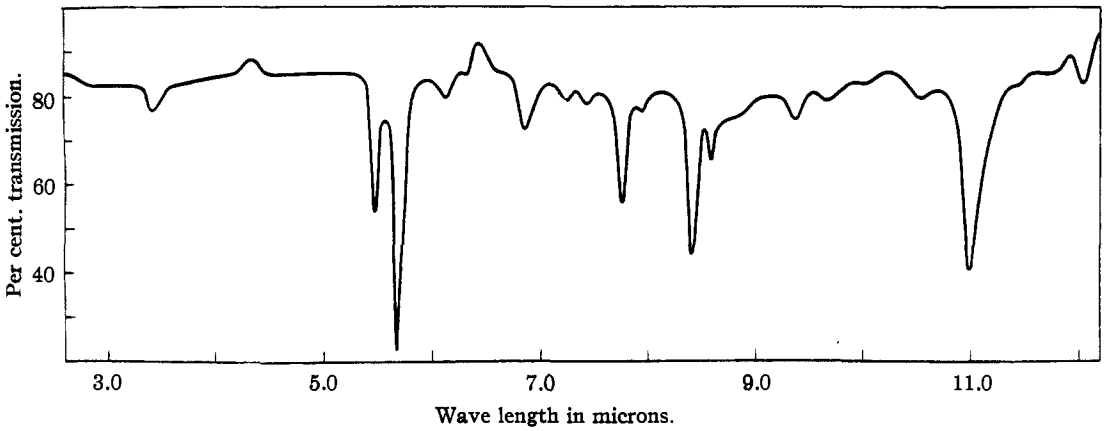


Fig. 3.—Infrared absorption spectrum of 3,5-diethylnaphthalene-1,2-dicarboxylic acid anhydride, 1.0% solution in carbon tetrachloride; absorption bands: 3.40μ, aliphatic carbon–hydrogen bond; 5.42 and 5.62μ, anhydride group.

nickel[9] added. (The ratio of Raney nickel to sulfur-containing anhydride was $10n$:1 by weight, where n = number of atoms of sulfur in the compound.) Desulfuration was carried out with vigorous stirring for five hours at the reflux temperature. Since the desulfurized product was found to be highly adsorbed on the finely divided nickel, Soxhlet extraction was employed in the isolation procedure. The nickel was allowed to settle, and the supernatant liquid was filtered through a Soxhlet thimble into the receiver of a Soxhlet extraction apparatus. The moist Raney nickel was transferred into the thimble with the aid of absolute ethanol and extracted for twenty-four hours. After evaporation of the solvent the residual monoester (a pale yellow solid) was dissolved in a solution of 2 g. of sodium hydroxide in 20 cc. of ethanol and 20 cc. of water, and the solution was refluxed for one-half hour. A small amount of inorganic matter was removed by filtration. Ethanol was completely removed by distillation at diminished pressure and the aqueous solution was heated for one hour at steam-bath temperature. Acidification with concentrated hydrochloric acid caused precipitation of the desulfurized anhydride, which exhibits a strong blue fluorescence in dilute ethanolic solution.

Compounds IV and VIa fail to decolorize dilute solutions of potassium permanganate in water and bromine in carbon tetrachloride.

(9) Prepared by the procedure of Mozingo, "Organic Syntheses," **21**, 15 (1941), and washed according to Pavlic and Adkins, THIS JOURNAL, **68**, 1471 (1946).

TABLE I

DESULFURIZED ANHYDRIDES

Compound	M. p., °C.[a]	Yield,[b] %	Formula	Analyses, % Calcd. C	Calcd. H	Found C	Found H
IV	153.5–158	49.7	$C_{14}H_{10}O_3$	74.33	4.46	74.14	4.54
V	159.5–162	39.0	$C_{16}H_{14}O_3$	75.57	5.55	75.85	5.56
VIa[c]	243.7–244.7	87.8	$C_{18}H_{12}O_3$	78.25	4.38	78.36	4.40
VIb[c]	214–216	67.4	$C_{18}H_{14}O_4$	74.50	4.67	74.25	4.60

[a] All desulfurized products crystallized as pale yellow needles from acetic acid. [b] Yields based on crude products. [c] Analytical sample dried for three hours at 110°; very hygroscopic.

Summary

1. The desulfuration reaction with Raney nickel has been applied to some aromatic sulfur-containing compounds.

2. A novel synthetic route is described for the preparation of certain naphthalene and phenanthrene derivatives.

CAMBRIDGE 38, MASSACHUSETTS RECEIVED JULY 29, 1949

[CONTRIBUTION FROM THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY]

Steroids Labeled with Isotopic Carbon: Cholestenone and Testosterone[1]

BY RICHARD B. TURNER*

Involvement of various endocrine secretions in the etiology of cancer has been recognized since 1916 when Lathrop and Loeb[2] observed that castration of female mice of certain strains results in a marked decrease in the incidence of spontaneous mammary cancer in these animals. Subsequently Lacassagne,[3] Gardiner,[4] and others found that prolonged administration of estrogens increases the incidence of neoplastic lesions of the uterus, testes, pituitary, adrenals and other organs, whereas in certain instances simultaneous administration of estrogen and testosterone propionate produces no such effect. Beneficial results of

* Harvard University Ph.D. 1942.

(1) This work was supported by funds provided by the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council. For preliminary reports see Turner, THIS JOURNAL, **69**, 726 (1947); *Science*, **106**, 248 (1947).

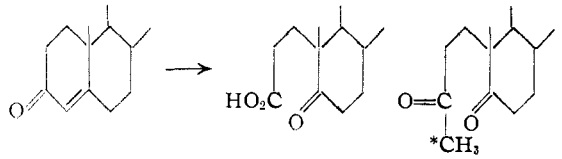
(2) Lathrop and Loeb, *J. Cancer Research*, **1**, 1 (1916).

(3) Lacassagne, *Compt. rend.*, **195**, 630 (1932); "Ergebnisse der Vitamin- und Hormonforschung," Vol. II, p. 258, Mellanby and Ruzicka, Academische Verlagagesellshaft, Leipzig, 1939.

(4) Gardiner, "Recent Progress in Hormone Research," Vol. I, Pincus, Academic Press, New York, N. Y., 1947, p. 217.

orchiectomy and of estrogen treatment in cases of prostatic malignancy have been noted by Huggins.[5] Heilman and Kendall[6] have observed the dramatic regression of a lymphosarcoma in mice treated with cortisone. A systematic study of steroid metabolism, with special reference to malignant growth, was begun in 1940 by Dobriner and his collaborators and has provided important additional information on the relation of endocrine disturbances to cancer.[7] Steroids labeled in the nucleus with isotopes of carbon are of obvious interest in connection with this work, and the synthesis of such compounds was accordingly undertaken in this Laboratory. Cholestenone (I), which contains the α,β-unsaturated carbonyl system characteristic of the principal active steroid hormones, other than estrone and estradiol, was chosen as a model for preliminary work.

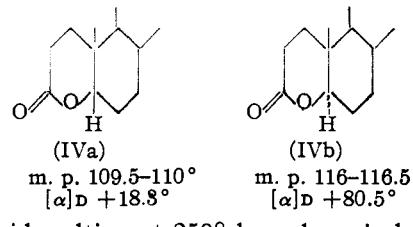
The plan originally devised for introduction of isotopic carbon involved oxidation of cholestenone to keto acid II, conversion of II (by treatment of the corresponding acid chloride with labeled diazomethane or dimethylcadmium) into a methyl ketone (III), and cyclization of the latter product.



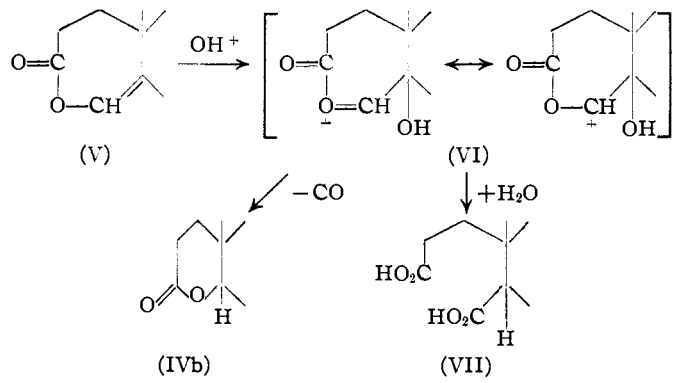
(I) (II) (III)

The first step in this series was carried out in 1906 by Windaus,[8] who obtained II in 20% yield by oxidation of cholestenone with potassium permanganate. Bolt[9] has reported that ozonization of cholestenone affords a 60% yield of the keto acid. Although yields no higher than 40% were realized in this Laboratory by repetition of Bolt's procedure, 80% of the theoretical amount of pure keto acid could be obtained consistently by the expedients of (a) hydrogen peroxide oxidation of the decomposed ozonide and (b) treatment of the residual neutral fraction with periodic acid.

The keto acid (II) furnishes an oily methyl ester when treated with diazomethane; on catalytic hydrogenation both acid and ester yield a mixture of the epimeric lactones IVa and IVb.[10] A substance of structure IV, m. p. 111°, and an unidentified acid melting at 250° have been isolated by Salamon[11] from the reaction of cholestenone with persulfuric acid. We have repeated Salamon's experiments and found that the neutral oxidation product, after thorough purification, melts at 116° and is identical with lactone IVb. The acidic material was characterized as dihydro Diels' acid (VII) by comparison of the free acid and of the dimethyl ester with authentic samples. Conversion of cholestenone into these products can be explained in terms of the initial formation of an unsaturated lactone (V).[12] This substance on further reaction with peracid can afford an intermediate VI, capable of transformation into IVb and into VII by simple electronic displacements accompanied by loss of carbon monoxide and by addition of water, respectively.

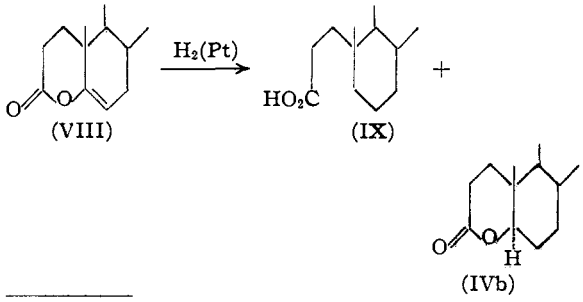


(IVa) (IVb)
m. p. 109.5–110° m. p. 116–116.5°
$[\alpha]_D$ +18.8° $[\alpha]_D$ +80.5°



(V) (VI) (IVb) (VII)

When keto acid II is treated with acetic anhydride and acetyl chloride,[13] a product is obtained to which structure VIII is assigned on the basis of the following evidence. Hydrolysis proceeds rapidly with quantitative regeneration of the keto acid. On catalytic hydrogenation VIII absorbs



(VIII) (IX) (IVb)

(5) Huggins and Hodges, *Cancer Research*, **1**, 293 (1941); Huggins, *J. Am. Med. Assoc.*, **131**, 576 (1946).

(6) Heilman and Kendall, *Endocrinology*, **34**, 416 (1944).

(7) A summary of this work has recently been published by Dobriner, *Acta de l'Union Internationale Contre le Cancer*, **6**, 315 (1948).

(8) Windaus, *Ber.*, **39**, 2008 (1906).

(9) Bolt, *Rec. trav. chim.*, **57**, 905 (1938); *cf.* Dorée and Gardiner, *J. Chem. Soc.*, **93**, 1328 (1908).

(10) Configurations provisionally assigned to the two lactones are based on correlation of the specific rotations of these substances with those of the corresponding carbocyclic compounds, coprostanone and cholestanone, and on the formation of IVb from enol-lactone VIII (below) by catalytic hydrogenation (*cf.* hydrogenation of cholesterol).

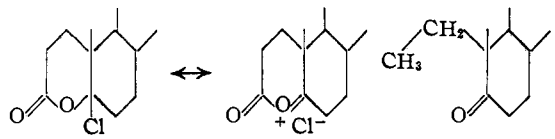
(11) Salamon, *Z. physiol. Chem.*, **272**, 61 (1941).

(12) *Cf.* Burckhardt and Reichstein, *Helv. Chim. Acta*, **25**, 1434 (1942); Böeseken and co-workers, *Rec. trav. chim.*, **50**, 827 (1931); *ibid.*, **52**, 874 (1933); *ibid.*, **55**, 786 (1936).

(13) *Cf.* Jacobs and Gustus, *J. Biol. Chem.*, **34**, 187 (1929); *ibid.*, **92**, 337 (1931).

1.4 molar equivalents of hydrogen and yields a mixture, from which two crystalline products, one acidic and the other neutral, can be isolated. The acidic product melted at 134–135.5° and proved identical with the desoxo acid (IX) obtained by Clemmensen reduction of II.[14] Identity of the neutral material, m. p. 114–116°, with lactone IVb was established by a mixed melting point determination and by comparison of infrared absorption curves of the two samples. A mixed melting point with lactone IVa was depressed to 85–90°.

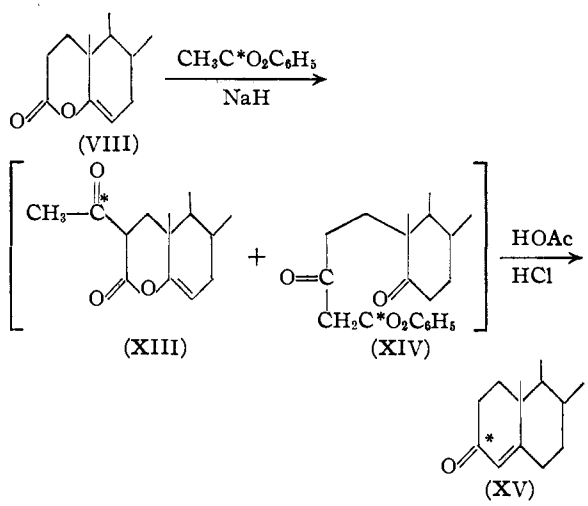
Attempts to prepare methyl ketone III from the keto acid (II) by way of the acid chloride were unsuccessful. When II, or the corresponding sodium salt, was treated with thionyl chloride in benzene solution, a crystalline product was obtained, the composition of which corresponds to that of the expected acid chloride. The substance, however, was recovered unchanged after standing overnight with a large excess of anhydrous diazomethane and could not be reduced with palladium-on-barium sulfate under conditions of the Rosenmund procedure. Hydrolysis with aqueous alcohol gave back the keto acid, whereas treatment with pyridine furnished enol-lactone VIII, though in poor yield. Examination of the infrared spectrum of the product revealed strong absorption at 5.65 μ (lactone C=O). A band of lower intensity occurs at 13.3 μ and may perhaps be attributed to C–Cl stretching. No bands were observed at 5.55 μ (acid chloride C=O) or in the region 5.7–6.0 μ (ketone C=O). These facts suggest a pseudo acid chloride structure (X) for this compound.[15]



(X) (XI) (XII)

Pyrolysis of the barium salt of keto acid II in the presence of barium acetate likewise failed to yield either methyl ketone III or cholestenone. Under these conditions the decarboxylated compound (XII) was the only isolable product.

A successful synthesis leading directly to cholestenone was achieved in the following way. The enol-lactone (VIII) was condensed with phenyl acetate in benzene solution in the presence of sodium hydride. Hydrolysis, decarboxylation and cyclization were effected simultaneously by treatment of the crude condensation product with dilute alkali or with concentrated hydrochloric acid in acetic acid. The neutral fraction was then separated and purified by chromatographic adsorption on alumina. The yield of cholestenone obtained by this procedure varied somewhat with the proportions of reactants as illustrated in Table I; the use of a large excess of phenyl acetate appeared to offer no advantage.



(VIII) (XIII) (XIV) (XV)

TABLE I

YIELD OF CHOLESTENONE

Enol-lactone, mmoles.	Phenyl acetate, mmoles.	NaH, mmoles.	% yield[a]
1.57	1.58	3.12	36
1.22	1.83	3.66	46
1.73	3.48	5.21	50
1.43	4.28	5.71	53

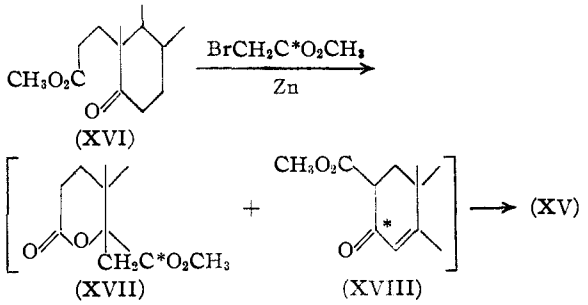
[a] Calculated on the basis of enol-lactone employed.

Both intermediates XIII and XIV can clearly yield cholestenone, and in either case the methyl-carbon of phenyl acetate will occupy position 4 in ring A. The carbonyl group of phenyl acetate, however, will be incorporated only by reaction of phenyl acetate with the enol-lactone anion to give XIII. The use of carboxyl-labeled phenyl acetate thus provides a method for distinguishing between the two reaction paths. This reagent was prepared by treatment of phenol with acetyl chloride containing C^{14} in the carbonyl group and possessed an activity of 4.20×10^4 counts/min./mmole. Condensation with VIII gave cholestenone-3-C^{14} with an activity of 3.79×10^4 counts/min./mmole. Hence, about 90% of the total cholestenone must be derived from intermediate XIII. Experiments with methyl-labeled phenyl acetate, prepared from radioactive methyl iodide by standard procedures, confirmed the hypothesis that the methyl group of phenyl acetate is the exclusive source of carbon atom 4 of the steroid. The ratio of the molar activity of cholestenone-4-C^{14} to that of the methyl-labeled phenyl acetate from which it was obtained was 1.04.

An alternate method for the synthesis of radioactive cholestenone is outlined in the accompanying chart. Keto acid II was converted into the corresponding methyl ester (XVI), which was then treated with zinc and methyl bromoacetate.

(14) Diels, Gädke and Körding, *Ann.*, **459**, 22 (1927); Tschesche, *ibid.*, **498**, 185 (1932).

(15) Displacement of the lactone carbonyl absorption from the normal position (5.73μ) observed in lactone IVb to shorter wave lengths is apparently the result of a relatively large contribution of form XI, which increases the double bond character of the carbonyl group. An analogous case has been described by Woodward and Kovach, THIS JOURNAL, **72**, Feb. (1950).



Direct acid hydrolysis of the crude Reformatsky product afforded considerable quantities of cholestenone, presumably derived from an intermediate of structure XVIII. Better yields resulted when conventional methods of cyclization, hydrolysis and decarboxylation were applied.[16] In the latter procedure two molar equivalents of methyl bromoacetate and one of keto ester gave cholestenone in 47% yield, based upon keto ester and corrected for recovered starting material. The yield referred to methyl bromoacetate, however, was only about 15%.

For incorporation of radioactive carbon carboxyl-labeled methyl bromoacetate was prepared by bromination of appropriately marked acetyl chloride followed by treatment of the resulting bromo acid chloride with anhydrous methanol.[17] The product possessed an activity of 4630 counts/min./mmole. and gave cholestenone with an activity of 4540 counts/min./mmole. Thus, within the limits of experimental error, all the cholestenone is produced by a cyclization involving the 2 methylene group of the original steroid and the carbonyl group of the methyl acetate residue. This conclusion accords with the fact that such a closure in the lactonic ester intermediate XVII[18] involves a 6-membered ring, whereas cyclization in the opposite sense (with resultant loss of activity) would involve a 4-membered ring intermediate.

The procedure already described for introduction of isotopic carbon by condensation with labeled phenyl acetate has been successfully extended to the preparation of marked testosterone. Ozonization of testosterone afforded a keto acid (*cf.* II), m. p. 147–148°, which on reaction with acetic anhydride and acetyl chloride yielded the corresponding enol-lactone, m. p. 202–202.5°. Condensation with carboxyl-labeled phenyl acetate (1.76×10^4 counts/min./mmole.) was carried out as described before. Although cyclization of the crude condensation product with hydrochloric acid and acetic acid gave poor results owing to the sensitivity of the 17-hydroxyl group to strong acids, no difficulty was experienced when dilute methanolic alkali was employed. The neutral material obtained in this way, after purification by chromatography, furnished testosterone-3-C^{14} with an activity of 1.45×10^4 counts/min./mmole. These results indicate that 82% of the testosterone is derived from an acetyl enol-lactone intermediate corresponding to XIII.

In addition to these experiments methyl iodide containing 56 atom % excess C^{13} was converted into phenyl acetate, which was employed in the synthesis of testosterone-4-C^{13} (2.73 atom % excess C^{13}; theoretical value, 2.94). Samples of both testosterone-4-C^{13} and testosterone-4-C^{14} have been submitted for use in physiological studies. The results of this work will be published elsewhere.

Acknowledgment.—The author is indebted to Mrs. Dorothy M. Voitle for technical assistance and to Drs. Warren W. Miller, Robert B. Loftfield, George B. Brown and T. F. Gallagher for isotope determinations. Radioactive methyl iodide was kindly furnished by Dr. M. Calvin and Dr. B. M. Tolbert; generous gifts of testosterone were made available through the courtesy of Dr. Caesar Scholz, Ciba Pharmaceutical Products Inc. and of Dr. Erwin Schwenk, Schering Corporation. Microanalyses were performed by Mr. S. M. Nagy of M. I. T.

Experimental[19]

Ozonization of Cholestenone.—A solution of 2.00 g. of cholestenone in 30 ml. of ethyl acetate and 30 ml. of glacial acetic acid was cooled to −10° in a salt-ice-bath. Two molar equivalents of ozone were passed into the solution, and after addition of 10 ml. of water and 1 ml. of 30% hydrogen peroxide the mixture was allowed to stand at room temperature for eighteen hours. A large quantity of ether was then added, and the acetic acid was removed by thorough washing with water. The ethereal solution was extracted 4 times with dilute sodium hydroxide solution, and the alkaline extracts were combined and acidified with dilute hydrochloric acid. The precipitated product was finally taken up in ether, washed with water and a saturated solution of sodium chloride, filtered through anhydrous sodium sulfate, and concentrated to small volume. Dilution with petroleum ether gave 1.66 g. of material melting at 151.5–152.5°. A second crop, 0.06 g., m. p. 147–149°, was obtained from the mother liquor.

The neutral fraction obtained after alkaline extraction (0.24 g.) was dissolved in 15 ml. of acetic acid and treated with a solution of 0.20 g. of periodic acid dihydrate in 5 ml. of 80% acetic acid. After standing at room temperature for two hours, the mixture was diluted with ether and washed with a large volume of water. The acidic product was isolated by alkaline extraction, and after crystallization from ether–petroleum ether furnished an additional 0.12 g. of keto acid, m. p. 137–142°. This fraction was combined with crop II above and on recrystallization from ether–petroleum ether gave 0.15 g. of product, m. p. 151–153°. The total yield of material melting above 151° was 1.81 g. (85%). Recrystallization from ether–petroleum ether gave a pure product, m. p. 154–154.5°, $[\alpha]_D$ + 34° (chloroform).

Hydrogenation of Keto Acid II.—The keto acid (3.00 g.) was dissolved in 20 ml. of acetic acid and hydrogenated in the presence of 1.00 g. of platinum oxide catalyst. At the end of forty-eight hours 96% of the theoretical amount

(16) *Cf.* Bachmann, Kushner and Stevenson, THIS JOURNAL, **64**, 974 (1942).

(17) Olsen, Hemingway and Nier, *J. Biol. Chem.*, **148**, 611 (1943).

(18) Compound XVII has been isolated from the Reformatsky reaction by R. D. H. Heard of McGill University. In view of Dr. Heard's interest in this problem, we have not undertaken investigation of any intermediate products of this reaction.

(19) All melting points are corrected.

of hydrogen had been absorbed. The catalyst was removed by filtration, and the filtrate was diluted with ether and washed with water and dilute sodium hydroxide solution. Only a faint turbidity was observed when the alkaline wash liquors were acidified. The ethereal solution of the reduction product was dried over anhydrous sodium sulfate, the ether was removed on the steam-bath, and the residue fractionally crystallized from petroleum ether. The following fractions were obtained: I, 810 mg., m. p. 107–108.5°; II, 120 mg., m. p. 100–104°; III, 700 mg., m. p. 68–76°; IV, 230 mg., m. p. 79–85°.

Lactone IVa.—This compound was obtained in pure form from fraction I by several recrystallizations from dilute methanol. The analytical sample melted at 109.5–110°, $[\alpha]D$ + 18.3° (chloroform).

Anal. Calcd. for $C_{26}H_{42}O_2$: C, 80.36; H, 11.41. Found: C, 80.40; H, 11.57.

Lactone IVb.—Fraction IV, after several recrystallizations from petroleum ether and from acetone, afforded a pure sample of lactone IVb, m. p. 116–116.5°, $[\alpha]D$ + 80.5° (chloroform).

Anal. Calcd. for $C_{26}H_{44}O_2$: C, 80.36; H, 11.41. Found: C, 80.17; H, 11.25.

The melting point of a mixture of the two lactones was depressed to 83–89°. Analogous results were obtained when the hydrogenation was carried out on the methyl ester (diazomethane) of II.

Preparation of Enol-lactone VIII.—A solution of 2.00 g. of keto acid II in 25 ml. of acetic anhydride (distilled from fused sodium acetate) and 10 ml. of freshly distilled acetyl chloride was heated to boiling under reflux for forty-four hours. The solvents were removed under reduced pressure, and the residue was dissolved in ether and washed successively with dilute sodium carbonate solution, water, and a saturated solution of sodium chloride. After filtering through anhydrous sodium sulfate, the solution was evaporated to dryness, and the residue was crystallized from dilute acetone. The product weighed 1.68 g. and melted at 92–93.5°. An additional 0.08 g., m. p. 90–92°, was obtained from the mother liquor; total crude yield, 92%.

The analytical sample was obtained as needles after 3 recrystallizations from dilute acetone, m. p. 94–94.5°, $[\alpha]D$ − 51° (chloroform).

Anal. Calcd. for $C_{26}H_{42}O_2$: C, 80.77; H, 10.95. Found: C, 81.00; H, 10.89.

Hydrolysis.—The enol-lactone (100 mg.) was suspended in 15 ml. of 4% sodium hydroxide and heated on the steam-bath until solution was complete. The product isolated by acidification and ether extraction weighed 104 mg. and melted at 152–153°. No melting point depression was observed when the substance was mixed with a sample of keto acid II.

Hydrogenation.—A solution of 387 mg. of enol-lactone in 2 ml. of acetic acid was stirred in an atmosphere of hydrogen in the presence of 100 mg. of prereduced platinum oxide catalyst. After about thirty minutes 1.4 molar equivalents of hydrogen had been absorbed, and the reaction was complete. The catalyst was removed by filtration, and the reduction product separated into acidic and neutral fractions by extraction of an ether solution with alkali.

The acidic fraction (150 mg.) was crystallized from acetone and from dilute acetone and gave a purified product melting at 134–135.5°, $[\alpha]D$ + 24.8° (chloroform). The material did not depress the melting point of a sample of the desoxo acid (m. p. 137.5–138°) of Diels, Gädke and Körding.[14]

The neutral material (228 mg.) was crystallized from petroleum ether and furnished 125 mg. of a product melting at 114–116°. A mixed melting point determination with lactone IVb showed no depression; the melting point of a mixture with lactone IVa was 85–90°.

Reaction of Keto Acid II with Thionyl Chloride.—A solution of 3.00 g. of keto acid in 10 ml. of dry benzene was cooled in an ice-bath and treated with 3 ml. of thionyl chloride, purified by the method described by Fieser.[20] After thirty hours at 5°, the solvent was removed under reduced pressure, and the product was triturated with petroleum ether and collected by filtration; yield 1.30 g. (42%), m. p. 136–137° (dec.). The analytical sample was prepared by recrystallization from methylene chloride–petroleum ether, m. p. 141–142° (dec.), $[\alpha]D$ + 30.9° (chloroform).

Anal. Calcd. for $C_{26}H_{43}O_2Cl$: C, 73.81; H, 10.27; Cl, 8.38. Found: C, 73.93; H, 10.46; Cl, 8.44.

Hydrolysis.—A small sample of pseudo acid chloride (X) was dissolved in dilute ethanol and heated on the steam-bath for two hours. The product obtained after crystallization from ether–petroleum ether melted at 152–153° and did not depress the melting point of a sample of keto acid II.

Reaction with Pyridine.—A solution of 150 mg. of pseudo acid chloride in 5 ml. of dry pyridine was heated under reflux for three hours. The mixture was then cooled, diluted with ether, and washed successively with water, dilute hydrochloric acid, water, dilute sodium hydroxide solution and saturated sodium chloride. After drying over anhydrous sodium sulfate, the solution was evaporated to dryness, and the residue crystallized from dilute acetone. The product melted at 93–94° and did not depress the melting point of a sample of enol-lactone VIII.

Pyrolysis of Barium Salt of Keto Acid II.—The keto acid (2.00 g., 4.92 mmoles.) was dissolved in 6 ml. of 1.0 *N* methanolic sodium methoxide and diluted with 25 ml. of water. An aqueous solution of 2.00 g. (excess) of barium hydroxide octahydrate was then added, and the resulting insoluble barium salt (2.32 g.) collected and dried in vacuum at 130°. The dry salt (1.00 g.) was ground in a mortar with 3.00 g. of barium acetate, and the mixture was heated to 300–330° at 0.01 mm. pressure. The oily material that distilled was purified by chromatographic adsorption on 15 g. of alumina, followed by crystallization from dilute acetone; yield 381 mg. (45%), m. p. 51.5–53°. The analytical sample was obtained by recrystallization from dilute acetone and melted at 55–55.5°, $[\alpha]D$ + 9.5° (chloroform).

Anal. Calcd. for $C_{25}H_{44}O$: C, 83.26; H, 12.30. Found: C, 83.40; H, 12.17.

The oxime, prepared as a derivative, was crystallized from ethanol and melted at 142.5–143°.

Anal. Calcd. for $C_{25}H_{45}ON$: C, 79.94; H, 12.07; N, 3.73. Found: C, 79.66; H, 11.82; N, 4.07.

Preparation of Cholestenone-3-C^{14}. Procedure A.—Sodium hydride (133 mg., 5.54 mmoles.), enol-lactone VIII (715 mg., 1.85 mmoles.) and carboxyl-labeled phenyl acetate (500 mg., 3.67 mmoles.), prepared as described below, were weighed into a flask containing 3.00 ml. of dry benzene. The flask was then attached to a buret system filled with dry nitrogen, and the contents were stirred magnetically at room temperature. After forty-two hours, evolution of hydrogen had ceased (122 ml.). The mixture was acidified with dilute hydrochloric acid and extracted with ether. The ether extract was washed with water and a saturated solution of sodium chloride, filtered through anhydrous sodium sulfate and concentrated under reduced pressure. Volatile products (phenol, phenyl acetoacetate, etc.) were then removed at 100° and 0.01 mm. pressure.

The oily residue obtained in this way was refluxed with 25 ml. of acetic acid and 3 ml. of concentrated hydrochloric acid for twenty-seven hours in a slow stream of nitrogen. The liberated carbon dioxide was collected in carbonate-free alkali and precipitated by the addition of barium chloride solution. The yield of barium carbonate was 238 mg. (1.21 mmoles.). After cooling, the reaction mixture was diluted with water and extracted several times with ether. The resulting ether extracts were combined, washed with water, dilute sodium hydroxide, saturated sodium chloride solution, filtered through anhy-

(20) Fieser, "Experiments in Organic Chemistry," 2nd ed., D. C. Heath and Co., Boston, Mass., 1941, p. 381.

drous sodium sulfate and evaporated to dryness. The residual oil was finally chromatographed on 12.0 g. of alumina and furnished 362 mg. (51%) of material melting at 78–80°. Recrystallization from acetone gave a pure sample, m. p. 80–80.6°, $[\alpha]D$ + 85.5° (chloroform), $\lambda_{max.}$ 241 mμ (log ε 4.22). A mixed melting point with an authentic sample of cholestenone showed no depression.

When methyl-labeled phenyl acetate (4.69 × 10^6 counts/min./mmole.) was employed in this procedure, cholestenone-4-C^{14}, 4.89 × 10^5 counts/min./mmole., was obtained.

Procedure B.—Keto acid II (1.00 g., 2.46 mmoles.) was converted into the corresponding methyl ester by treatment with an ethereal solution of diazomethane. The ester did not crystallize and was dissolved directly in a mixture of 10 ml. of anhydrous benzene and 10 ml. of dry ether. To this solution there were added 0.47 ml. (2.03 equivalents) of carboxyl-labeled methyl bromoacetate, 2.00 g. of granulated zinc activated by the procedure of Fieser and Johnson,[21] and a small crystal of iodine. The mixture was then refluxed with stirring in an atmosphere of dry nitrogen; the reaction commenced after about thirty minutes, and 1.00-g. portions of zinc were added at forty-five-minute intervals until 5 additions had been made.

After standing overnight at room temperature, the solution was decanted, and the excess zinc was washed several times with benzene and with ether containing small amounts of acetic acid. The organic fractions were combined, washed with water, dried over anhydrous sodium sulfate and concentrated to dryness. The residue was then dissolved in 10 ml. of dry benzene, 1.00 g. of calcium chloride was added, and a stream of dry hydrogen chloride was passed through the solution for twenty minutes.[16] At the end of this time water was added, and the benzene layer was separated, washed with water, dried and concentrated. The material obtained in this way was redissolved in 5 ml. of dry benzene. Sodium hydride (120 mg., 5.00 mmoles.) was added, the flask was connected to a buret system filled with dry nitrogen, and the reaction mixture was stirred magnetically at room temperature. At the end of forty-eight hours evolution of hydrogen had ceased. The cyclization product was isolated by acidification and ether extraction, and after refluxing for twenty-two hours with 25 ml. of acetic acid and 5 ml. of concentrated hydrochloric acid (nitrogen), the material was separated into acidic and neutral fractions.

Crystallization of the acidic product from ether–petroleum ether afforded 335 mg. (0.82 mmole.) of a substance, m. p. 149–150°, that was identified as keto acid II by comparison with an authentic sample. The neutral fraction was chromatographed on alumina and furnished 298 mg. (0.78 mmole.) of crude cholestenone, m. p. 75–77°. A pure sample melting at 79–80° was obtained by recrystallization from acetone.

In a separate experiment on the same scale, in which inactive methyl bromoacetate was employed, the crude Reformatsky product was treated directly with hot acetic acid and hydrochloric acid in an atmosphere of nitrogen. After refluxing for twenty-four hours, the reaction mixture yielded 420 mg. (1.03 mmoles.) of keto acid II, m. p. 149–151°, and 195 mg. (0.51 mmole.) of crude cholestenone, m. p. 75–77°.

Ozonization of Testosterone Benzoate.—Testosterone benzoate (2.25 g.) was ozonized (3 molar equivalents) at −10° in a mixture of 30 ml. of ethyl acetate and 30 ml. of acetic acid. Water (10 ml.) and 30% hydrogen peroxide (1.5 ml.) were added, and the solution was allowed to stand overnight at room temperature. The mixture was then diluted with a large volume of water and extracted with ether. The acidic product was isolated by extraction with successive portions of cold 1% sodium hydroxide, which were immediately acidified with dilute hydrochloric acid to prevent hydrolysis of the benzoate group. Additional acidic material could be obtained by treatment of the residual neutral fraction with periodic acid as described previously. The total oily acid obtained in this way was crystallized 3 times from dilute methanol and gave 1.53 g. (65%) of keto acid melting at 143–146°. Further recrystallization from dilute methanol furnished a pure sample, m. p. 147–148°, $[\alpha]D$ + 79° (chloroform).

Anal. Calcd. for $C_{25}H_{32}O_5$: C, 72.79; H, 7.82. Found: C, 72.83; H, 7.84.

Preparation of the Enol-lactone.—The keto acid (1.28 g.) obtained in the preceding experiment was refluxed for forty-eight hours with 10 ml. of acetic anhydride (distilled from fused sodium acetate) and 10 ml. of freshly distilled acetyl chloride. The solvents were removed under reduced pressure, and the crystalline residue was dissolved in methylene chloride and ether, washed with dilute sodium hydroxide, water and a saturated solution of sodium chloride, filtered through anhydrous sodium sulfate and concentrated to small volume. Dilution with petroleum ether gave 975 mg. (80%) of glistening plates, m. p. 199–200°. Several recrystallizations from methylene chloride–petroleum ether yielded an analytical sample melting at 202–202.5°, $[\alpha]D$ −19° (chloroform).

Anal. Calcd. for $C_{25}H_{36}O_4$: C, 76.11; H, 7.66. Found: C, 75.98; H, 7.74.

Preparation of Testosterone-3-C^{14}.—Condensation of the enol-lactone derived from testosterone benzoate with carboxyl-labeled phenyl acetate was effected by essentially the same procedure as was used for enol-lactone VIII. Sodium hydride (150 mg., 6.25 mmoles.), phenyl acetate (540 mg., 3.98 mmoles.), enol-lactone (790 mg., 2.00 mmoles.) and 8 ml. of anhydrous benzene were stirred in an atmosphere of dry nitrogen for four days, at the end of which time no further hydrogen was evolved. After acidification with dilute hydrochloric acid, the condensation product was extracted with ether and methylene chloride, washed, dried and finally concentrated. The volatile material was removed at the oil-pump, and the residue was refluxed under nitrogen with 1.00 g. of potassium hydroxide in 100 ml. of 75% aqueous methanol for eighteen hours. The solution was then acidified with acetic acid and evaporated to dryness under diminished pressure.

The crude product was taken up in ether and washed with dilute sodium hydroxide solution. The residual neutral material, after chromatography on alumina, furnished 275 mg. (48% based on enol-lactone) of a compound melting at 148–150°. Crystallization from ether–petroleum ether gave a pure sample, m. p. 153–154°, $[\alpha]D$ + 110 (chloroform), $\lambda_{max.}$ 241 mμ (log ε 4.21), that did not depress the melting point of an authentic specimen of testosterone. Testosterone-3-C^{14} acetate, m. p. 139–140°, was prepared as a derivative.

The same procedure was employed for the preparation of testosterone-4-C^{14} and testosterone-4-C^{13} from the appropriate methyl-labeled phenyl acetates.

Preparation of Labeled Phenyl Acetate.—(a) Carboxyl-labeled sodium acetate (2.85 g.) prepared by the method of Sakami, Evans and Gurin[22] was covered with 10 ml. of dry benzene and treated with 5.0 g. (1.2 equivalents) of thionyl chloride in 5.0 ml. of dry benzene. The thionyl chloride solution was added slowly, and the reaction mixture was cooled in ice. Evolution of sulfur dioxide subsided after about ten minutes. The ice-bath was then removed, and the mixture was allowed to stand at room temperature for one and one-half hours. Phenol (5.0 g.) was added, and the solution was heated to boiling under an efficient reflux condenser for four and one-half hours. The reaction mixture was cooled, diluted with water and extracted with ether. The ether phase was washed with dilute sodium hydroxide, dried and fractionated. The yield of material boiling at 82.5–83° (14 mm.) was 4.32 g. (91%); $n^{25}D$ 1.5038.

(b) **Methyl-labeled sodium acetate** was prepared from radioactive methyl iodide by carbonation of the corresponding Grignard reagent with inactive carbon dioxide.[22] The dry salt was then converted into phenyl acetate by the

(21) Fieser and Johnson, THIS JOURNAL, **62**, 575 (1940).

(22) Sakami, Evans and Gurin, *ibid.*, **69**, 1110 (1947); Ruben, Allen and Nahinsky, *ibid.*, **64**, 3050 (1942).

method described in procedure (a). Methyl-labeled phenyl acetate was prepared from $C^{13}H_3I$ in the same way.

Persulfuric Acid Oxidation of Cholestenone.—Cholestenone (8.8 g.) in 350 ml. of glacial acetic acid was treated with a solution of 26.4 g. of potassium persulfate and 29.0 g. of 96% sulfuric acid as described by Salamon.[11] The mixture was diluted with 530 ml. of acetic acid and allowed to stand at room temperature for one week with frequent shaking.

The sulfuric acid was neutralized by the addition of 50% potassium hydroxide, the salts were removed by filtration, and the filtrate concentrated to small volume. The crude oxidation product was then separated into acidic and neutral fractions by alkaline extraction from ether.

Crystallization of the neutral material from dilute acetone gave 2.25 g. of a product, m. p. 114–116°, that did not depress the melting point of a sample of lactone IVb. The acidic fraction was esterified with diazomethane and chromatographed on alumina. The early eluates furnished 450 mg. of crystalline material melting at 119–123°, which on repeated crystallization from ether gave a pure substance, m. p. 124.5–125.5°, identical in all respects with dihydro Diels ester.[23] Alkaline hydrolysis of the dimethyl ester afforded the free acid, m. p. 252–253°, identical with dihydro Diels acid (VII).

Summary

Two methods have been devised for the introduction of isotopic carbon in ring A of steroids. The best procedure involves condensation of an unsaturated ring A lactone with phenyl acetate and has been applied to syntheses of cholestenone-3-C^{14}, testosterone-3-C^{14}, testosterone-4-C^{14} and testosterone-4-C^{13}.

(23) Windaus, *Ber.*, **52**, 170 (1919).

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3-Azabenzanthrone Dyes[1]

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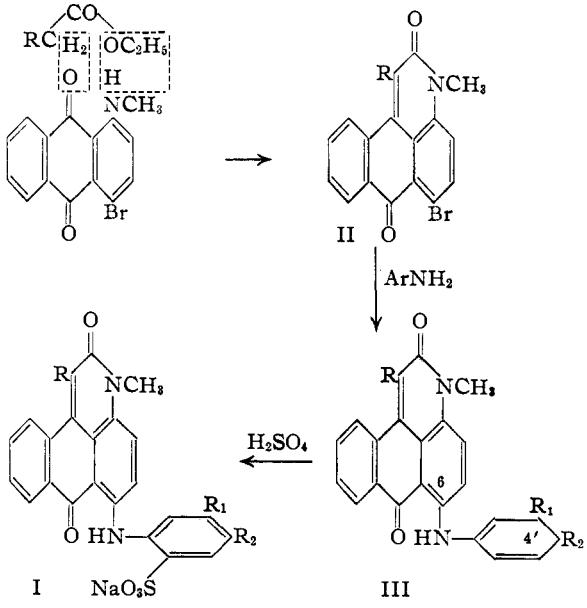
The longest-known representative of dyes containing the 3-azabenzanthrone nucleus is Alizarin Rubinol R (I, R, R_1 = H, R_2 = CH_3),[5,6] but there are four other Alizarin Rubinols[6]; the entire series is characterized by the absence of a substituent in position 1 (R = H). Two other commercial dyes, belonging to the Brilliant Alizarin Light Red Class, have a substituent group in this position. In order to learn the effect of a variety of substituent groups at several positions in the molecule on light absorption in the visible portion of the spectrum, a considerable number of dyes has been prepared.

Most of these dyes have arylamino groups in the 6-position, with different substituents in position 1. The reactions used in their preparation are largely found in the patent literature.[7–13]

Substances of the general formula II, in which R = C_2H_5OOC, CH_3CO and C_6H_5CO, were prepared by treating 4-bromo-1-methylaminoanthraquinone with ethyl malonate,[7] with ethyl acetoacetate[7,14] and with ethyl benzoylacetate,[7] respectively; while substance II, R = C_6H_5, was formed by cyclization of 4-bromo-1-phenylacetylaminoanthraquinone. Substance II, R = CN, was formed by the action of potassium cyanide on 4-bromo-1-(N-chloroacetyl-N-methylamino)-anthraquinone; in the patent literature an alternative procedure, which starts with ethyl cyanoacetate, is given.[13]



The bromoazabenzanthrone (II) was then condensed with the appropriate aromatic amine[8,13] to give the "dye base" (III), which was sulfonated[9,13] to form the dye, I. Care was taken to use carefully purified intermediates throughout; most of the dye bases (III) were not sulfonated until they had been recrystallized enough

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(1) The modern aza nomenclature has been adopted on account of its marked superiority over the older naming systems. In the previous paper[2] the substances were called "Anthrapyridones."

(2) Allen and Wilson, *J. Org. Chem.*, **6**, 594 (1945).

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(5) "Colour Index," 1st Edition (1924), No. 1091.

(6) Fierz-David, "Künstliche Organische Farbstoffe," Verlag von Julius Springer, Berlin, 1926, p. 624.

(7) German Patent 578,995; *Frdl.*, **19**, 1964 (1934).

(8) German Patent 580,283; *ibid.*, **19**, 1969 (1934).

(9) German Patent 581,161; *ibid.*, **20**, 1335 (1935).

(10) German Patent 633,308; *C. A.*, **30**, 7870 (1936).

(11) German Patent 658,114; *ibid.*, **32**, 4798 (1938).

(12) French Patent 837,591; *Chem. Zentr.*, **110**, Part 2, 531 (1939).

(13) German Patents 655,650, 655,651; *ibid.*, **109**, Part 1, 3700 (1938).

(14) U. S. Patent 1,891,317; *C. A.*, **27**, 1892 (1933).