[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

Steroid Analogs Lacking Ring C. V. Some Analogs of Testosterone and Androstenedione[1]

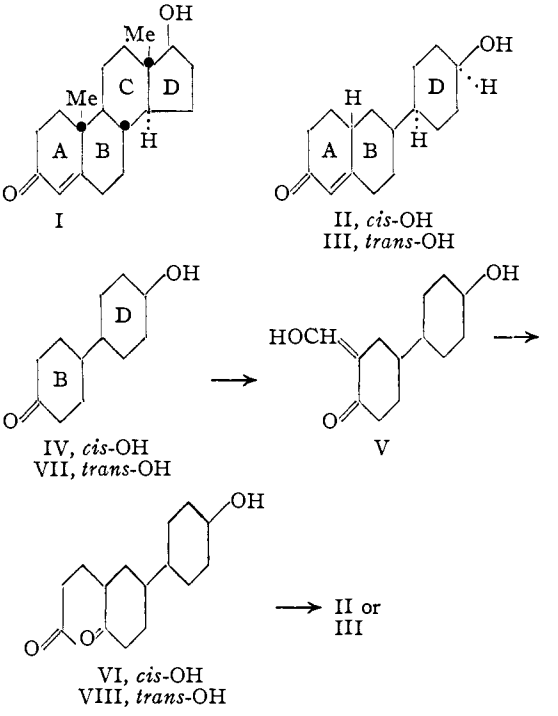
BY A. L. WILDS, CARL H. HOFFMAN[2] AND TILLMON H. PEARSON[3]

RECEIVED JUNE 23, 1954

Some analogs (II, III, XII) of testosterone, epi-testosterone and andostenedione have been prepared lacking ring C. These resulted from application of improved Robinson–Mannich base procedures to the hydroxymethylene derivatives of 4-(*trans*-4′-hydroxycyclohexyl)-cyclohexanone (VII), the *cis* isomer (IV) and bicyclohexyl-4,4′-dione (IX). Preliminary experiments on the preparation of the *trans* analog having the angular methyl group (XV) were also carried out using a procedure which gave improved, but still low yields in preparing 10-methyl-Δ^{1-9}-octalone-2 (XIII). Despite the preliminary results reported earlier indicating the diketone XII to be a weakly active androgen,[4] it now appears that none of these analogs possesses dependable androgenic activity, although they may give some of the associated physiological effects.

Following the discovery of Dodds and co-workers[5] that simple synthetic compounds possess the estrogenic activity of the female sex hormone estradiol, research in many laboratories led to numerous active analogs of this type. In 1944 Masson[6] estimated that of some 460 compounds tested for estrogenic activity nearly half were found active to some extent. Still more potent compounds have been prepared since that time.[7]

In contrast, little is known regarding simpler analogs of other types of steroid hormones, including the androgens. To no small degree this is due, in our opinion, to the greater obstacles in the way of synthesis of such analogs of the non-aromatic steroids, and especially to the greater stereochemical difficulties which must be overcome. Considerable attention has been devoted to androgen analogs in the perhydrohexestrol series.[8] Although the crystalline analog of testosterone with the appropriate configuration still has not been described, apparently no compound with dependable androgenic activity has yet been reported in this or any other non-steroid series, so far as we are aware.[9,10] This situation contrasts with that for the steroids themselves, where some androgenic activity is associated with at least thirty androstane derivatives showing significant variations in structures.[11]



In view of this situation we started in 1940 to prepare analogs (II, III, XII) of testosterone (I), its 17α-epimer and androstenedione, lacking ring C. The methods for these syntheses were developed over a period of years and have been described in earlier papers, including that reporting similar analogs of progesterone and desoxycorticosterone.[12]

The *cis*- and *trans*-hydroxy ketones (IV and VII) in the bicyclohexyl series suitable for attachment of ring A were described in the preceding papers of this series.[1,13] Applying to these the methods developed in Part II,[12] the hydroxymethylene group was first introduced. It was observed that the ultraviolet absorption spectra for the crude hy-

(1) Paper IV, A. L. Wilds, T. H. Pearson and C. H. Hoffman, THIS JOURNAL, **76**, 1737 (1954).

(2) Wisconsin Alumni Research Foundation Research Assistant, 1946–1947; Ciba Pharmaceutical Products, Inc., Fellow, 1948–1949.

(3) Wisconsin Alumni Research Foundation Research Assistant, 1949–1951.

(4) A. L. Wilds, C. H. Shunk and C. H. Hoffman, THIS JOURNAL, **71**, 3266 (1949).

(5) J. W. Cook, E. C. Dodds, C. L. Hewett and W. Lawson, *Proc. Roy. Soc.* (*London*), **B114**, 272 (1933); E. C. Dodds, L. Golberg, W. Lawson and R. Robinson, *ibid.*, **B127**, 140 (1939).

(6) G. Masson, *Rev. Can. Biol.*, **3**, 491 (1944); see also U. V. Solmssen, *Chem. Revs.*, **37**, 481 (1945).

(7) *Cf.* K. Miescher, *Helv. Chim. Acta*, **27**, 1727 (1944); C. W. Shoppee, *Ann. Repts.*, **44**, 190ff (1947).

(8) (a) P. G. Carpenter, Ph.D. Thesis, University of Wisconsin, 1941; (b) J. F. Lane and E. S. Wallis, THIS JOURNAL, **65**, 994 (1943); (c) R. T. Major, K. Folkers and C. C. Christman, U. S. Patent 2,350,-361 (June 6, 1944); (d) H. E. Ungnade and A. Ludutsky, *J. Org. Chem.*, **10**, 307 (1945); (e) W. Schoeller, H. Inhoffen, K. Steinruck and O. Hoess, U. S. Patent 2,392,864 (Jan. 15, 1946); (f) H. E. Ungnade and P. W. Tucker, THIS JOURNAL, **71**, 1381 (1949); (g) A. J. Birch and S. M. Mukherji, *J. Chem. Soc.*, 2531 (1949).

(9) (a) J. G. Cook and R. Robinson, *ibid.*, 391 (1941); (b) J. Heer and K. Miescher, *Helv. Chim Acta*, **30**, 786 (1947); note, however, that these androstane analogs of the doisynolic acids do not have the configuration of the most active estrogenic analogs, see K. Miescher, *Experientia*, **5**, 6 (1949); (c) M. Stoll, M. Hinder and L. Ruzicka, *Helv. Chim. Acta*, **31**, 1176 (1948); (d) E. C. Kornfeld, THIS JOURNAL, **70**, 1373 (1948); (e) J. C. Sheehan and C. D. Laubach, *ibid.*, **72**, 2478 (1950); (f) F. C. Novello and M. E. Christy, *ibid.*, **73**, 1267 (1951); (g) A. J. Birch and H. Smith, *J. Chem. Soc.*, 1882 (1951).

(10) The claims in the patent of Schoeller, *et al.* (ref. 8e) must be discounted until convincing evidence is published, in view of their claims for activity apparently irrespective of configuration.

(11) See for example (a) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publ. Corp., New York, N. Y., 3rd ed., 1949, pp. 374–380; (b) A. L. Wilds and N. A. Nelson, THIS JOURNAL, **75**, 5366 (1953).

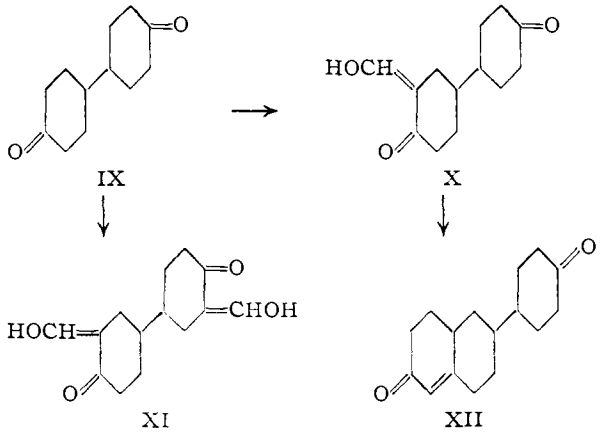
(12) A. L. Wilds and C. H. Shunk, *ibid.*, **72**, 2388 (1950).

(13) A. L. Wilds, C. H. Shunk and C. H. Hoffman, *ibid.*, **76**, 1733 (1954).

droxymethylene ketones (V) frequently showed a shoulder at 310 mμ in addition to the maximum at 278 mμ. While bis-hydroxymethylene derivatives would be expected to absorb around 310 mμ, the previous conclusion that these are not formed was confirmed, when it was found that addition of one or more equivalents of hydrochloric acid resulted in a sharp maximum at 278 mμ. Thus, the ultraviolet spectra at varying *p*H of these relatively strongly acidic hydroxymethylene ketones parallel those for dihydroresorcinol,[14] and the 310 mμ shoulder is due to ionization which is suppressed by mineral acid.

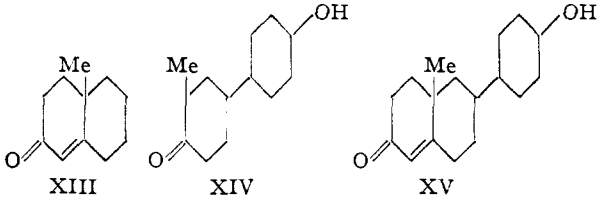
Reaction of the hydroxymethylene derivatives with the methiodide of 1-diethylamino-3-butanone gave mixtures presumably containing the diketones VI or VIII (or the corresponding cyclic ketols). In the *trans*-hydroxy series the crystalline intermediate could be isolated, but it was preferable to convert the mixture to the α,β-unsaturated ketones II or III by the action of alkali. In this way, after purification by chromatography, the crystalline analogs of testosterone in the *cis*- and *trans*-hydroxy series were obtained. Only one of the two possible stereoisomers was obtained in each series, in 21–34% over-all yield from the hydroxy ketone (IV, VII).

When the same series of reactions was applied to the diketone IX, more difficulty was encountered in the first step. Even with equimolar amounts of diketone and ethyl formate, the alkali-soluble hydroxymethylene derivative X was accompanied by some bis-hydroxymethylene derivative XI as well as much unreacted diketone. With a higher ratio of ethyl formate the crystalline bis derivative XI was isolated. As anticipated, the ultraviolet spectrum (λ_{max} 280.5 mμ, ε 18,700) showed that this had the symmetrical structure XI rather than being disubstituted in the same ring.



By using the crude mono derivative X for alkylation and cyclization, it was possible to isolate the crystalline androstenedione analog XII, although in only 10% over-all yield from IX. The same diketone was obtained in low yield by oxidation of the *trans*-hydroxy derivative III.

Some preliminary experiments also were carried out exploring the synthesis of analogs such as XV having the angular methyl group between rings A and B. In using the Robinson–Mannich base synthesis for this purpose, it is no longer possible to activate the ketone, and low yields usually result even with uncomplicated examples. Thus, du Feu, McQuillin and Robinson[15] showed that the product from 2-methylcyclohexanone was indeed the angularly methylated octalone XIII, but obtained this in only 19% yield based on the ketone (or 38% yield based on the Mannich base). We have



modified the procedure to obtain 26–30% yields of the octalone XIII from 2-methylcyclohexanone, using triphenylmethylsodium in pyridine solution as the enolizing agent. The necessary ketone XIV was prepared by methylating the hydroxymethylene derivative V (*trans*-hydroxyl) with elimination of the formyl group. The product of reaction of XIV with the Mannich base methiodide was a mixture, from which was obtained impure material containing about 40% of the unsaturated ketone, judged by the absorption maximum at 238 mμ. The structure is formulated as XV from this and by analogy with XIII.

These compounds were tested for physiological activity in the Department of Zoology under the direction of Drs. R. K. Meyer and Elva G. Shipley. In the first series, using the comb growth test in day-old chicks, injecting an oil solution daily for seven days and weighing the combs on the eighth day, the diketone XII gave no activity at 1.5 mg. total dose (11% increase) but gave a large response at 2.5 mg. (83% increase above the controls). A second test at 2.5 mg. on slightly less pure material gave 26% increase. Under comparable conditions 0.012 mg. of testosterone propionate gave a 40% increase. In a later series of tests, however, no activity was observed with XII at 3.5 mg. in chicks, and in young castrate rats at 7 mg. (1 mg. per day for 7 days) no increase in seminal vesicle or prostate gland weights resulted, although there was a 32% increase in the levator ani muscle.[16] (The negative androgenic results in the rat are to be compared to a positive response obtained with 0.035 mg. of testosterone propionate.) That the initial activities probably were not due to impurities was indicated by negative results in the chick with 3.0 mg. doses for impure solid or the oily residues from preparing XII. We must now conclude that no androgenic activity has been demonstrated for this diketone,[4] although the possibility of other types of activity still remains.

The initial chick tests with the *trans*-hydroxy derivative III were borderline at 1.5 mg. (45 and 22% increase). Later tests with the *trans*-acetate of III were negative at 3.0 mg. (12% decrease).

(14) *Cf.* H. Bastron, R. E. Davis and L. W. Butz, *J. Org. Chem.*, **8**, 515 (1943), with the reinterpretation of E. K. Blout, V. W. Eager and D. C. Silverman, THIS JOURNAL, **68**, 566 (1946).

(15) E. C. du Feu, F. J. McQuillin and R. Robinson, *J. Chem. Soc.*, 53 (1937).

(16) This increase may indicate some nitrogen retention effect.

From the configuration alone, it might be considered that the *trans*-derivative III corresponds to the less potent 17α-testosterone (17 OH-*trans* to 8–14 bond) rather than to the natural 17β hormone.[17] On the basis of conformation, however, the *trans* derivative having the hydroxyl equatorial would more nearly approximate the 17β-isomer. In any case assays on the *cis* derivative II were desirable. This isomer gave a slight decrease in comb weight at 3.0 mg., the highest practical dose. The acetate of II also was inactive at a dose of 3.3 mg. Preliminary tests with the crude methyl derivative XV (*trans*-hydroxyl), at 4.0 mg. total dose gave 39% decrease in comb weight in chicks.

Thus, at the present time, no consistent androgenic activity has been demonstrated in these analogs lacking ring C.[18] It is still possible that such activity may yet be found in this series, however, when closer analogs are prepared with the angular methyl group and the correct configuration, or with related compounds having a five-membered ring D, the precursors of which were reported earlier.[19]

Experimental[20]

2-Hydroxymethylene-4-(*trans*-4'-hydroxycyclohexyl)-cyclohexanone (V). (a) **From the Keto Alcohol (VII).**—In a three-necked flask fitted with a stirrer and reflux condenser and furnished with a nitrogen atmosphere, a suspension of sodium methoxide was prepared from 0.987 g. of sodium followed by removal of the excess methanol and addition of two portions of dry thiophene-free benzene, which were also removed by distillation. The resulting solid was suspended in 60 ml. of dry thiophene-free benzene, stirred at room temperature and a mixture of 190 ml. of benzene, 2.12 g. of ethyl formate and 2.805 g. of the *trans*-hydroxy ketone VII[13] (m.p. 126–129°) added in one portion. The mixture was stirred for 18 hours and chilled in ice. Water was added and the aqueous layer separated. The benzene layer was washed once with approximately 0.5% potassium hydroxide solution. The alkaline aqueous layers were combined, chilled in ice and made acid to congo red. Extraction with five to seven portions of chloroform and evaporation of an aliquot ($^1/_{10}$, 332 mg.)[21] to constant weight indicated the total yield of alkali-soluble material to be 3.32 g. (104%) of light yellow oil. The ultraviolet absorption spectrum in 95% ethanol showed a maximum at 284 mμ (ϵ 7090) and a shoulder at 310 mμ. In acid solution (one drop of concentrated hydrochloric acid per 10 ml. of 95% ethanol) the spectrum exhibited a maximum at 278 mμ (ϵ 8150) without the 310 mμ shoulder and in dilute ethanolic potassium hydroxide (1.8×10^{-4} *N*) a maximum at 314 mμ (ϵ 12,600). In other runs, using alcohol alone, before the value of adding acid was discovered, the maxima ranged from 279 mμ (ϵ 6070) to 312 mμ (ϵ 9330), and the position of the maximum varied with the concentration of the solution.

In order to minimize decomposition the hydroxymethylene derivative was used in the next step as soon as possible. In one run a small amount (6%) of granular solid was obtained, m.p. 98–102° dec., λ_{max} 279 mμ (ϵ 7000 in 95% alcohol and ϵ 7700 in acidified alcohol).

(b) **From the Keto Benzoate.**—In a similar way a suspension of alcohol-free sodium methoxide (from 730 mg. of sodium) in 40 ml. of benzene, was treated with a solution of 2.25 g. (7.5 mmoles) of the *trans*-ketobenzoate[13] (m.p. 151.5–153.5°), in 40 ml. of dry thiophene-free benzene, 6.7 g. (90 mmoles) of ethyl formate and 10 ml. of purified dioxane. After 1.5 hours in an ice-bath and 16 hours at room temperature, water was added and the product isolated as before. Evaporation of an aliquot (1/22.5; 81 mg.) to constant weight indicated the total yield of alkali-soluble material to be 1.82 g. of yellow oil. The ultraviolet absorption spectrum in alcohol showed a maximum at 280 mμ (ϵ 8260).[22]

2-(γ-Ketobutyl)-4-(*trans*-4'-hydroxycyclohexyl)-cyclohexanone (VIII).—To a solution of 310 mg. of sodium in 15 ml. of anhydrous methanol in a nitrogen atmosphere was added a solution of 2.43 g. of the oily 2-hydroxymethylene-4-(*trans*-4'-hydroxycyclohexyl)-cyclohexanone from 2.124 g. of *trans*-keto alcohol (VII) in 20 ml. of dry methanol, while the mixture was stirred and cooled in an ice-bath. A solution of 1-diethylamino-3-butanone methiodide (prepared from 4.64 g. of the Mannich base)[23] in 15 ml. of dry methanol was added in one portion and stirring was continued for 17 hours with cooling during the first 2 hours. The mixture was then decanted into 500 ml. of saturated sodium chloride solution and extracted thoroughly with chloroform. The chloroform layers were washed with sodium bicarbonate solution, excess dilute hydrochloric acid, water and filtered. An aliquot (2/25.3, 275 mg.) evaporated to constant weight indicated 3.48 g. of neutral material to be present.

Since attempts to crystallize portions of the neutral oil failed, a solution of 0.48 g. of the oil in 10 ml. of ether was adsorbed on a column of 20 g. of acid-washed alumina. No crystalline material was eluted with 100 cc. of ether, but 2% methanol in ether (75 cc.) eluted 438 mg. Recrystallization of a portion from ether gave 88 mg., m.p. 79.5–81.0°. Two more recrystallizations from ether–petroleum ether (b.p. 40–60°) gave small rosettes of white needles, m.p. 81.0–82.0°. This evidently was the ketobutyl derivative VIII which had lost the formyl group.

Anal. Calcd. for $C_{18}H_{30}O_2$: C, 72.1; H, 9.84. Found: C, 72.1; H, 9.77.

A second crop of 70 mg., 76–78°, and an additional 41 mg., m.p. 72–76°, and 13 mg., m.p. 65–68°, were obtained for a total of 212 mg. (53% from VII).

6-(*trans*-4'-Hydroxycyclohexyl)-Δ^{1-9}-octalone-2 (III).—A solution of 3.48 g. of the oily γ-ketobutyl derivative VIII in 324 ml. of methanol, through which was bubbling a slow stream of nitrogen, was treated with 12 g. of potassium hydroxide in 24 ml. of water, and the mixture was held at room temperature for 2.5 hours. It was then decanted into 500 ml. of saturated salt solution, acidified with dilute hydrochloric acid and extracted thoroughly with chloroform. An aliquot (25/415, 178 mg.) when evaporated to constant weight indicated 2.95 g. of oil. This had an absorption maximum at 238 mμ (ϵ 5080) indicating a 30% purity (based on ϵ for pure III, or 33% maximum possible over-all yield from the *trans*-hydroxy ketone VII).

The oil was dissolved in 40 ml. of acetone–ether (1:2), adsorbed on 100 g. of acid-washed alumina and eluted with a 1:3 ratio of the same solvents. From fractions 7–11 (each fraction 25 cc.) by recrystallization from acetone was obtained 564 mg., m.p. 103–118°; further recrystallization from petroleum ether–acetone gave 368 mg. of octalone derivative, m.p. 118–123°, corresponding to a 14% over-all yield from the *trans*-hydroxy ketone VII. In other runs it was possible to isolate part of the product by direct crystallization. The purest sample of the *trans*-octalone derivative III was obtained after chromatography and crystallization from ethyl acetate–cyclohexane as heavy prisms, m.p. 127–127.5°, λ_{max}^{EtOH} 238 mμ (ϵ 16,900) and 310 mμ (90).

Anal. Calcd. for $C_{18}H_{24}O_2$: C, 77.4; H, 9.74. Found: C, 77.7; H, 9.84.

(17) See ref. 11a, p. 325.

(18) Further tests with the progesterone and desoxycorticosterone analogs reported in Part II have given negative results in the sodium retention test for 6-(4'-acetoxyacetylcyclohexyl)-Δ^{1-9}-octalone-2 (isomer A, formula XV of ref. 12) and the 6-(4'-acetoxyacetylphenyl) derivative (XIII) at 120γ, while desoxycorticosterone acetate was active at 3γ. The 6-(4'-acetylcyclohexyl) (XIV, isomer A) and 6-(4'-acetylphenyl) (XII) derivatives showed no progestational activity in the rabbit at 30 and 40 mg., respectively. XIV at 1 mg. and XII, XIII and XV at 0.5 mg. were inactive in the glycogen deposition test in mice (cortisone active at 10γ).

(19) A. L. Wilds and T. L. Johnson, THIS JOURNAL, **67**, 286 (1945).

(20) All melting points are corrected; those marked micro m.p. were taken on a calibrated microscope hotstage. Microanalyses were carried out by John Belew, Ernest Blades, Bennett Buell, Gerald Gilbert, Richard Hunt, Virginia Miller and Edward Shiner.

(21) Since many of the products in this work were viscous oils, it was quicker and more convenient to get a constant weight on a small amount rather than the whole product. Such aliquots were concentrated to constant weight using the water aspirator and steam-bath.

(22) The value of ϵ at 228 mμ was 105. Since methyl benzoate exhibits a maximum of 228 mμ (ϵ 11,600) this would indicate that less than 1% of the benzoate group remained in the hydroxymethylene compound.

(23) The Mannich base was prepared essentially as described previously [A. L. Wilds and C. H. Shunk, THIS JOURNAL, **65**, 469 (1943)] except most of the material used had $n^{25}D$ 1.4302–1.4310.

The semicarbazone, prepared in pyridine-alcohol in 99% yield, was recrystallized from ethanol, m.p. 238–239° dec. (sample inserted at 230°); λ_{max}^{EtOH} 268 mμ (ε 16,800). The melting point was appreciably lower when the sample was inserted at lower temperatures.

Anal. Calcd. for $C_{17}H_{27}O_2N_3$: C, 66.9; H, 8.91. Found: C, 66.7, 67.0; H, 8.55, 8.71.

By cyclization of 69 mg. of the solid γ-ketobutyl derivative VIII as described above, 34 mg. of the octalone III, m.p. 123–125°, and 21 mg. of semicarbazone, m.p. 228–231° (inserted at 225°), were obtained for a total yield of 80%.

6-(*trans*-4′-Acetoxycyclohexyl)-Δ^{1-9}-octalone-2.—A mixture of 276 mg. of acetyl chloride, 159 mg. of the octalone III and 2.5 ml. of anhydrous pyridine was heated on the steam-bath for 5 minutes, then water and ether were added and the washed ether solution was concentrated to 199 mg. of a yellow oil. All attempts at direct crystallization failing, the oil was dissolved in 17 ml. of anhydrous ether, adsorbed on 6 g. of alumina and eluted with ether (20-cc. fractions).

Fraction 3–7 crystallized from cyclohexane to give 57 mg. (31%), m.p. 98–103°. Further recrystallizations gave the acetate as thick needles with the m.p. 102–103.5°.

Anal. Calcd. for $C_{18}H_{26}O_3$: C, 74.4; H, 9.03. Found: C, 74.3; H, 9.00.

6-(*cis*-4′-Hydroxycyclohexyl)-Δ^{1-9}-octalone-2 (II).—Using procedures similar to those in the *trans* series, 710 mg. of the *cis*-hydroxy ketone IV[1] (m.p. 90–91.5°) was converted to the hydroxymethylene derivative using sodium methoxide from 350 mg. of sodium and 3.25 g. of ethyl formate in 19 ml. of benzene. The weight of alkali-soluble oil (V) was 806 mg.; λ_{max}^{EtOH} 288 mμ (ε 5980). This was treated with sodium methoxide from 98 mg. of sodium and the methiodide from 1.55 g. of 1-diethylamino-3-butanone in a total of 60 ml. of methanol. The neutral oil VI (950 mg.) was cyclized with 3.4 g. of potassium hydroxide in 7 ml. of water and 91 ml. of methanol under nitrogen for 2 hours at room temperature. The oily product (807 mg., λ_{max}^{EtOH} 239 mμ, ε 8580) was adsorbed in ether solution on 20 g. of alumina. The crystalline fractions (3–11) eluted with 50 cc. of ether containing 0.5 to 2% methanol, were combined (444 mg.) and recrystallized from cyclohexane, giving 306 mg. (34% over-all yield from the hydroxyketone), m.p. 125–130°. Further recrystallization from cyclohexane–ethyl acetate gave the *cis*-octalone derivative II as colorless prisms, m.p. 131.3–132.3°; λ_{max}^{EtOH} 238 mμ (ε 17,900). A mixture with the *trans*-octalone derivative III melted at 107–115°.

Anal. Calcd. for $C_{16}H_{24}O_2$: C, 77.4; H, 9.74. Found: C, 77.4; H, 9.73.

The acetate was prepared and chromatographed on alumina as described for the *trans* derivative. The resulting oil, which could not be crystallized, was used for physiological assay.

Crude 2-Hydroxymethylene-4-(4′-ketocyclohexyl)-cyclohexanone (X).—To the alcohol-free sodium methoxide prepared as described above from 0.94 g. (0.041 mole) of sodium was added 60 ml. of dry benzene and 3.05 g. (0.041 mole) of ethyl formate, and the mixture was stirred at room temperature for 0.5 hour. After cooling in ice, 8.0 g. (0.031 mole) of bicyclohexyl-4,4′-dione (IX)[13] (m.p. 115–116°) in 50 ml. of benzene was added and stirring continued for 2 hours at 5° and 12 hours at room temperature. After isolating the product as described for the *trans*-hydroxy derivative V, 3.87 g. (48%) of the starting diketone, m.p. 110–113°, was recovered from the neutral fraction. The oily, alkali-soluble fraction amounted to 4.83 g., λ_{max}^{EtOH} 279.5 mμ (ε 8,900 based on mol. wt. 222).

An attempt to prepare the anilino derivative gave a small amount of material melting at 240–250° which probably was the impure bis-anilinomethylene derivative (see below). The copper enolate was prepared from 193 mg. of oil in 10 ml. of benzene by shaking with a saturated solution of 1 g. of cupric acetate in water. After 15 minutes shaking the mixture was filtered, and the residue washed with benzene and water. This material (116 mg.), 250–254° dec., probably was derived in part from bis-hydroxymethylene derivative (Found: Cu, 14.4. Calcd. for mono derivative, 12.5; for bis-derivative 20.4).

The benzene solution was washed with water, dried and concentrated to 115 mg. of a light green solid, 210–212° dec., which was recrystallized from benzene–ether to give 30 mg. of light olive-green needles, **the copper enolate of 2-hydroxymethylene-4-(4′-ketocyclohexyl)-cyclohexanone.**

Anal. Calcd. for $C_{26}H_{34}O_6Cu$: C, 61.7; H, 6.77; Cu, 12.5. Found: C, 62.3; H, 6.77; Cu, 11.9.

When the ratio of sodium methoxide and ethyl formate to diketone was higher, the recovery of neutral material was reduced but the proportion of bis-hydroxymethylene derivative increased. With a ratio of 2 moles of sodium methoxide and 3 moles of ethyl formate to 1 mole of diketone, only 2% of neutral material remained and the alkali-soluble fraction was crystalline, micro m.p. 120–130°; recrystallization from ethyl acetate gave 21% of **3,3′-bis-hydroxymethylene-bicyclohexyl-4,4′-dione (XI)**, m.p. 150–153° (softening from 135°). Further recrystallization gave the bis derivative as shining leaflets, m.p. 154.5–155.5°; λ_{max}^{EtOH} 280.5 mμ (ε 18,700).

Anal. Calcd. for $C_{14}H_{18}O_4$: C, 67.2; H, 7.25. Found: C, 67.2; H, 6.95.

The bis-anilinomethylene derivative, prepared from 88 mg. of the solid, 100 mg. of aniline and 8 ml. of methanol at room temperature for one hour (77% yield, micro m.p. 280–320°), was purified by dissolving in pyridine, diluting with water and cooling. The yellow solid had no definite m.p. but decomposed rapidly at 300–320° (darkening at 280–300°).

Anal. Calcd. for $C_{26}H_{28}O_2N_2$: C, 78.0; H, 7.05. Found: C, 77.5; H, 6.86.

6-(4′-Ketocyclohexyl)-Δ^{1-9}-octalone-2 (XII).—A solution of 4.25 g. of the oily hydroxymethylene derivative X, described above, in a total of 80 ml. of dry methanol was alkylated using sodium methoxide from 0.41 g. of sodium and methiodide from 5.43 g. of 1-diethylamino-3-butanone. After removing a small amount of solid material, m.p. 183–186° (dec. from 150°), the product was isolated with chloroform. The neutral oil amounted to 4.22 g. Chromatography on acid-washed alumina did not give solid material.

A solution of 2.03 g. of this oil in a total of 200 ml. of methanol was cyclized under nitrogen with 8.2 g. of potassium hydroxide in 16 ml. of water as described for the *trans*-hydroxy derivative VIII, allowing 2.3 hours at room temperature. After isolation of the neutral fraction, evaporation of an aliquot indicated the weight of oil to be 1.13 g. This was adsorbed from benzene–ether (2:1) on 30 g. of acid-washed alumina and eluted. From fractions 4–6 (25 cc. each, benzene–ether 1:1) totalling 232 mg. was obtained by recrystallization from ethyl acetate–petroleum ether (b.p. 40–60°), 203 mg., m.p. 85–87° (softening from 83°) and 29 mg., m.p. 80–86°. Further recrystallization from cyclohexane or mixtures of ethyl acetate or acetone with petroleum ether gave dense rosettes of constant m.p. 88.5–89.5°; λ_{max}^{EtOH} 238 mμ (ε 16,450) and 304 mμ (83).

Anal. Calcd. for $C_{16}H_{22}O_2$: C, 78.0; H, 9.00. Found: C, 77.9; H, 8.72.

The disemicarbazone, prepared in alcohol–pyridine solution in 97% yield and too insoluble for recrystallization from the usual solvents, was purified by digestion with hot water and boiling alcohol, m.p. 256–257° dec. (sample inserted at 255°).

Anal. Calcd. for $C_{18}H_{28}O_2N_6$: C, 60.0; H, 7.83. Found: C, 59.9; H, 7.47.

In this and other runs the over-all yield of the octalone derivative XII was 8–11% from crude hydroxymethylene derivative X or from bicyclohexyl-4,4′-dione (IX) if corrected for the recovered diketone in the first step.

(XII) by Oxidation of the *trans*-Hydroxyoctalone Derivative (III).—The theoretical amount of bromine (0.127 g.) in 1 ml. of glacial acetic acid was added slowly to a solution of 186 mg. of the hydroxyoctalone III (m.p. 123–125°) in 5 ml. of glacial acetic acid with stirring and while holding the temperature at 15–19°. Then 0.104 g. (100% excess) of chromium trioxide in 1 ml. of 90% acetic acid–10% water solution was added during 0.5 hour and the solution allowed to stand for an additional 1.5 hours while holding the temperature at 13–19° by occasional cooling. Excess methanol (5 ml.) was added, and after 15 minutes the mixture was diluted and extracted thoroughly with benzene. The latter extracts were concentrated, treated with 0.72 g. of sodium iodide in 5 ml. of absolute alcohol, warmed for 2 hours and again extracted, washing with sodium sulfite. The resulting neutral oil (162 mg.) was adsorbed on 4 g. of acid-washed alumina from chloroform–ether (1:1); the first four fractions (5 cc.) eluted by the same solvent pair (145 mg.), crystallized slowly from ethyl acetate–petroleum ether

affording 6 mg. (3%), micro m.p. 86–88° (softening from 85°). A mixture with the sample prepared from bicyclohexyl-4,4'-dione gave no m.p. depression. No crystalline product could be isolated when the Oppenauer oxidation (aluminum *t*-butoxide, cyclohexanone, toluene, 0.5 hour at reflux) was used with III.

2-Methyl-4-(*trans*-4'-hydroxycyclohexyl)-cyclohexanone (XIV).—In a three-necked flask, fitted with a stirrer and under a nitrogen atomosphere, sodium methoxide was prepared from 435 mg. of sodium in 30 ml. of anhydrous methanol. To this was added the oily hydroxymethylene compound V prepared from 1.22 g. of the hydroxyketone VII, in 50 ml. of benzene. The homogeneous solution was refluxed 15 minutes, then 25 ml. of methyl iodide was added and refluxing continued. Additional methyl iodide was added after 12 and 22 hours and refluxing was continued for a total of 34 hours. The reaction mixture tested neutral to moist litmus. Isolation of the neutral fraction gave 1.329 g. of neutral oil. The alkaline washes gave 118 mg. (8%) of alkali-soluble oil. The neutral fraction was refluxed for two hours with a mixture of 100 ml. of methanol, 25 ml. of water and 20 ml. of concentrated hydrochloric acid. In this way 1.01 g. of neutral product was obtained and 126 mg. of alkali-soluble oil (corresponding to 9% O-methylation). The neutral product was dissolved in 50 ml. of ethyl acetate, adsorbed on 70 g. of alumina and eluted with 50-cc. portions of ethyl acetate. Fractions 4–11 (1006 mg.) were combined and recrystallized from ethyl acetate–cyclohexane giving 526 mg. (40%) of crystalline methyl derivative, m.p. 62–68°. Further recrystallizations from cyclohexane or ethyl acetate–cyclohexane gave thin plates melting at 72–76°, evidently a mixture of the two possible C-2 diastereoisomers of XIV.

Anal. Calcd. for $C_{13}H_{22}O_2$: C, 74.2; H, 10.54. Found: C, 74.4; H, 10.58.

Conversion of 2-Methylcyclohexanone to 10-Methyl-Δ^{1-9}-octalone-2 (XIII).—A solution of 10 g. (89 mmoles) of 2-methylcyclohexanone in 67 ml. of pyridine in a three-necked flask fitted with a stirrer and under nitrogen was treated with an ether solution of 180 ml. of 0.5 *N* triphenylmethylsodium solution (90 mmoles). Most of the ether was removed under reduced pressure with stirring, maintaining the nitrogen atmosphere, and a solution of the methiodide (prepared from 12.8 g. of 1-diethylamino-3-butanone) in 50 ml. of pyridine was added during 13 minutes, keeping the mixture at approximately 25°. Stirring was continued at room temperature for 2 hours, then a mixture of 22 g. of potassium hydroxide, 25 ml. of water and 225 ml. of methanol was added. After one hour stirring, the mixture was decanted into 800 ml. of saturated sodium chloride solution, concentrated hydrochloric acid was added and the mixture was extracted with ether. The extracts were dried over anhydrous sodium sulfate and evaporated to an oil. Addition of cold alcohol, trituration and filtration yielded 19.2 g. of triphenylmethane. The filtrate was concentrated to an oil and distilled giving 1.02 g. (10%) of recovered 2-methylcyclohexanone, b.p. 60–64° at 20 mm. Further distillation gave 10-methyl-Δ^{1-9}-octalone-2 in two fractions: 2.62 g. (18%), b.p. 82–88° at 0.2 mm. and 1.13 g. (8%) boiling slightly higher. The purity of each fraction was determined by conversion to the semicarbazone. The first fraction gave 2.38 g., m.p. 201–203.5°, and additional 0.64 g., m.p. 200–202°, for a total of 86%. The second fraction yielded 0.98 g. (64%), m.p. 201–203.5° (du Feu, McQuillin and Robinson reported m.p. 203.5–204°).[15]

Crude 10-Methyl-6-(*trans*-4'-hydroxycyclohexyl)-Δ^{1-9}-octalone-2 ((XV).—A solution of 905 mg. of 2-methyl-4-(*trans*-hydroxycyclohexyl)-cyclohexanone (XIV) (m.p. 65–70°) in 7 ml. of dry pyridine was treated with 18 ml. of 0.5 *N* triphenylmethylsodium solution in ether as described above for 2-methylcyclohexanone, the solvent removed and the enolate treated slowly with the methiodide from 740 mg. of 1-diethylamino-3-butanone in 11 ml. of pyridine. After three hours at room temperature and the further cyclization treatment with potassium hydroxide as above, the product was isolated as before. The neutral portion was adsorbed on 70 g. of alumina from benzene, the triphenylmethane being eluted by the same solvent. Fractions 9–28 (eluted with 50-cc. portions of ether–benzene 1:1, ether and ethyl acetate) were combined, and again adsorbed from ether onto 25 g. of alumina. Fractions 11–22 (eluted with ethyl acetate) were adsorbed from ether onto 30 g. of alumina and eluted with 25-cc. portions of ether, ether–ethyl acetate 2:1 to 1:2. Fractions 22–32, which had λ_{max}^{EtOH} 238 mμ were combined to give 155 mg. of impure yellow oil, which from the ultraviolet absorption (λ_{max} 238 mμ, ϵ 6800) appeared to contain approximately 40% of the octalone derivative XV. This material was used for physiological testing.

MADISON 6, WISCONSIN

[CONTRIBUTION FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH AND THE DIVISION OF PURE CHEMISTRY OF THE NATIONAL RESEARCH COUNCIL OF CANADA[1]]

The Infrared Spectra of Ketosteroids Below 1350 Cm.$^{-1}$

BY R. NORMAN JONES, F. HERLING[2a] AND E. KATZENELLENBOGEN[2b]

RECEIVED APRIL 7, 1954

In the infrared spectra of ketosteroids several bands can be distinguished between 1350 and 650 cm.$^{-1}$, the position of which serves to characterize the location of the ketone group and its relationship to neighboring centers of unsaturation. Many of these bands also can be recognized in the spectra of diketones, keto-esters and keto-alcohols unless submerged beneath more intense absorption associated with the other functional substituents. Some of these characteristic frequencies may be perturbed by substitution in ring C.

Ketosteroids are most effectively characterized by the intense infrared absorption band between 1800 and 1650 cm.$^{-1}$ associated with the C=O stretching vibration, and the influence of the molecular structure on the position and intensity of this band has been a subject of detailed study.[3–5] Ketosteroids also absorb characteristically between 1475 and 1350 cm.$^{-1}$ due to bands associated with methyl and methylene groups vicinal to the carbonyl group.[6] The spectra of ketosteroids between 1350 and 650 cm.$^{-1}$ have not hitherto been surveyed for group vibrations specific to the ketone group, although it has been recognized that these compounds do possess prominent bands in this region of the spectrum.[7] Such a survey now has been carried out and several new bands have been identified.

Experimental Methods and Results

The spectra were determined on Perkin–Elmer model 12 and model 21 spectrometers. Many of the data are based

(1) Published as Contribution No. 3465 from The Laboratories of the National Research Council of Canada, and No. XXI in the series "Studies in Steroid Metabolism."

(2) (a) Mrs. H. Kaelber; (b) Mrs. K. J. Mysels.

(3) R. N. Jones and F. Herling, *J. Org. Chem.*, **19**, 1252 (1954).

(4) K. Dobriner, E. Katzenellenbogen and R. N. Jones, "Infrared Absorption Spectra of Steroids—An Atlas," Interscience Publishers, Inc., New York, N. Y., 1953.

(5) R. N. Jones, D. A. Ramsay, D. S. Keir and K. Dobriner, THIS JOURNAL, **74**, 80 (1952).

(6) R. N. Jones and A. R. H. Cole, *ibid.*, **74**, 5648 (1952).

(7) A. R. H. Cole, R. N. Jones and K. Dobriner, *ibid.*, **74**, 5571 (1952).