index, n_D^{25}, was 1.4244. When frozen in Dry Ice–acetone, the melting point was approximately −30°. The optical rotation of this alcohol, $[\alpha]_D^{25}$, was −6.0°, c = 4.56 in alcohol. Attempted preparations of the 3,5-dinitrobenzoate and phenylurethane of the alcohol led only to uncrystallizable oils. The hydrogen phthalate[24] melted at 74–75°. l-2-Octyl hydrogen phthalate melts at 75°.[21]

SYRACUSE 10, N. Y.

(24) J. F. Goggans, Jr. and J. E. Copenhaver, *J. Am. Chem. Soc.*, 61, 2909 (1939).

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, MCGILL UNIVERSITY]

Ring D Steroid Oximes and the Beckmann Rearrangement

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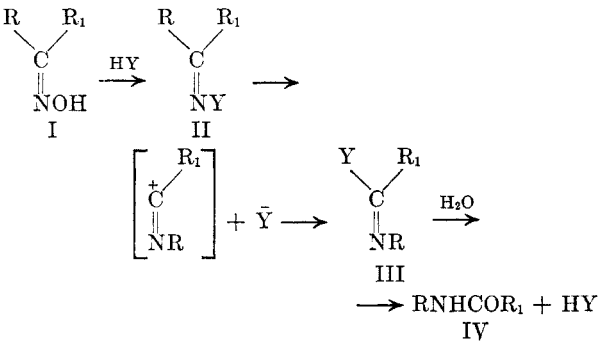
Whereas simple ring D and ring A steroid oximes yield the corresponding oxime acetates on subjection to mild acetylating conditions, 3β-hydroxyandrostan-16,17-dione 16-oxime gives a pure crystalline compound which was characterized by its chemical properties and behavior as an intermediate in a Beckmann Rearrangement. Rearrangement could be completed under appropriate, though equally mild, conditions. Reasons for the unexpected rearrangement under such mild conditions are advanced.

The mechanism of the Beckmann Rearrangement still stimulates considerable experimental investigation despite the ninety years that have elapsed since the original discovery of the reaction. Mesenheimer, Kuhara, and Chapman have laid the foundations of our knowledge and outlined the generalizations for understanding this reaction.[3] As stated by various authors,[4,5] Beckmann Rearrangement with a variety of acid reagents essentially involves ester formation between an oxime (I) and the reagent HY. By means of ionization a process of *trans* interchange then takes place yielding an imidoyl ester (III) from the oxime ester (II). The amide (IV), the end-product of the reaction, arises from (III) by ill-defined steps, one of which undoubtedly is hydrolytic, and the acid reagent is regenerated.



A recent paper by Stephen and Staskun[6] departs from the classical formulations of this reaction and proposes a new mechanism for amide formation under entirely anhydrous conditions. Our contribution in this field arose during the course of investigations on the structure of Heard's lactone.[7] These investigations involved an attempt to prepare 3β-acetoxy-16-acetoximinoandrostan-17-one (VI) from 3β-hydroxyandrostan-16,17-dione 16-oxime (V) by allowing the latter to stand overnight in a mixture of acetic anhydride and pyridine at room temperature. We have acetylated the oximes of cholestanone, testosterone, and epiandrosterone under these same conditions. However, while many stable acetates of alpha oximino ketones have been prepared,[8] the product obtained from V was not VI, as expected, but has been formulated as having the structure VII.

After recrystallization from anhydrous ether VII was obtained as colorless feathery needles. While stable in the dark, it gradually turns yellow on exposure to light. It has an absorption peak in the ultraviolet at 223 mμ as compared with the peak at 240 mμ exhibited by V. This hypsochromic shift of 17 mμ could not be explained by mere acetylation of the oxime hydroxyl—as in VI—, especially since we observed that similar acetylation of testosterone oxime causes a bathochromic displacement of the absorption maximum of 5 mμ. (Table I). When dissolved in alcohol the absorption

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(2) Present address: Du Pont of Canada, Ltd., Montreal.

(3) See, for example, B. Jones, *Chem. Revs.*, **35**, 335 (1944).

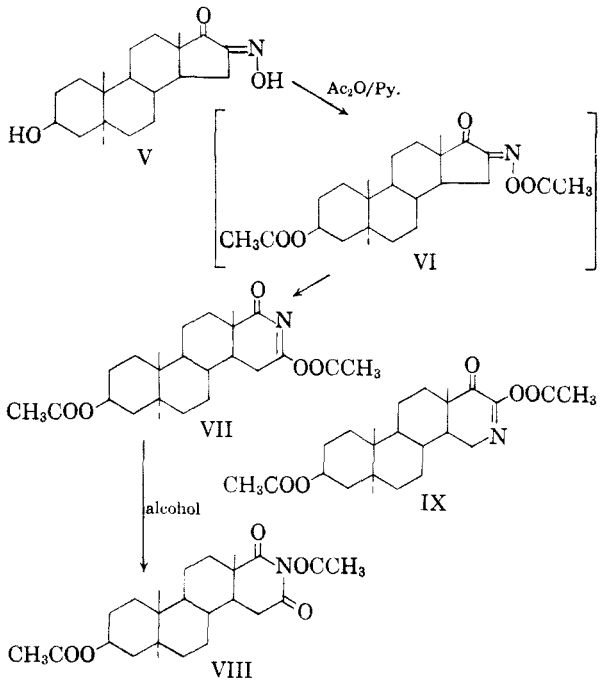
(4) D. E. Pearson and F. Ball, *J. Org. Chem.*, **14**, 118 (1949).

(5) C. R. Hauser and D. S. Hoffenberg, *J. Org. Chem.*, **20**, 1482 (1955); also (3) and (4).

(6) H. Stephen and B. Staskun, *J. Chem. Soc.*, 980 (1956).

(7) R. D. H. Heard, *J. Am. Chem. Soc.*, **60**, 493 (1938).

(8) A. H. Blatt, and R. P. Barnes, *J. Am. Chem. Soc.*, **56**, 1148 (1934); **57**, 1331 (1935); **58**, 1903 (1936).



band at 223 mμ fades gradually at a rate which, as measured spectrophotometrically, is dependent on the temperature and on the presence and concentration of added hydrochloric acid (Table II and Fig. I).

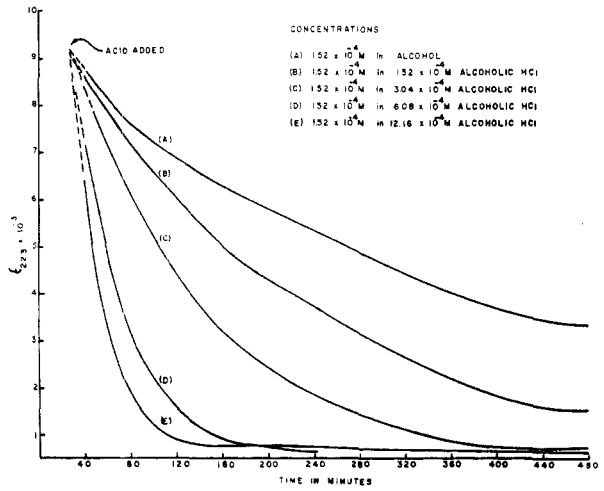
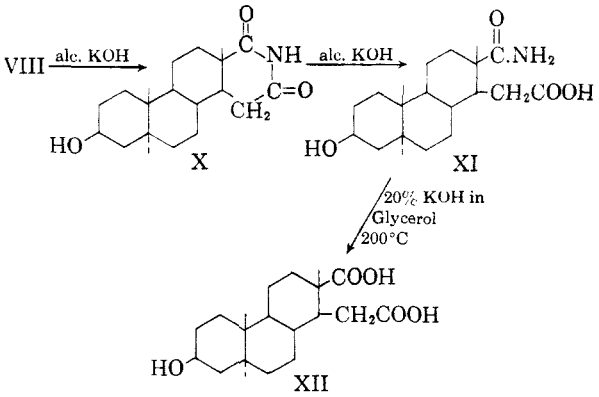


Fig. 1. Rearrangement of VII at room temperature in alcohol and alcoholic hydrochloric acid. The rearrangement was followed at 223 mμ in a Beckmann DU Quartz Spectrophotometer with photomultiplier attachment. Zero Time is the time of addition of the alcohol to VII. Aqueous hydrochloric acid was subsequently added at the time indicated to give the stated concentrations

The structure VII corresponds to that of an intermediate in the Beckmann Rearrangement of the alpha oximino ketone (V)—or, more exactly, of its diacetate (VI)—and presupposes that the starting oxime consists of a single geometric isomer. The alternate isomeric orientation of the oxime hydroxyl should yield a compound of structure IX which does not correspond to the chemical behavior of the material isolated. That such ring D oximes consist of single isomers is indicated by the work of Regan and Hayes[9] on the behavior of estrone oxime 3-methyl ether and 3β-acetoxy-5-androsten-16,17-dione 16-oxime in a formal Beckmann Rearrangement. This preferred orientation of the oxime hydroxyl is to be expected in this region of the molecule and is further indicated in a comparison of the melting points of the acetates of ring D and ring A oximes (Table I). In the case of testosterone and cholestanone oximes acetylation yields a mixture of the oxime acetate isomers, as indicated by the wide range of melting points of the products. The 3β - acetoxy - 17 - acetoximino - androstane, on the other hand, has a very sharp melting point and must consist of a single isomer.

The proof of the structure of VII rests on its easy rearrangement in alcohol, especially under the influence of heat and acid conditions, as compared to its stability in anhydrous ether from which it could be crystallized. The oily neutral product, formulated as VIII, obtained by refluxing VII in alcohol, could be converted by stepwise alkaline hydrolysis into 3β-hydroxy-16,17-*seco*-androstan-16,17-imide (X), 3β-hydroxy-16,17-*seco*-androstan-16,17-dioic acid 17-amide (XI), and the known 3β-hydroxy - 16,17 - *seco* - androstan - 16,17 - dioic acid (XII). The tertiary nature of the semi-amide XI is borne out by its resistance to alkaline hydrolysis, which could only be effected by heating to 200° in a solution of 20% potassium hydroxide in glycerol, as well as its failure to react with cold nitrous acid. This inertness is well known to be characteristic of C_{17} carboxyl derivatives.[9]



Much controversy has existed as to the actual occurrence of the imidoyl ester type of structure, such as VII, during the course of Beckmann Rearrangement. Such a formulation was given by Kuhara[10] in the rearrangement of *O*-benzenesulfonylbenzophenone oxime but was later disputed by Chapman.[11] Over the years, however, indirect

(9) B. M. Regan and F. N. Hayes, *J. Am. Chem. Soc.*, **78**, 639 (1956).

(10) M. Kuhara, K. Matsumiya, and N. Matsunami, *Mem. Coll. Sci. Kyoto*, **1**, 25 (1914).

(11) A. W. Chapman and C. C. Howis, *J. Chem. Soc.*, 808 (1933).

TABLE I[a]

SOME STEROID OXIMES AND THEIR ACETATES

Oxime	Acetate
17-Oximino-3β-acetoxy-androstane M.p. 184–186° Calcd. for $C_{21}H_{33}O_3N$: C, 72.59; H, 9.51; N, 4.03. Found: C, 72.66, 72.50; H, 9.78, 9.83; N, 4.16	17-Acetoximino-3β-acetoxy-androstane M.p. 180–180.5° Calcd. for $C_{23}H_{35}O_4N$: C, 70.95; H, 8.99; N, 3.59. Found: C, 70.93, 71.04; H, 9.31, 9.11; N, 3.71
Cholestanone oxime M.p. 200–201° (Kofler) Reported[18] m.p. 196°	3-Acetoximino cholestane M.p. 124–138° (Kofler) Calcd. for $C_{29}H_{49}O_2N$: C. 78.55; H, 11.06; N, 3.13. Found: C, 78.41, 78.36; H, 11.08, 11.00; N, 3.21.
Testosterone Oxime M.p. 222–223° (Kofler) Reported[19] m.p. 221–222.5°. λ_{max}. 241 mμ (ε 19,520)	17β-Acetoxy-3-acetoximino-4-androstene M.p. 113–131° (Kofler) Calcd. for $C_{23}H_{33}O_4N$: C, 71.32; H, 8.52; N, 3.61. Found: C, 71.01, 71.21; H, 8.75, 8.80; N, 3.68. λ_{max}. 246 mμ (ε 23,976)

[a] Spectra were done in alcohol at a concentration of $2 \times 10^{-5}M$.

evidence has accumulated for the occurrence of such imidoyl esters as intermediates[12] and Coleman and Pyle[13] have isolated in imidoyl chloride in the rearrangement of benzophenone oxime by phosphorus pentachloride. The isolation of a pure crystalline imidoyl acetate in these studies, together with the associated shift in the absorption maximum, is further unequivocal evidence as to the structural changes taking place during the course of the Beckmann Rearrangement. In addition, the properties of the final product of the overall rearrangement—the oil obtained by refluxing VII in 95% alcohol—suggest that it is the *N*-acetyl imide rather than the free imide (X). In fact the free imide is only produced after prolonged hydrolysis of the oil.

The occurrence here of the Beckmann Rearrangement under conditions so mild that they should properly yield only the oxime acetate (VI) is attributed, partly at least, to the strained nature of ring D of the steroid nucleus and to the steric hindrance prevailing in this region of the molecule. In this context it is known, for example, that hindered ketones often yield rearranged products on attempted oximation.[14] It should be emphasized, however, that the acetate of a simple ring D oxime (epiandrosterone oxime) was isolated under those same conditions which led to rearrangement in the case of the ring D alpha oximino ketone (V). It is presumed that rearrangement under such mild conditions is an inherent property of the alpha oximino ketone grouping when present in a strained ring system Further work would be necessary to establish this.

(12) P. Oxley and W. F. Short, *J. Chem. Soc.*, 1514 (1948); B. B. Lampert and F. G. Bordwell, *J. Am. Chem. Soc.*, **73**, 2369 (1951); C. W. Kenner, A. R. Todd, and R. F. Webb, *J. Chem. Soc.*, 1231 (1956).

(13) G. H. Coleman and R. E. Pyle, *J. Am. Chem. Soc.*, **68**, 2007 (1946).

(14) F. Greer and Pearson, D. E. *J. Am. Chem. Soc.*, **77**, 6649 (1955).

EXPERIMENTAL[15]

3β-Hydroxyandrostan-16,17-dione 16-oxime (V) was prepared in a manner similar to that of Huffman.[16] It was purified, however, by dissolving 2.95 g. of crude product in a mixture of 40 ml. methanol and 10 ml. water and refluxing in the presence of charcoal. After removal of the charcoal the hot filtrate was concentrated by evaporation and then allowed to crystallize. This furnished pure material in 70% over-all yield. M.p. 220° (Kofler). Reported[16] m.p. 218–219.5°. Ultraviolet spectrum in ethanol; λ_{max}. 240 mμ (ε 9570).

Acetylation of (V) One hundred milligrams of V was dissolved in a mixture of 1 ml. of pyridine (freshly distilled from barium oxide) and 1 ml. of acetic anhydride (twice distilled from fused sodium acetate; b.p. 137–138°) and allowed to stand overnight. The solution was then poured with stirring into 50 ml. of 3*N* hydrochloric acid. After filtration the white precipitate was washed with dilute hydrochloric acid and water. It was then dried on suction and in a vacuum desiccator over calcium chloride. This yielded 102 mg. of colorless powdery material, m.p. 161.5–163°. Recrystallization from hot anhydrous ether (Merck) gave 82 mg. of VII as long feathery crystals, m.p. 163–165°. Ultraviolet spectrum in ethanol; λ_{max}. 223 mμ (ε 9431).

Anal.[17] Calcd. for $C_{23}H_{33}O_5N$: C, 68.48; H, 8.18; N, 3.47. Found: C, 68.49, 68.39; H, 8.38, 8.23; N, 3.38.

Crystallization from Merck Reagent ether yielded material in much lower yield, m.p. 154–164°. The compound (VII) is quite stable in the dark (unchanged after 1.5 years) but turns yellow within one week when exposed to light. It is particularly unstable in solution in alcohol where the absorption peak at 223 mμ gradually disappears—especially in the presence of acid or on heating (Fig. 1 and Table II).

Preparation and acetylation of other oximes. Oximes were prepared in the usual manner by refluxing in alcohol for 2 hr. in the presence of excess hydroxylamine hydrochloride and sodium acetate. Acetylating conditions identical to those used in the preparation of VII were employed and the

(15) Unless otherwise stated all melting points were carried out on the Fisher-Johns apparatus and are uncorrected.

(16) M. N. Huffman and M. H. Lott, *J. Biol. Chem.*, **207**, 431 (1954).

(17) Microanalyses were performed by E. Thommen, Thannerstrasse 45, Basel, Switzerland.

(18) L. Ruzicka and A. Wettstein, *Helv. Chim. Acta*, **18**, 1264, (1935).

(19) D. P. Dodgson and R. D. Haworth, *J. Chem. Soc.*, 67 (1952).

authenticity of the acetates was established by alkaline hydrolysis to the starting oximes.

Conversion of VII to VIII. Five hundred milligrams of VII was dissolved in 50 ml. of 95% alcohol and refluxed. Samples were taken at one-hour intervals and after appropriate dilution measured in the spectrophotometer at 223 mμ. As can be seen from Table II the absorption peak disappears within 3 hr. After 6 hr. the solvent was evaporated yielding a pale yellow oil which could not be crystallized. The oil was insoluble in aqueous sodium carbonate and could not be extracted out of an ether solution with alkali.

TABLE II

REARRANGEMENT OF VII IN BOILING ALCOHOL

Time, hours	ϵ_{223}
0	9,400
1	1,410
3	427
4	427

Alkaline hydrolysis of VIII. To 500 mg. of the oil dissolved in 50 ml. of 95% alcohol 2.5 g. of potassium hydroxide was added and the solution refluxed for 20 hr. On cooling, the solution was evaporated *in vacuo* and the residue dissolved in 50 ml. of water. Ether extraction removed 60 mg. of yellow oil. Addition of concentrated hydrochloric acid to the clear aqueous phase precipitated a mixture of X and XI.

Isolation of 3β-hydroxy-16,17-seco-16,17-dioic acid 17-amide (XI). To the acidified aqueous suspension 60 ml. of chloroform was added and the whole shaken in a separatory funnel during which X passed into the chloroform phase. After separation, the aqueous suspension was filtered and the precipitate washed thoroughly with water and dried. This yielded 220 mg. of material, m.p. 214–218°. After 6 recrystallizations from methanol followed by drying *in vacuo* over phosphorous pentoxide at 120° needle crystals melting at 218.5–220.5° were obtained. The compound was completely insoluble in chloroform, benzene, ether, and ethyl acetate but was soluble in dilute aqueous sodium carbonate. Titration indicated that it was a monobasic acid. When dissolved in cold acetic acid and treated with a cold solution of sodium nitrite, no evolution of nitrogen was evident which confirmed the tertiary nature of the amide grouping.

Anal. Calcd. for $C_{19}H_{31}O_4N$: C, 67.65; H, 9.46; N, 4.16. Found: C, 67.67, 67.61; H, 9.36, 9.40; N, 4.25.

Isolation of 3β-hydroxy-16,17-seco-androstan-16,17-imide (X). The chloroform solution obtained in the isolation of XI was washed free of acid and dried over sodium sulfate. Evaporation of the chloroform solution *in vacuo* yielded 136 mg. of a yellowish flaky solid which melted at 100–110°. This was dissolved in 4 ml. methanol and a few drops of water added which removed the yellow impurity. The solution was then centrifuged and the supernatant removed. The concentration of the methanol solution was then adjusted by boiling and adding water so that on cooling the material crystallized out as fine colorless needles. After drying *in vacuo* at 120° over phosphorus pentoxide for 1.5 hr. 113 mg. of X melting at 180–182.5° was obtained. It was readily soluble in dilute aqueous sodium carbonate. On refluxing a sample in 10% aqueous potassium hydroxide for 24 hr. it was converted into XI.

Anal. Calcd. for $C_{19}H_{29}O_3N$: C, 71.48; H, 9.09; N, 4.38. Found: C, 71.55, 71.92; H, 8.97, 9.16; N, 4.72.

Conversion of XI to 3β-hydroxy-16,17-seco-androstan-16,17-dioic acid (VII). Forty milligrams of the pure semi-amide (XI) was added to 5 ml. of a solution of 20% potassium hydroxide in glycerol. The flask was placed in an oil bath and the temperature gradually raised to 200° whereupon a copious evolution of bubbles of a basic gas took place. After 2.5 hours heating was stopped. The material came out of solution on cooling but on dilution with water a clear, slightly yellow, solution was obtained. This was acidified and after standing for 2 hr. it was filtered and washed well with water. It was dried on suction and finally *in vacuo* over phosphorus pentoxide at 120°. This yielded 20 mg. of material which melted at 234–237° with previous softening at 225°. After one recrystallization from methanol the melting point was 237.5–238.5°. It titrated as a dibasic acid and sodium fusion indicated the absence of nitrogen. On admixture with an authentic sample of XII there was no depression in the melting point.

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[CONTRIBUTION FROM U. S. DEPARTMENT OF AGRICULTURE]

Separation of Aliphatic Disulfides and Trisulfides by Gas-Liquid Partition Chromatography

JOHN F. CARSON AND FRANCIS F. WONG

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The polar stationary phases, Carbowax and Reoplex, and a nonpolar phase, Apiezon M, have been compared in the separation of aliphatic disulfides and trisulfides by gas-liquid partition chromatography. Mixtures of disulfides and trisulfides can be separated at 150° without decomposition. The polar phases are particularly useful for separating unsaturated disulfides from the corresponding saturated compounds.

This paper reports the application of gas-liquid partition chromatography to the separation and isolation of some simple aliphatic disulfides and trisulfides in connection with a study of the volatile components of onions.[1] A number of investigators have studied the separation of thiols and sulfides. Sunner, Karrman, and Sunden[2] reported quantitative separation of a number of thiols by gas-liquid partition chromatography, and Ryce and Bryce[3]

(1) Presented at the Joint Symposium of Analytical and Petroleum Chemistry, American Chemical Society meeting, New York, September 1957.

(2) S. Sunner, K. J. Karrman, and V. Sunden, *Mikrochim. Acta*, 1144 (1956).

(3) S. A. Ryce and W. A. Bryce, *Anal. Chem.*, **29**, 925 (1957).