progesterone is about five times as soluble in petroleum ether as in 35% alcohol, while a simple experiment using crystalline material revealed that androstenedione is twice as soluble in 35% alcohol as in petroleum ether. Using these figures, the following procedure was devised.

The sample, containing about 200 units, is taken up in 100 cc. of absolute alcohol, and 42 cc. of water is added. It is extracted with two 50-cc. portions of petroleum ether. The petroleum ether is extracted with 58 cc. of 70% alcohol and discarded. The combined alcoholic extracts are diluted to 400 cc. with water, extracted with five 80-cc. portions of petroleum ether and discarded. The ether is evaporated to 50 cc., 200 cc. of 35% alcohol is added and a total of five extractions with 50-cc. portions of petroleum ether are made. The hormone is stored in alcohol after evaporation of the petroleum ether. There is no loss of progestational activity within the limits of the assay method. A preparation so purified was assayed for androgens. There was no significant result at a dose level of 1 rabbit unit (seminal vesicles averaged 13 mg. in 4 rats), or at 5 units (16 mg., 4 rats). At 10 rabbit units per rat, the vesicles from 4 animals averaged 28 mg.

(10) Wintersteiner and Allen, *J. Biol. Chem.*, **107**, 321 (1934).

**Isolation of Progesterone.**—For purpose of characterization a sample of progesterone was isolated in crystalline form. About 100 units, purified by the 35% alcohol method, was distilled at 180° (air-bath) at 0.001 mm. pressure, and then crystallized several times from dilute methanol. The top fraction was stout prisms melting at 128°.[10,11] The second fraction melting at 110–113° appeared to be a mixture of prisms and the lower-melting needle form. This was confirmed by conversion to the common dioxime which darkened at 220° and melted at 238°.[10] The total of recovered crystalline hormone was 14 mg.

**Recovery of Cholestenone.**—The main by-product is cholestenone which remains in the petroleum ether after the hydrochloric acid extraction. The solvent is washed with water and then evaporated on a steam-bath. The residue is taken up in 25 cc. of acetone and set in the refrigerator to crystallize. About 25 g. of cholestenone is recovered; m. p. 79–80°. Further crystallization is best made from 50% acetone–methanol.

## Summary

Detailed directions are given for a simple and inexpensive method of making progesterone from cholesterol. The physiological properties of the product are described.

(11) Allen and Wintersteiner, *Science*, **80**, 190 (1934).

MADISON, WISCONSIN RECEIVED FEBRUARY 3, 1939

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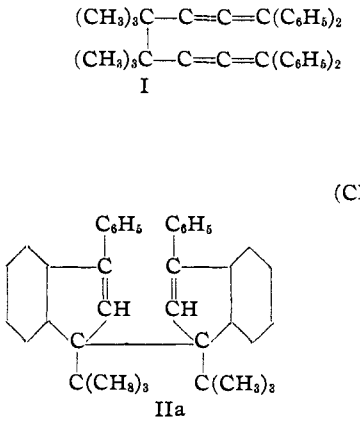
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

# Rearrangements of Tetraaryldiallenes. XII.[1] The Synthesis of 2,8-Diphenylchrysene

BY C. S. MARVEL AND W. J. PEPPEL

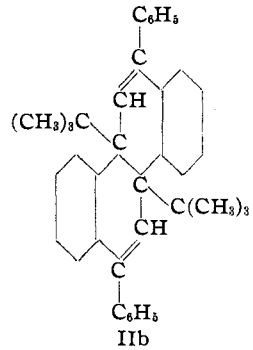
It has been shown[2] that the tetraaryldiallene, 1,1,6,6-tetraphenyl-1,3-di-*t*-butylhexatetraene-1,2,4,5 (I) is isomerized by acids and by the addition and subsequent removal of sodium to give two new $C_{38}H_{38}$ hydrocarbons (isomers A and B, respectively) of unknown structures. Neither of these rearrangement products can be the diindenyl[2d] (IIa) which might be formed by simple ring closure in a manner analogous to the formation of indenes from phenylallenes.[3] In the present communication evidence is submitted to show that neither of these rearrangements of the tetraaryldiallene (I) produces the chrysene structure (IIb)




I




IIa




IIb

by simultaneous closure of two six-membered rings.

2,8-Diphenyl-6b,12b-dihydrochrysene (V) and 2,8-diphenylchrysene (VI) were synthesized by the following reactions.

(1) For the eleventh communication in this series see Marvel, Mueller and Peppel, THIS JOURNAL, **60**, 410 (1938).

(2) (a) Farley and Marvel, *ibid.*, **58**, 29 (1936); (b) Althausen and Marvel, *ibid.*, **54**, 1174 (1932); (c) Stampfli and Marvel, *ibid.*, **53**, 4057 (1931); (d) Halley and Marvel, *ibid.*, **54**, 4450 (1932); Goebel and Marvel, *ibid.*, **55**, 3712 (1933).

(3) Vorländer and Seibert, *Ber.*, **39**, 1024 (1906); Ziegler, Grabbe and Ulrich, *ibid.*, **57**, 1983 (1924).