of β-benzamidopropionitrile and the solution was heated under reflux for four days. The solvent was evaporated and the residue was extracted with 50 ml. of 1 *N* sodium hydroxide. The extract was made acidic with dilute hydrochloric acid and was concentrated to dryness under reduced pressure. The resulting solid was extracted with absolute ethanol which, in turn, was evaporated, and the residue was recrystallized from 10 ml. of water. There was obtained 0.2 g. of 5-β-benzamidoethyltetrazole. Approximately 9 g. of starting material was recovered.

5-β-Aminoethyltetrazole (I) Hydrochloride.—5-β-Benzamidoethyltetrazole (1.5 g.) suspended in 25 ml. of dilute hydrochloric acid was heated under reflux for 6 hours. Benzoic acid, which precipitated on cooling, was removed by filtration. The filtrate was evaporated to dryness under reduced pressure. After recrystallization from ethanol–ether, 5-β-aminoethyltetrazole hydrochloride was obtained in quantitative yield as prisms; m.p. 128–129°; pK'_a 5.0, 10.0 (66% dimethylformamide); no $\lambda_{max} > 210$ mμ; mol. wt., 152 (by titration).

Anal. Calcd. for $C_3H_7N_5 \cdot HCl$: C, 24.09; H, 5.39; Cl, 23.70. Found: C, 24.34; H, 5.95; Cl, 23.88.

THE LILLY RESEARCH LABORATORIES
INDIANAPOLIS 6, INDIANA

Biosynthesis and Characterization of 11β-Hydroxytestosterone[1]

BY L. R. AXELROD AND G. ARROYAVE[2]

RECEIVED JUNE 15, 1953

The introduction of an hydroxyl group at the 11-position of some steroid compounds by adrenal gland preparations has been reported. Hechter, *et al.*,[3] demonstrated this bioöxidation in the perfused adrenal gland. Other investigators[4,5] have obtained essentially the same results using brei, homogenates and more purified preparations.

Various steroids of the C_{21} and C_{19} types have been shown to undergo this bioöxidation. Consequently, it was considered of interest to investigate the potentiality of the adrenal gland to introduce a C_{11}-hydroxyl group in steroids which are recognized as major secretory products of other endocrine glands, since normally these compounds are present in the circulation and constitute potential substrates for this adrenocortical enzymatic system.

As a representative of these naturally occurring steroids, testosterone was perfused in homologous blood through isolated beef adrenal glands freshly obtained from the abattoir. In other experiments testosterone was incubated with fresh beef adrenal gland brei and homogenates. For the latter experiments the tissue was suspended in a modified Krebs–Ringer phosphate buffer at *p*H 7.4 (calcium ions were omitted), containing sodium fumarate and ATP as cofactors[6] and incubation was continued for two hours at 37° under an atmosphere of 95% oxygen and 5% carbon dioxide. The steroids were extracted from the incubation medium by dialysis according to a technique developed in these laboratories[7] and separated by paper chromatography.

Among several steroids isolated from the perfused medium and incubation mixture, one has been characterized as 11β-hydroxytestosterone (I), hitherto unreported in the literature.

The compound was purified by paper chromatography and crystallized twice from methanol–ether–pentane. White crystals were obtained which melted at 234.5–235.5°; $[\alpha]^{25}D$ 142° (2 mg. in 1.00 ml. of methanol). The infrared absorption spectrum of I is shown in Fig. 1.

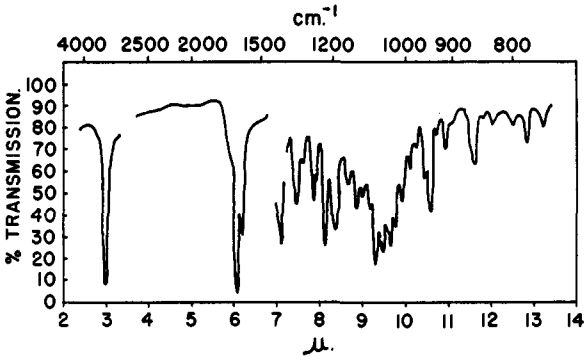


Fig. 1.—Infrared absorption spectrum of 11β-hydroxytestosterone: cell, 0.025 mm.; temp., 23°; concn., 3 mg. in 2 drops of Nujol.

Some characteristics of the compound are presented in Table I.

TABLE I

SOME CHARACTERISTICS OF 11β-HYDROXYTESTOSTERONE

Test	Result	Responsible structure
Modified Zimmerman	Blue	C_3-keto group[8,9]
Modified Lund	Orange	Δ^4-3-keto group[9]
Ultraviolet absorption maxima, mμ	242	α,β-Unsaturated ketone
Triphenyltetrazolium chloride	No reaction	Absence of reducin side chain
Rf value, benzene–formamide	0.07	.....

Chromic acid oxidation of I yielded adrenosterone (II) which was characterized by comparison with an authentic sample, showing the same chromatographic behavior in two different solvent systems, and identical color spot tests, sulfuric acid chromogen absorption spectrum, and ultraviolet

TABLE II

CHARACTERISTICS OF THE OXIDATION PRODUCTS OF 11β-HYDROXYTESTOSTERONE

Compound	Modified Zimmerman reaction	Modified Lund reaction	Ultraviolet absorption max., mμ	*Rf* value, benzene–formamide
II (adrenosterone)	Purple[a]	Orange	238	0.56
III	Blue	Orange	238	.24

[a] Indicative of a C_{17}-keto group.[8,9]

(1) This investigation was supported principally by a research grant from the Jane Coffin Memorial Fund for Medical Research and is based in part on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York.

(2) John Simon Guggenheim Memorial Foundation Fellow.

(3) O. Hechter, R. Jacobsen, R. Jeanloz, H. Levy, C. N. Marshall, G. Pincus and V. Schenker, THIS JOURNAL, **71**, 3261 (1949), and *Arch. Biochem.*, **25**, 457 (1950).

(4) K. Savard, A. A. Green and L. A. Lewis, *Endocrinology*, **47**, 418 (1950).

(5) M. Hayano and R. I. Dorfman, *J. Biol. Chem.*, **201**, 175 (1953).

(6) M. Hayano, R. I. Dorfman and R. V. Yamada, *ibid.*, **193**, 175 (1951).

(7) L. R. Axelrod and A. Zaffaroni, unpublished data.

(8) C. D. Kochakian and G. Stidworthy, *J. Biol. Chem.*, **199**, 607 (1952).

(9) L. R. Axelrod, *ibid.*, in press.

absorption maxima of a methanol solution. The characteristics are summarized in Table II.

The melting point was the same as that of an authentic sample (222.5–224°) and the mixed melting point showed no depression.

Acetylation of I followed by chromic acid oxidation and hydrolysis yielded a new compound III which on simultaneous paper chromatography with the original steroid I and adrenosterone (II) was shown to possess an *Rf* value intermediate between the two. The results of color spot tests and spectrophotometric studies on this compound III are presented in Table II. The presence of an α,β-unsaturated ketone is demonstrated by its ultraviolet absorption maximum at 238 mμ. Further chromic acid oxidation of III yielded adrenosterone. Table III illustrates the characteristics of the sulfuric acid chromogen absorption spectra of compounds I–III.

TABLE III

CONCENTRATED SULFURIC ACID CHROMOGEN ABSORPTION SPECTRA

Compound	Maxima, mμ	O.D.	γ/ml.
11-β-Hydroxytestosterone (I)	295	1.80	30
	385	0.28	
Adrenosterone (II)	280	1.35	25
11-Ketotestosterone ? (III)	285	1.80	30
	355	0.08	

The elementary analysis and the degradation to adrenosterone (II) proved that I is a steroid containing 19 carbons and 3 oxygens, with the oxygen functions at positions 3, 11 and 17, and that the oxygen function on carbon 3 was thereby established to be a Δ^4-3 keto structure. This was substantiated by the characteristic color spot tests and the spectrophotometric data.

The nature of the oxygen moieties on carbons 11 and 17 was deduced from the results of the chromic acid oxidation of the acetate of I followed by hydrolysis to III and the subsequent oxidation of III to adrenosterone (II). The fact that III was chromatographically and qualitatively different from the original steroid was interpreted as indicating that the 11-oxygenated function is a β-oriented hydroxyl group, since the closely related compounds 11α-hydroxytestosterone and 11-ketotestosterone would have remained unchanged upon this treatment. The fact that III was not adrenosterone, but yielded adrenosterone upon oxidation proved that the oxygen function on carbon 17 is an hydroxyl radical. Finally, the β-orientation of this hydroxyl group at position 17 in testosterone was taken as presumptive evidence that the same configuration is present in the biosynthetic product I derived from it.

A brief outline of the procedures is given in the experimental section.

Experimental

Paper Chromatography.—Two chromatographic systems were applied: benzene–formamide[10] and methylcyclohexane–propylene glycol.[9] When chromatographic positions were to be compared for the purpose of identification, quantities of steroids between 25 and 50 γ per cm. width of paper were applied. All chromatograms were run at constant temperature (26°). The position of the spots was determined by cutting strips 0.3 cm. in width from the middle and the sides of the chromatogram and developing with modified Zimmerman reagent[5,9] and modified Lund reagent[9] or by using a fluorescent scanner.[11]

Extraction and Isolation of 11β-Hydroxytestosterone. (a) **Dialysis.**—The blood perfusate or incubation mixture was mixed with 1 part water and 1 part absolute methanol, introduced into Visking tubing (1 inch diameter) and dialyzed against 40% methanol in 250-ml. cylinders. The dialysate was collected each day for 8 days, pooled, brought to 10% methanol by volume with water and extracted five times with 15% by volume of C.P. chloroform.

(b) **Chromatography.**—Aliquots of the chloroform extract were chromatographed using the system benzene–formamide. Whatman No. 1 filter paper strips 17 cm. wide were impregnated with formamide by dipping them in a mixture of formamide and absolute methanol (1:1 by volume), removing the excess of solvents by blotting between two sheets of filter paper and evaporating off the methanol by fanning for 2 to 3 minutes in the air. In a 12-hour chromatogram, run under the conditions described above, 11β-hydroxytestosterone separated from other steroids present in the mixture moving to a position 9 to 12 cm. from the starting line. The steroid was eluted from the paper with absolute methanol and the eluate was evaporated down to dryness at 45° *in vacuo* under a stream of nitrogen. The dry residue was dissolved in warm ether by the addition of a few drops of methanol, pentane was added and the mixture was allowed to stand in the refrigerator overnight. Crystals were obtained m.p. 234.5–235.5°. Recrystallization from the same solvent mixture caused no change in the melting point.

Anal.[12] Calcd. for $C_{19}H_{28}O_3$: C, 74.97; H, 9.27. Found: C, 75.40; H, 9.10.

Chromic Acid Oxidation.—This was done essentially according to the technique described by Zaffaroni, *et al.*[13] Two mg. of I was treated with 1 ml. of 90% acetic acid and 2.5 mg. of chromic anhydride. The mixture was stoppered and allowed to stand at room temperature (24°) for 16 hours. Three ml. of distilled water was then added followed by extraction with four 1-ml. portions of chloroform. The chloroform extract was evaporated to dryness at 45° under nitrogen. Aliquots were taken for the different characterization tests.

Oxidation and Hydrolysis of I Acetate.—One and a half milligrams of I, thoroughly dried, was treated in a 10 × 75 mm. test-tube with 1 ml. of pyridine and 1 ml. of acetic anhydride. The tube was stoppered and the mixture allowed to stand at room temperature (approximately 24°) for 14 hours. The solution was then evaporated to dryness at room temperature under a stream of nitrogen. Several small portions of absolute methanol were added to aid the evaporation of the reagents. The residue was then submitted to chromic acid oxidation as previously described. Extraction of the oxidized acetate from the reaction mixture was carried on with three 0.5-ml. portions of ethyl acetate and three 0.5-ml. portions of chloroform. The combined extracts were evaporated to dryness at 45° under a stream of nitrogen. The residue, containing the acetate, was then treated with 1 ml. of 0.1 *N* sodium hydroxide through which nitrogen had been previously passed in order to displace the dissolved oxygen. The reaction mixture was covered with an atmosphere of nitrogen, stoppered tightly and allowed to stand for 14 hours at room temperature. After addition of four times its volume of distilled water, the free steroid was extracted with four 1-ml. portions of chloroform. The chloroform extract was evaporated to dryness *in vacuo* at 45° under a stream of nitrogen and chromatographed in benzene–formamide. Oxidation of III to adrenosterone was done using the same technique previously described for the chromic acid oxidation of I.

Absorption Spectra of Sulfuric Acid Chromogens.—The technique previously described by Zaffaroni, *et al.*,[14] was applied. A 90 to 100 γ dry sample of the steroid was treated with 3 ml. of concentrated sulfuric acid. After 2 hours standing at room temperature the absorption spec-

(10) R. Burton, A. Zaffaroni and E. H. Keutman, *J. Biol. Chem.*, **188**, 765 (1951).

(11) W. J. Haines and N. A. Drake, *Federation Proc.*, **9**, 180 (1950).

(12) Analysis was performed by Dr. D. Ketchum, Rochester, N. Y.

(13) A. Zaffaroni and R. Burton, *J. Biol. Chem.*, **188**, 749 (1951).

(14) A. Zaffaroni, THIS JOURNAL, **72**, 3999 (1950).

trum between 220 and 600 mμ was read in a DU Beckman spectrophotometer.

DEPARTMENT OF RADIATION BIOLOGY AND
DEPARTMENT OF BIOCHEMISTRY
SCHOOL OF MEDICINE AND DENTISTRY
UNIVERSITY OF ROCHESTER
ROCHESTER, NEW YORK

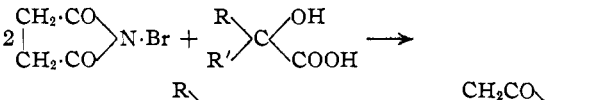
Action of N-Bromosuccinimide on Aliphatic α-Hydroxy Acids

BY MOHAMED ZAKI BARAKAT AND MOHAMED FATHY ABD EL-WAHAB

RECEIVED MAY 4, 1953

It has already been shown that N-bromosuccinimide functions as an oxidizing agent, *e.g.*, it converts primary and secondary alcohols into the corresponding aldehydes and ketones, respectively,[1,2] and in many cases the action is highly selective. Fieser and Rajagopalan have reported high selectivity in the oxidation of the 7α-hydroxyl group of cholic acid and the 6β-hydroxyl group of cholestane-3β,5α,6β-triol by use of N-bromosuccinimide. Whereas 3-hydroxyl groups usually resist attack by N-bromosuccinimide in aqueous acetone, methyl 3α-hydroxy-9α,11α-oxidocholanate[3] is oxidized to the 3-ketone. Selective oxidation of a 3-acyl derivative of methyl cholate[4] to the 7-ketone can be accomplished in high yield with N-bromosuccinimide.

There appears to have been no report on the action of this reagent with α-hydroxy acids. We have shown that N-bromosuccinimide reacts readily on heating in an aqueous solution with aliphatic α-hydroxy acids, *e.g.*, glycolic, lactic, mandelic and benzilic acids, yielding aldehydes or ketones containing one carbon atom less, *e.g.*, formaldehyde, acetaldehyde, benzaldehyde and benzophenone, respectively. Evolution of carbon dioxide and bromine was demonstrated in all cases. Succinimide has been isolated in the reaction with lactic and benzilic acids.



$$2\,\mathrm{CH_2(CO)_2N{\cdot}Br} + \mathrm{RR'C(OH)COOH} \longrightarrow \mathrm{RR'C{=}O} + \mathrm{CO_2} + \mathrm{Br_2} + 2\,\mathrm{CH_2(CO)_2NH}$$

R = R′ = H : glycolic acid ⟶ formaldehyde
R = CH_3, R′ = H : lactic acid ⟶ acetaldehyde
R = C_6H_5, R′ = H : mandelic acid ⟶ benzaldehyde
R = R′ = C_6H_5 : benzilic acid ⟶ benzophenone

Compared with the fatty acids the corresponding α-hydroxy acids possess higher dissociation constants, and this may explain why such a reaction takes place; an analogous case may be the degradation of aliphatic dicarboxylic acids, *e.g.*, oxalic acid,[5] by N-bromosuccinimide in aqueous medium at room temperature.

The conversion of benzilic acid to benzophenone when treated with N-bromosuccinimide provides a new route to pass from α-diketones, *e.g.*, benzil, to aromatic ketones, *e.g.*, benzophenone.

Experimental

Action of N-Bromosuccinimide on Aliphatic α-Hydroxy Acids. (1) **Isolation of Aldehydes.** (a).—N-Bromosuccinimide (1.78 g., 2 moles) and glycollic acid (0.38 g., 1 mole) or lactic acid (0.43 cc., 1 mole) or mandelic acid (0.76 g., 1 mole) in distilled water (20 cc.) were refluxed in the apparatus previously described (Schonberg, Moubasher and Mostafa[6]) in a stream of carbon dioxide for 20 minutes. The receiver contained an ice-cold solution of 2,4-dinitrophenylhydrazine sulfate (0.6 g.) in alcohol (20 cc.). Yellow or orange crystals deposited and were recrystallized from the proper solvent (ligroin, alcohol and ethyl acetate) to give the 2,4-dinitrophenylhydrazone of formaldehyde, acetaldehyde and benzaldehyde, respectively, in 50% yields, identified by their m.p. and mixed m.p. with authentic samples.

(b) **Formation of Bromine, Carbon Dioxide and Succinimide in the Degradation.**—N-Bromosuccinimide (1.78 g.) and lactic acid (0.43 cc.) in distilled water (20 cc.) were heated for 20 minutes; the mixture was then concentrated by heat to a small volume (about 2 cc.) and allowed to cool; the colorless crystals which deposited were pressed on a porous plate and recrystallized from benzene. They were proved to be succinimide by m.p. and mixed m.p. (yield 0.5 g.).

The evolution of bromine and carbon dioxide during the degradation was demonstrated by passing the gases evolved during the reaction, first into 10% silver nitrate solution acidified with nitric acid and then into baryta water. A yellowish-white precipitate of silver bromide deposited, while the baryta water became turbid.

(2) **Isolation of Ketones.**—It is sufficient to describe one example in detail to illustrate the procedure.

N-Bromosuccinimide (1.78 g., 2 moles) and benzilic acid (1.14 g., 1 mole) in distilled water (100 cc.) were refluxed for 30 minutes. The reaction started after heating for 2 minutes with evolution of bromine vapor. The N-bromosuccinimide and benzilic acid gradually dissolved and an oil began to separate. At the end of the reaction, the mixture was allowed to cool and extracted with ether. The aqueous layer was concentrated to a small volume (about 5 cc.) and on standing deposited colorless crystals of succinimide, which after recrystallization from benzene were identified by m.p. and mixed m.p. (yield 0.6 g.).

The ethereal layer was dried over anhydrous sodium sulfate for 12 hours, filtered and concentrated to yield an oil which soon crystallized. The solid was recrystallized from aqueous alcohol to give benzophenone (m.p. and mixed m.p.) in 85–90% yield. The evolution of carbon dioxide during the reaction was demonstrated as above.

Acknowledgment.—The authors thank Dr. M. M. El-Sadr for his interest in this work and acknowledge their gratitude to the National Aniline Division, New York 6, New York, for supplying N-bromosuccinimide.

(1) L. F. Fieser and S. Rajagopalan, THIS JOURNAL, **71**, 3935 (1949); *ibid.*, **71**, 3938 (1949).

(2) M. Z. Barakat and G. M. Mousa, *J. Pharm. and Pharmacol.*, **4**, 115 (1952).

(3) L. F. Fieser, H. Heymann and S. Rajagopalan, THIS JOURNAL, **72**, 2306 (1950).

(4) L. F. Fieser and S. Rajagopalan, *ibid.*, **72**, 5530 (1950).

(5) M. S. Barakat, *J. Pharm. and Pharmacol.*, **4**, 882 (1952).

(6) Schönberg, Moubasher and Mostafa, *J. Chem. Soc.*, 176 (1948).

BIOCHEMISTRY DEPARTMENT
IBRAHIM PASHA EL-KEBIR UNIVERSITY
FACULTY OF MEDICINE
ABBASSIA, CAIRO, EGYPT

The High Field Conductance of Aqueous Solutions of Ammonia at 25°[1]

BY DANIEL BERG AND ANDREW PATTERSON, JR.

RECEIVED JULY 6, 1953

The high field conductance of aqueous solutions of ammonia, between 1.3 and 1.5 × 10^{-3} *M*, has been measured at 25.00° relative to potassium chloride. The high field conductance data are

(1) Contribution No. 1166 from the Department of Chemistry, Yale University.