experimental techniques have been given in earlier publications.[2,3]

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Summary

A comparative study has been made of the infrared absorption spectra between 1660 and 1780 cm.$^{-1}$ of ketosteroids containing a carbonyl group at positions 11 and 12.

A non-conjugated ketone group at position 11 gives rise to a maximum at 1710–1713 cm.$^{-1}$ in carbon disulfide solution. In 3,11- and 11,20-diketosteroids the maxima associated with the two carbonyl groups are too close to be separated, but there is no evidence of interaction effects causing displacements of the bands. In the 11,17-diketosteroids the band attributed to the 11-ketone group occurs at the normal position (1710–1713 cm.$^{-1}$) but the band attributed to the 17-ketone group is displaced from 1742–1745 to 1748–1754 cm.$^{-1}$.

Similar data are given also for 12-ketosteroids and the significance of these observations in the elucidation of steroid structure is discussed.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

The Dehydration of 22-Phenyl-3-methoxy-22-hydroxy-bisnor-5-cholene

BY F. W. HEYL, M. E. HERR AND A. P. CENTOLELLA

The reaction of 3-acetoxy-bisnor-5-cholenaldehyde with phenylmagnesium bromide, yielding 22-phenyl-3,22-dihydroxy-bisnor-5-cholene (I) as well as the preparation of several analogous 22-phenyl-3-alkoxy-22-hydroxy-bisnor-5-cholenes from stigmasteryl ethers have been reported.[1,2] The problem of introducing a double bond into the C-20,22 position of the side chain involves the difficulties usually encountered in the dehydration of a secondary alcohol.

For the purpose of studying this dehydration reaction, it was decided to use 22-phenyl-3-methoxy-22-hydroxy-bisnor-5-cholene (III) which was prepared from stigmasteryl methyl ether as previously reported,[2] and also by refluxing the monotosylate (II) prepared from 22-phenyl-3,22-dihydroxy-bisnor-5-cholene (I), with methanol, whereby the same methoxy compound (III) is obtained and the position of this tosyl group is established.

The dehydration of this 3-methoxy-22-ol (III) was accomplished by using a variation of the method of Wuyts[3] in which the alcohol was refluxed in toluene in the presence of a catalytic amount of *p*-toluenesulfonic acid and a trace of phenol. The desired 22-phenyl-3-methoxy-bisnor-5,20-choladiene (IV) was obtained in good yields.

The 5,6 dibromo compound of the diene (IV) was ozonized directly to pregnene-3β-ol-20-one methyl ether (V) which was isolated as the semicarbazone in 62% yields. Acid hydrolysis of the semicarbazone resulted in a 92% yield of pregnenolone methyl ether (V). This compound was converted to pregnene-3β-ol-20-one acetate (VI), the immediate precursor of progesterone, according to the recently described method of Huffman and Lott.[4]

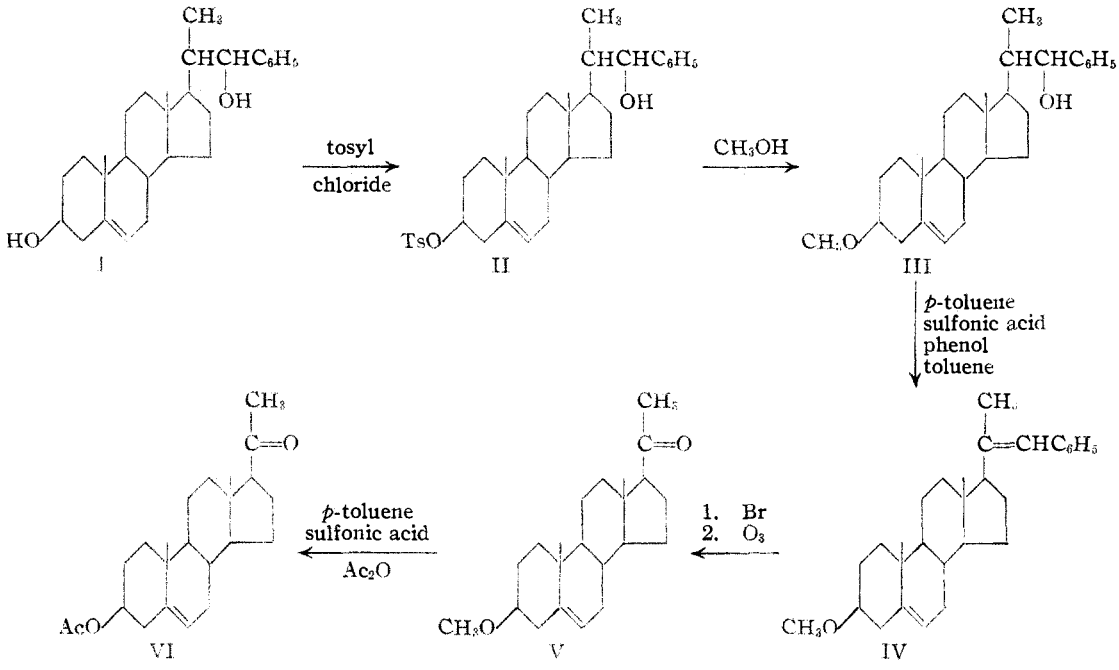
Experimental[5]

22-Phenyl-3,22-dihydroxy-bisnor-5-cholene-3-tosylate (II). —To 3.14 g. of diol (I) dissolved in 25 ml. of dry pyridine was added 2.2 g. (1½ moles) of *p*-toluene-sulfonyl chloride. After standing twenty-four hours at 37° it was poured into ice and sodium bicarbonate (1 g.) and the tosyl ester extracted with benzene. The solution was dried over sodium sulfate and upon removing the benzene and remaining pyridine *in vacuo* there was obtained a quantitative yield of crystalline residue. Recrystallization from benzene–hexane gave 2.85 g. of silky needles, m. p. 157–159° (dec.). Working up the mother liquor gave a second crop of slightly lower melting tosyl ester.

Anal. Calcd. for $C_{35}H_{46}O_4S$: C, 74.69; H, 8.24; S, 5.7. Found: C, 74.82; H, 7.98; S, 5.7. $[\alpha]^{25}D$ −38.9 (79.1 mg. made up to 10 ml. in chloroform, $\alpha^{25}D$ −0.308, *l*, 1 dm.).

When the above esterification was carried out at room temperature most of the diol was recovered. When the

(1) Heyl, Centolella and Herr, THIS JOURNAL, **69**, 1957 (1947).
(2) Centolella, Heyl and Herr, *ibid.*, **70**, 2953 (1948).
(3) Wuyts, *Bull. soc. chim. Belg.*, **26**, 304 (1912).
(4) Huffman and Lott, *J. Biol. Chem.*, **172**, 793 (1948).
(5) Analyses and rotations by members of the Upjohn microanalytical group. Melting points are as read on Fisher-Johns melting point apparatus.



reaction was allowed to proceed twenty-four hours at 55–58° little of the desired tosyl ester could be obtained. Considerable material resulted which was insoluble in ether. When recrystallized from large volumes of acetone high melting fractions separated. One melting at 272–275° contained no sulfur and was not investigated further.

22-Phenyl-3-methoxy-22-hydroxy-bisnor-5-cholene (III).—The above tosyl ester was refluxed for three hours with an amount of methanol sufficient to dissolve it and upon cooling an almost quantitative yield of crystals separated out. Recrystallized from methanol-chloroform, it melted at 205–206°, and, when mixed with the 3-methoxy-ol (III) previously reported,[2] there was no m. p. depression.

Anal. Calcd. for $C_{29}H_{42}O_2$: C, 82.43; H, 10.02. Found: C, 81.93; H, 9.9. $[\alpha]^{25}D$ −45.7 (0.09623 g. made up to 10 ml. with chloroform, $\alpha^{25}D$ −0.44, *l*, 1 dm.).

22-Phenyl-3-methoxy-22-chloro-bisnor-5-cholene.—0.42 g. of 22-phenyl-3-methoxy-22-hydroxy-bisnor-5-cholene was dissolved in 15 ml. of hot benzene and to this was added a slight excess of thionyl chloride and the mixture was refluxed for one hour. After removal of most of the solvent the chloro compound crystallized out. Recrystallized from hexane, it melted at 189–190°.

Anal. Calcd. for $C_{29}H_{41}OCl$: Cl, 8.04. Found: 8.20.

22-Phenyl-3-methoxy-22-acetoxy-bisnor-5-cholene.—250 mg. of 22-phenyl-3-methoxy-22-hydroxy-bisnor-5-cholene was dissolved in 20 ml. of dry pyridine and 5 ml. of acetic anhydride added. The mixture was refluxed one-half hour, cooled, and poured, with stirring, into a mixture of 15 ml. of concentrated hydrochloric acid and ice. This was extracted with ether, washed with saturated sodium bicarbonate solution and water, and dried over magnesium sulfate. Upon evaporating the solvent and recrystallizing the residue from chloroform–methanol the compound melted at 216–217°.

Anal. Calcd. for $C_{31}H_{44}O_3$: C, 80.12; H, 9.54. Found: C, 80.24; H, 9.59.

22-Phenyl-3-methoxy-bisnor-5,20-choladiene (IV).—500 mg. of 22-phenyl-3-methoxy-22-hydroxy-bisnor-5-cholene was dissolved in 200 ml. of toluene by heating and 20 mg. of phenol was added. The mixture was heated until the phenol was in solution and 50 mg. of *p*-toluene sulfonic acid monohydrate was added. The solution was refluxed eighteen hours, using a water trap. After cooling the solution was washed with five per cent sodium hydroxide and with water until neutral and the toluene was removed *in vacuo*. The crystalline residue was dissolved in 50 ml. of boiling benzene and, after cooling, the clear solution was passed over a column of 15 g. of activated alumina (Fisher adsorption), and the product eluted with benzene in 40 ml. fractions. The first four fractions in several runs weighed 305–370 mg. (60–75%) and were nicely crystalline. Recrystallized from methanol–chloroform the melting point was 166–169°.

Anal. Calcd. for $C_{29}H_{40}O$: C, 86.11; H, 9.97. Found: C, 86.45; H, 10.47.

Pregnene-3β-ol-20-one Methyl Ether Semicarbazone.—400 mg. of the diene (IV) was dissolved in 25 ml. of chloroform and, while cooling in an ice-bath, 0.158 g. of bromine in 5 ml. of chloroform was added dropwise with mechanical stirring. The ice cooled solution was ozonized for thirty minutes (*ca.* 7.2 mg. of ozone per minute). The solvent was removed *in vacuo* below 30° and the residue taken up in 20 ml. of glacial acetic acid and debrominated by careful addition of 0.5 g. of zinc dust. The product was extracted with ether, washed with 5% sodium hydroxide and then water till neutral. After drying the ether solution over sodium sulfate the solvent was removed to yield 0.34 g. of oily crystalline material having a strong odor of benzaldehyde. Upon refluxing this residue with 30 ml. of methanol, 3 ml. of water, 0.4 g. of semicarbazide hydrochloride and 0.4 g. of sodium acetate for one hour, the semicarbazone of V precipitated. After cooling, filtering and washing with water and a little methanol, 240 mg. of the semicarbazone was obtained, m. p. 224–228°. Recrystallized from methanol–chloroform it melted at 235–237°. A mixed m. p. with a sample of semicarbazone made from an authentic sample of pregnene-3β-ol-20-one methyl ether prepared according to the method of Butenandt and Gross[6] showed no depression.

Anal. Calcd. for $C_{23}H_{37}O_2N_3$: N, 10.84. Found: N, 10.76.

Pregnene-3β-ol-20-one Methyl Ether (V).—240 mg. of the semicarbazone was taken up in 50 ml. of methanol and 25 ml. of 5 *N* sulfuric acid was added. After refluxing one-half hour and diluting with 125 ml. of water the prod-

(6) Butenandt and Gross, *Ber.*, **70**, 1448 (1937).

uct was extracted with ether. The ether solution was washed with water till neutral and dried over sodium sulfate. Upon removing the ether 192 mg. of nicely crystalline product was recovered, m. p. 118–121°. When recrystallized from a small volume of dilute acetone it melted at 124–125° and did not depress the m. p. of an authentic sample of V.

Anal. Calcd. for $C_{22}H_{34}O_2$: C, 79.94; H, 10.37. Found: C, 79.68; H, 10.45.

Pregnene-3β-ol-20-one Acetate (VI).—80 mg. of pregnene-3β-ol-20-one methyl ether and 40 mg. of *p*-toluene sulfonic acid monohydrate was covered with 4 ml. of redistilled acetic anhydride and heated on a steam cone with stirring for one-half hour. The mixture was cooled and poured into ice-water with stirring, and allowed to stand two hours. The product was extracted with ether, washed with sodium bicarbonate solution and water and dried over sodium sulfate. Upon removing the solvent and recrystallizing the residue three times from dilute methanol the product melted at 143–144° and did not depress the melting point of an authentic sample of VI.

Summary

1. The secondary carbinol, 22-phenyl-3-methoxy-22-hydroxy-bisnor-5-cholene (III), has been dehydrated in yields of 60–75% and the resulting diene (IV) has been degraded to give pregnene-3β-ol-20-one methyl ether (V).

2. The mono tosylate mentioned previously[1] has been shown to be 22-phenyl-3,22-dihydroxy-bisnor-5-cholene-3-tosylate (II).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLLEGE OF MEDICINE, NEW YORK UNIVERSITY]

A Method for the Determination of Organic Compounds in the Form of Isotopic Derivatives.[1,2] I. Estimation of Amino Acids by the Carrier Technique

BY ALBERT S. KESTON, SIDNEY UDENFRIEND[3] AND R. KEITH CANNAN

In a preliminary communication[4] we have outlined a general method for the estimation of organic compounds in the form of isotopically labelled derivatives. The method is of particular value for the estimation of the components of mixtures of related compounds for which individual chemical methods of analysis are not available. We have applied it to the estimation of certain of the amino acids which are present in hydrolysates of proteins.

The mixture is treated with a reagent containing a stable or a radioactive isotope under such conditions that the components which are to be estimated are quantitatively converted into derivatives of the reagent. If any one of these derivatives can be separated quantitatively from the others, it can then be directly estimated[5] with the precision and sensitivity characteristic of isotopic measurements. In the analysis of mixtures of amino acids, however—and, indeed, of most biological materials—the quantitative separation of a single component is not always attainable. In this situation, the isotopic method may be applied with the aid of carrier or indicator techniques.[4,5]

In the carrier method, an overwhelming excess, W moles, of the unlabelled derivative of the desired constituent is added to the mixture and is then separated and purified to constant molal isotope concentration, Cc. If Cr be the molal isotope concentration of a pure isotopic derivative which has been prepared with the same sample of the reagent, then the amount of the isotopic derivative (w) which was present in the mixture is

$$w = Cc(w + W)/Cr$$

When relatively large amounts of the carrier are added, this equation reduces to

$$w = WCc/Cr$$

The carrier principle gives the method wide scope and flexibility. It makes it possible to carry out estimations of very minute amounts of the compound of interest and yet to operate with milligram quantities of material. It is, moreover unnecessary to seek a quantitative recovery of the compound in pure form. What is essential is the rigorous purification of the carrier from significant amounts of isotopic impurities. To accomplish this large losses of the product may be accepted. Finally, in the analysis of optically active compounds, the use of a large excess of a racemic carrier ensures the estimation of the total of both of the active forms. This is a significant advantage in the analysis of proteins in which indeterminate degrees of racemization may have occurred during hydrolysis. If, on the other hand, one wishes to estimate only one of the isomers the corresponding carrier may be used.

The validity and precision of the method which has been outlined depend primarily on, (a) the completeness of the reaction between the isotopic reagent and the compound which is to be estimated, (b) the rigorous purification from radioactive contaminants of the carrier after addition to and isolation from the reaction mixture and (c) the precision of the measurements of Cc and Cr.

(1) Taken from a thesis submitted in December, 1947, by Sidney Udenfriend to the Faculty of the Graduate School in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Chemistry.

(2) This work was done under a grant from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council.

(3) Present address: Department of Biological Chemistry, Washington University School of Medicine, St. Louis, Mo.

(4) A. S. Keston, S. Udenfriend and R. K. Cannan, THIS JOURNAL, **68**, 1390 (1946).

(5) A. S. Keston, S. Udenfriend and M. Levy, *ibid.*, **69**, 3151 (1947).