Summary

1. Several intermediates for the synthesis of vitamin A and vitamin A analogs have been synthesized and characterized.
2. 1-[Cyclohexen-1′-yl]-3-methyl-3-epoxybutyne-1 and related epoxides have been synthesized and their physical and chemical properties studied.

CAMBRIDGE 39, MASSACHUSETTS
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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE GLIDDEN COMPANY, SOYA PRODUCTS DIVISION]

Sterols. V. The *i*-Cholesterylamines

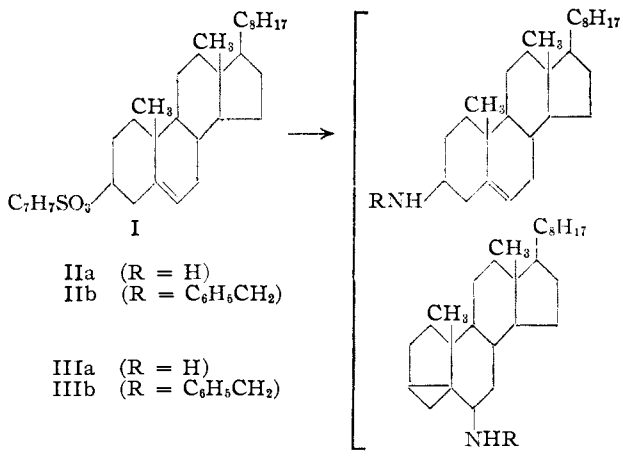
BY PERCY L. JULIAN, ARTHUR MAGNANI, EDWIN W. MEYER AND WAYNE COLE

In connection with transformations involving replacement reactions at the C_3 position of $\Delta^{5,6}$ unsaturated steroids, it was decided to explore carefully the possible role of *i*-steroids as intermediates in such conversions. The formation of an *i*-steroid has been shown to take place readily when the 3-*p*-toluenesulfonate of a $\Delta^{5,6}$-steroid is treated with an appropriate reagent in the presence of a proton acceptor[1]; however, in the absence of the latter a simple replacement seems to occur.

Our attempts to replace the 3-*p*-toluenesulfonate group by amino groups resulted in varying yields of 3-amino steroids, the relative basicity of the reagent employed strongly influencing the course of the reaction. It seemed logical to assume that we might be encountering the hitherto unknown *i*-steroid amines.

The unexpected ether solubility of the hydrochlorides of the *i*-steroid amines obscured at first their presence among the reaction products. Advantage was taken, however, of this property for their separation and characterization. This communication reports a study of certain *i*-cholesterylamines.

When cholesteryl *p*-toluenesulfonate (I) was heated with ammonia at about 98°, there was obtained not only cholesterylamine (IIa)[2] but an isomeric amine, *i*-cholesterylamine (IIIa), which was the predominant product. The separation of the isomeric cholesterylamines was greatly facilitated by the ether solubility of *i*-cholesterylamine hydrochloride. This hydrochloride, which melted at 212–214°, gave the crystalline *i*-cholesterylamine (IIIa), m. p. 77–79°. Both the *i*-amine and its hydrochloride were dextrorotatory, possessing specific rotations of +34° and +20°, respectively, in contrast to the negative rotation of cholesterylamine and its hydrochloride.



IIa (R = H)
IIb (R = $C_6H_5CH_2$)

IIIa (R = H)
IIIb (R = $C_6H_5CH_2$)

The reaction of cholesteryl *p*-toluenesulfonate (I) and benzylamine was found to proceed in an analogous fashion. Here again two isomeric amines were formed. The reaction mixture was separated into two fractions on the basis of the ether solubilities of the amine hydrochlorides. From the ether-insoluble hydrochloride, there was isolated benzylcholesterylamine (IIb), a levorotatory crystalline solid which melted at 115–117°. The purified ether-soluble hydrochloride which melted at 217–218° and possessed a specific rotation of −27° gave benzyl-*i*-cholesterylamine (IIIb), a viscous liquid which could not be crystallized. Unlike the hydrochloride, the free base was dextrorotatory, $[\alpha]_D$ + 12°.

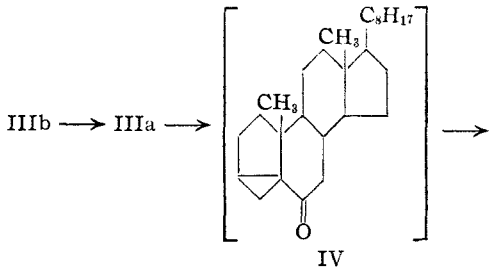
In order to prove the constitution of benzylcholesterylamine, benzyl-*i*-cholesterylamine and *i*-cholesterylamine, these amines were degraded by alkaline decomposition of the respective chloroamines followed by acid hydrolysis.[3] Upon treatment with an ethereal solution of hypochlorous acid, benzylcholesterylamine formed an N-chloro derivative which when decomposed with sodium ethoxide followed by acid hydrolysis gave cholesterylamine, identified as the acetyl derivative.[2] In a similar fashion benzyl-*i*-cholesterylamine was degraded. The product of this degradation, *i*-cholesterylamine, was identified as the crystalline hydrochloride. Further degradation of this hydrochloride via the N-chloro derivative gave a neutral product which, in spite of the inability to crystallize it, was *i*-cholestenone (IV), for upon treatment with hydrochloric acid in acetic acid in the known way,[4] it readily yielded 3(β)-chlorocholestane-6-one. Thus the position

(1) (a) Stoll, *Z. physiol. Chem.*, **207**, 147 (1932); (b) Beynon, Heilbron and Spring, *J. Chem. Soc.*, 907 (1936); (c) Wallis, Fernholz and Gephardt, THIS JOURNAL, **59**, 137 (1937).

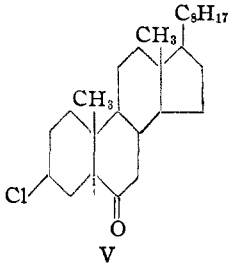
(2) Windaus and Adamla, *Ber.*, **44**, 3051 (1911).

(3) *Cf.* Hellerman and Sanders, THIS JOURNAL, **49**, 1742 (1927).

(4) Ford, Chakravorty and Wallis, *ibid.*, **60**, 413 (1938).



IV



V

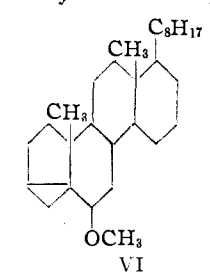
of the substituent amino group is adequately demonstrated in each instance.

During the investigation of the formation of benzylcholesterylamine and benzyl-*i*-cholesterylamine it was noted that as the reaction time increased the yield of benzyl-*i*-cholesterylamine decreased while that of benzylcholesterylamine increased. This suggested that the *i*-steroid amine was either the precursor of the C_3 substituted amine or the source of a common intermediate. The validity of this hypothesis was proved by the conversion of benzyl-*i*-cholesterylamine into benzylcholesterylamine with benzylamine in the presence of benzylammonium *p*-toluenesulfonate. Furthermore, reaction of benzyl-*i*-cholesterylamine hydrochloride with aniline gave cholesterylaniline (VII). Both of these transformations failed to proceed in the absence of an ammonium salt. This type of acid catalysis is in harmony with the conditions necessary for the "rearrangement" of *i*-steroid ethers.[5] The analogy of the *i*-steroid amines to the *i*-steroid ethers is strengthened by the nature of the reaction of *i*-cholesteryl methyl ether (VI) with benzylamine or aniline. In the presence of an ammonium salt, these reagents gave benzylcholesterylamine (IIb) and cholesterylaniline (VII), respectively. The attempted conversions failed in the absence of the ammonium salt. It is important to note that benzyl-*i*-cholesterylamine and *i*-cholesteryl methyl ether, in the presence of the proton donor, react more sluggishly with benzylamine than with aniline, an amine with a smaller basic dissociation constant. This may be explained through consideration of the following equilibria

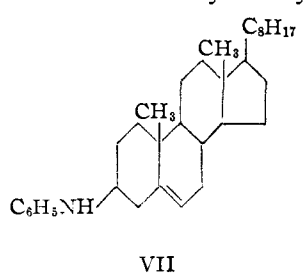
$$RNH_3^+ + i\text{-CholNHCH}_2C_6H_5 \rightleftharpoons RNH_2 + i\text{-Chol}\overset{\overset{H^+}{\cdot\cdot}}{N}HCH_2C_6H_5$$

$$RNH_3^+ + i\text{-CholOCH}_3 \rightleftharpoons RNH_2 + i\text{-Chol}\overset{\overset{H^+}{\cdot\cdot}}{O}CH_3$$

As the basic strength of RNH_2 decreases, the concentration of the steroid ammonium and steroid oxonium ions should increase. Thus if these ions underwent conversion to the C_3 substituted amines, which appears plausible, the "rearrangement" should be more readily effected with amines of low basic strength. In any given instance, the equilibrium may be shifted to the right by an increase in concentration of RNH_3. This fact has been of value in effecting the reactions of benzyl-*i*-cholesterylamine and *i*-cholesteryl methyl ether with benzylamine.



VI



VII

Experimental[6]

Cholesterylamine (IIa) and *i*-Cholesterylamine (IIIa).—A mixture of 15.0 g. of cholesteryl *p*-toluenesulfonate and 18.0 g. of liquid ammonia was placed in a glass-lined steel bomb and heated with steam at about 98° for fifteen hours. After cooling the bomb to room temperature, the excess ammonia was allowed to evaporate and the residue was shaken with ether and 10% sodium hydroxide solution. Upon shaking the ethereal layer with excess 5% hydrochloric acid there separated a white, gelatinous hydrochloride which was centrifuged, washed with ether and dried. (The ether layer and washings which contained the *i*-amine hydrochloride was saved.) The crude, insoluble hydrochloride (4.0 g.) was shaken with 10% sodium hydroxide and ether. The water-washed ether layer gave, upon removal of solvent, 2.6 g. (23%) of white, waxy crystals of cholesterylamine which melted at 89–94°; $[\alpha]^{29}D$ −26° (115 mg. made up to 10 ml. with chloroform, α −0.30°, *l*, 1 dm.). For identification, a sample was converted to N-cholesterylacetamide,[3] m. p. 238–242°.

The ether solution which was separated from the insoluble cholesterylamine hydrochloride was washed with 5% hydrochloric acid and with water, and then concentrated to about 25 ml. After dilution with 50 ml. of acetone, the *i*-cholesterylamine hydrochloride slowly crystallized. The acetone-washed and dried hydrochloride weighed 7.6 g. (64%), m. p. 176–182°. Two crystallizations from ether–acetone raised the melting point to 212–214°; $[\alpha]^{29}D$ +20° (151.9 mg. made up to 5.1 ml. with chloroform, α +0.60°, *l*, 1 dm.).

Anal. Calcd. for $C_{27}H_{47}N\cdot HCl$: N, 3.32; Cl, 8.40. Found: N, 3.20; Cl, 8.13.

This hydrochloride was soluble in ether, benzene or methanol, but relatively insoluble in water or acetone.

The free amine was prepared from the salt by shaking a mixture of 1.0 g. of *i*-cholesterylamine hydrochloride, 100 ml. of ether and 10 ml. of 10% sodium carbonate solution. The clear ether layer was washed twice with sodium carbonate solution, four times with distilled water and then dried and concentrated to a white wax which could be crystallized directly from pentane or sublimed *in vacuo*. At about 1 × 10^{-2} mm., the amine vaporized

(5) Wagner-Jauregg and Werner, *Z. physiol. Chem.*, **213**, 119 (1932).

(6) Analyses by Mr. R. Schroeder of this Laboratory, Dr. T. S. Ma of the University of Chicago and Mr. C. W. Beazley of Micro-Tech Laboratories, Skokie, Illinois.

from a bath held at 115° and crystallized in the receiver as white rosettes, m. p. 77–79°. Recrystallization from pentane did not change the melting point; $[\alpha]^{30}D$ +34° (371 mg. made up to 3.64 ml. with chloroform, α +3.42°, l, 1 dm.).

Anal. Calcd. for $C_{27}H_{47}N$: C, 84.08; H, 12.28; N, 3.63. Found: C, 84.10; H, 12.00; N, 3.44.

A sample of the *i*-cholesterylamine was reconverted to the hydrochloride, m. p. 212–214°. The amine failed to give a crystalline derivative with benzaldehyde, but with acetic anhydride in pyridine it gave N-*i*-cholesterylacetamide which crystallized from ether as white prisms, m. p. 142–143°.

Anal. Calcd. for $C_{29}H_{49}ON$: C, 81.43; H, 11.55; N, 3.27. Found: C, 81.35; H, 10.87; N, 3.16.

Benzycholesterylamine (IIb) and Benzyl-*i*-cholesterylamine (IIIb).—A solution of 100 g. of cholesteryl-*p*-toluenesulfonate in 200 ml. of benzylamine was refluxed for two hours, chilled and poured into ether. The benzylammonium *p*-toluenesulfonate (48.7 g.) was filtered and washed with ether. The combined ethereal filtrate was concentrated, steam distilled and the residue was dissolved in ether. The white, gelatinous precipitate which formed upon shaking the water-washed ether solution with 10% hydrochloric acid was separated by centrifugation. The hydrochloride, after dissolving in a small volume of ethanol, was decomposed with 10% sodium hydroxide solution. The free base was then extracted with ether, washed free of alkali and dried. The solid remaining after removal of ether crystallized from acetone yielding 19.6 g. of crude benzylcholesterylamine melting at 110–115°. Several recrystallizations from acetone gave colorless prisms melting at 115.5–117°; $[\alpha]^{33}D$ −25° (164.2 mg. made up to 5 ml. with chloroform, α −0.83°, l, 1 dm.).

Anal. Calcd. for $C_{34}H_{53}N$: C, 85.83; H, 11.22. Found: C, 86.02; H, 10.88.

The amine formed an acetyl derivative melting at 153–154°, a picrate melting at 195–198° (dec.) and a benzenesulfonamide melting at 151–153°.

The ether solution and washings separated from the gelatinous hydrochloride were washed with water, dried and concentrated to a solid residue (40.9 g.). The residue when crystallized from chloroform–acetone gave 34.5 g. (40%) of benzyl-*i*-cholesterylamine hydrochloride melting at 217–218° (dec.), $[\alpha]D$ −27° (126 mg. made up to 5 ml. with chloroform, αD −0.68°, l, 1 dm.).

Anal. Calcd. for $C_{34}H_{53}N$: C, 85.83; H, 11.23. Found: C, 85.42, 85.51; H, 11.34, 10.98.

Upon refluxing the solution of cholesteryl *p*-toluenesulfonate in benzylamine for twenty-two hours, the yield of benzylcholesterylamine was increased to 52%; however, the yield of benzyl-*i*-cholesterylamine was decreased to 10%. A further increase in reflux time (forty-six hours) complicated matters with the formation of considerable tribenzylamine,[7] an amine which also forms an ether and water insoluble hydrochloride.

Degradation of Benzylcholesterylamine.—A 4.75-g. sample of benzylcholesterylamine was dissolved in 100 ml. of dry ether, cooled to −5° and treated with 50 ml. of an ethereal solution of hypochlorous acid[8] (0.0152 g./ml.). The amine which had separated from solution on chilling, dissolved and soon a solid separated. After five minutes at room temperature (solid dissolved on warming) the ether solution was washed with 20 ml. of cold 8% aqueous sulfuric acid, 20 ml. of cold 5% sodium hydroxide solution and finally water until free of alkali. (A small quantity of the N-chloramine was separated as a white solid, m. p. 119–124°.) The dried ether solution was treated with a solution of 1.0 g. of sodium in 100 ml. of ethanol. Sodium chloride separated. The ether was removed by distillation and the remaining solution was refluxed for thirty minutes. The mixture was then steam distilled after the addition of 60 ml. of 1:5 hydrochloric acid. Benzaldehyde was evident in the distillate (gave 1.62 g. of the 2,4-dinitrophenylhydrazone, m. p. 237–238°). The residue was made basic with 50 ml. of 10% sodium hydroxide and extracted with ether. The water-washed ether layer was shaken with an aqueous solution of *p*-toluenesulfonic acid and the resulting precipitate was filtered, washed with ether and dried. The dry cholesterylammonium *p*-toluenesulfonate weighed 4.4 g. and melted at 276–278°.

One gram of this salt was decomposed in ethanol with dilute sodium hydroxide. The amine was taken up in ether, washed with water, dried and concentrated. It was then treated with 1 ml. of acetic anhydride in 20 ml. of ether to yield 0.7 g. of once-recrystallized (ethanol) N-cholesterylacetamide, m. p. 238–240° which gave no depression with that previously described.

Degradation of Benzyl-*i*-cholesterylamine.—A solution of 5.12 g. of benzyl-*i*-cholesterylamine hydrochloride in 100 ml. of ether was shaken with 10% sodium hydroxide, washed with water and dried. This solution was then treated with 50 ml. of hypochlorous acid solution as described above. After treatment with sodium ethoxide, the ethanol solution was refluxed for seventy-five minutes (it no longer liberated iodine from an acidified potassium iodide solution). After the addition of 60 ml. of 1:5 hydrochloric acid, the mixture was refluxed for forty-five minutes and steam distilled (benzaldehyde). The residue, a brown gum, was taken up in ether and washed with 10% sodium hydroxide solution, 10% hydrochloric acid and water. The gum remaining after removal of solvent from the dried ether solution was crystallized from acetone; 2.7 g. of white solid. A 2.2-g. sample recrystallized from chloroform–acetone gave 2.0 g. of material melting at 201–205°. Several recrystallizations from the same solvent mixture and one from ether–acetone raised the melting point to 212–215°. This substance gave no depression in melting point when mixed with a sample of *i*-cholesterylamine hydrochloride.

Degradation of *i*-Cholesterylamine.—A solution of 1.05 g. of *i*-cholesterylamine hydrochloride (material prepared from the *i*-benzyl compound as described above) in ether was shaken with 10% sodium hydroxide solution, washed with water and dried. It was then treated at −10° with 6.2 ml. of an ether solution of hypochlorous acid (0.021 g./ml.). After five minutes, the solution was washed with 5% sodium hydroxide solution, water and dried. Titration of the iodine liberated by 1.0 ml. of the ether solution from acidified potassium iodide indicated that the conversion to the N-chloramine had taken place in 84% yield. The ether solution was then poured into a solution of 0.5 g. of sodium in 25 ml. of ethanol, the ether removed by distillation, and the remainder refluxed for thirty minutes. The mixture was then diluted with 100 ml. of cold water and acidified with dilute hydrochloric acid. After standing overnight, the mixture was extracted with ether. The residue remaining after removal of ether from the washed and dried solution could not be crystallized from methanol or acetone even after seeding with *i*-cholestenone. Thus it was taken up in 10 ml. of warm glacial acetic acid, chilled and treated with 2 ml. of concentrated hydrochloric acid. The solid which crystallized upon scratching was filtered, washed with methanol and dried; 0.5 g., m. p. 126–131°. Recrystallization from methanol raised the melting point to 130–133°. A mixture of this material with an authentic specimen of 3(β)-chlorocholestane-6-one[9] showed no depression in melting point.

Rearrangement of Benzyl-*i*-cholesterylamine to Benzylcholesterylamine.—A solution of 7.5 g. of benzyl-*i*-cholesterylamine and 5.0 g. of benzylammonium *p*-toluenesulfonate in 20 ml. of benzylamine was refluxed for twenty-three hours. The golden-yellow solution was poured into ether and the benzylammonium *p*-toluenesulfonate (5.0 g.) was separated and washed with ether. The ether filtrate was concentrated and steam distilled.

(7) *Cf.* Nozaki, THIS JOURNAL, **64**, 2920 (1942).

(8) Goldschmidt, *Ber.*, **46**, 2728 (1913).

(9) Windaus and Dalmer, *ibid.*, **52**, 162 (1919).

Upon shaking an ethereal solution of the residue with 10% hydrochloric acid, a gelatinous precipitate formed. The hydrochloride was separated by centrifugation, washed three times with ether and decomposed in ethanol solution with 10% sodium hydroxide solution. The amine was extracted with ether and washed with water. The residue remaining after removal of solvent from the dried solution was crystallized from acetone yielding 3.4 g. (56.5%) of fine, white crystals melting at 115–117°. These gave no depression in melting point when mixed with a sample of benzylcholesterylamine.

The ether washings of the gelatinous hydrochloride gave 1.5 g. of unchanged benzyl-*i*-cholesterylamine hydrochloride melting at 216–218° (dec.).

Reaction of Benzyl-*i*-cholesterylamine Hydrochloride with Aniline.—A solution of 3.0 g. of benzyl-*i*-cholesterylamine hydrochloride in 15 ml. of aniline was refluxed for four hours. Upon cooling, the mixture set to a semi-solid mass. It was then digested with ethanol and chilled. The solid was separated, washed with ethanol and dried. The dry material, 2.3 g. (85%) of white plates, melted at 189–191°. A sample of the compound gave no depression in melting point when mixed with cholesterylaniline.[10]

In a similar experiment in which the free amine, benzyl-*i*-cholesterylamine, was employed, the amine was recovered unchanged.

Reaction of *i*-Cholesteryl Methyl Ether with Benzylamine.—A solution of 3.0 g. of *i*-cholesteryl methyl ether[1a] and 3.0 g. of benzylammonium *p*-toluenesulfonate in 20 ml. of benzylamine was refluxed for twenty-two hours. The solution was poured into water and extracted with ether. Upon shaking the ethereal layer with dilute hydrochloric acid an insoluble hydrochloride separated. The hydrochloride was centrifuged, washed three times with ether and then decomposed in ethanol with 10% sodium hydroxide. An ether extract of the free amine was washed with water, dried and concentrated. Upon crystallization from acetone, the yellow residue yielded 1.5 g. of white prisms melting at 115–118°. This material showed no depression in melting point when mixed with a sample of benzylcholesterylamine.

Upon heating a 1.0-g. sample of the *i*-ether with 5 ml. of benzylamine in a closed tube at 240° for eighteen hours, the *i*-ether was recovered unchanged.

Reaction of *i*-Cholesteryl Methyl Ether with Aniline.—A mixture of 0.6 g. of carefully purified *i*-cholesteryl methyl ether and 5 ml. of freshly distilled aniline containing a few mg. of *p*-toluenesulfonic acid was refluxed from an oil-bath at 190° for two hours and then allowed to cool. The semi-solid mass was slurried with 10 ml. of methanol, filtered, washed with methanol and dried. The resulting cholesterylaniline, 0.65 g. of white flakes, melted at 190°. This gave no depression in melting point when mixed with a sample of cholesterylaniline prepared in the known manner.[10]

In a similar experiment, in which the *p*-toluenesulfonic acid was omitted, the *i*-ether was recovered unchanged.

(10) Lieb, Winkelmann and Koeppl, *Ann.*, **509**, 214 (1934).

Summary

1. Cholesteryl *p*-toluenesulfonate reacts with ammonia and with primary aliphatic amines to give 6-amino-*i*-cholestenes accompanied by some 3-amino-5-cholestenes.

2. *i*-Cholesterylamine and benzyl-*i*-cholesterylamine are described and their structures proved by stepwise degradation to the known 3(β)-chlorocholestan-6-one.

3. Benzyl-*i*-cholesterylamine can be transformed into benzylcholesterylamine by treatment with benzylamine and benzylammonium toluenesulfonate. The necessity of the presence of the salt in this reaction points to an ionic mechanism for the conversion of the *i*-steroid into the normal steroid and further corroborates the known acid-catalyzed reactivity of the *i*-steroids.

CHICAGO, ILLINOIS RECEIVED NOVEMBER 28, 1947

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY OF THE UNIVERSITY OF CHICAGO]

Preparation of 17-Ketosteroids from Enol Acetates of 20-Ketosteroids[1a]

BY CHARLES W. MARSHALL, THEODORE H. KRITCHEVSKY, SEYMOUR LIEBERMAN[1b] AND T. F. GALLAGHER[1b]

The 20-ketosteroids are obtainable in good yield from accessible natural products. It appeared to us that oxidation of the enol acetates might offer a promising procedure for the preparation of 17-ketosteroids from these substances and we have accordingly investigated this problem using four different 20-ketosteroids. The general reactions are summarized in the partial formulations of Fig. 1. The enol acetates were prepared by the method of Bedoukian[2] and from the three pregnane derivatives studied, only one enol acetate was obtained. The structure of the product was proved by ozonolysis to the corresponding 17-ketosteroid. From 3(β)-hydroxy-20-ketoallopregnane, two stereoisomeric enol acetates were obtained which must be regarded as *cis* and *trans* isomers about the double bond from C-17 to C-20, since both compounds upon ozonolysis followed by saponification yielded isoandrosterone. The compound with higher melting point has been arbitrarily designated as the *trans*-form. For preparative purposes, isolation of the enol acetate is

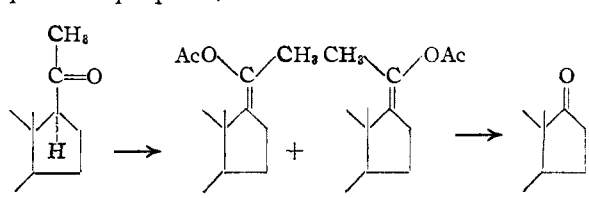


Fig. 1.

(1a) The work described in this paper was supported in part by a grant from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council and in part by grants from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago. Presented before the Midwest Regional Meeting of the American Chemical Society, June, 1947.

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(2) Bedoukian, THIS JOURNAL, **67**, 1430 (1945).