changed at 204–209° and the substance melted at 212–215°; $[\alpha]_D^{18}$ +86.5° (*c*, 0.4108); $[M]_D$ +301°; infrared: $\nu_{max.}^{KBr}$ 3600, 1740, 1257, 1240, 1023 (axial acetoxy) cm.$^{-1}$.

Etiocholane-3,11,17-trione (IX). A. To a solution of 93 mg. of authentic 3α,11β,17α,21-tetrahydroxypregnan-20-one (X) in 3 ml. of glacial acetic acid a solution of 237 mg. of chromium trioxide in 0.4 ml. of glacial acetic acid and 0.1 ml. of water was added and the mixture was left for 16 hr. at room temperature. The reaction mixture was processed in the usual manner and the residue was dissolved in a mixture of ethyl acetate and ether. After two weeks in the refrigerator, 22 mg. of IX m.p. 128–131° separated.

B. A solution of 15 mg. of 3β,11β-dihydroxyetiocholan-17-one (VIIIa) in 0.5 ml. of pyridine was added to a suspension of 20 mg. of chromium trioxide in 0.2 ml. of pyridine and the mixture was left for 16 hr. at room temperature. The product was recovered in the usual manner and was crystallized as above to yield 2 mg. of IX, m.p. 129–131°, infrared: $\nu_{max.}^{KBr}$ 1745, 1705 cm.$^{-1}$.

3α,11β-Dihydroxyethicholan-17-one (XIa). A. The side chain of IIa was cleaved with sodium bismuthate and the steroid was recovered as previously described. The residue was crystallized from ethyl acetate to produce XIa.

B. Authentic 3α,11β,17α,21-tetrahydroxypregnan-20-one was oxidized as above to yield XIa.

The infrared spectra of both samples were identical. The recrystallized sample showed a m.p. 239–241°; infrared: $\nu_{max.}^{KBr}$ 3620, 1715, 1031 (equatorial hydroxyl).

3α,20β,21-Triacetoxy-17α-hydroxypregnan-11-one (XIIb). The sirupy triacetate IIb, 40 mg., was dissolved in pyridine, 0.5 ml., and oxidized for 16 hr. at room temperature with a suspension of 70 mg. of chromium trioxide in 0.7 ml. of pyridine. The reaction mixture was processed as previously described to yield 29 mg. of XIIb, m.p. 197–200°. Recrystallization from ethyl acetate-neohexane raised the m.p. to 202–203°; $[\alpha]_D^{20}$ +104° (*c*, 0.6561); $[M]_D$ +513°; infrared; $\nu_{max.}^{KBr}$ 3600, 1738, 1698, 1245, 1026 (equatorial acetoxy) cm.$^{-1}$.

3α,17α,20β,21-Tetrahydroxypregnan-11-one (XIIa). A solution of 10 mg. of potassium bicarbonate in 0.1 ml. of water was added to a solution of 10 mg. of XIIb in 0.5 ml. methanol. The air was replaced with nitrogen and the mixture was left for 16 hr. at room temperature. After the addition of a drop of acetic acid the volatile components were removed *in vacuo* and the residue dissolved in ethyl acetate. The ethyl acetate solution was washed with water and dried over sodium sulfate. On concentration the solution gave 4.5 mg. of XIIa, m.p. 240–245°; $[\alpha]_D^{21}$ +28.4° (*c*, 0.2047); $[M]_D$ +104°; infrared: $\nu_{max.}^{KBr}$ 3550, 1700, 1045 cm.$^{-1}$.

3β,11β-Dihydroxyandrostan-17-one (XIII). A. A solution of 30 mg. of IVa in 15 ml. of methanol was treated with 6 ml. of a stock solution of sodium metaperiodate[3i,1b] and was left for 120 min. in the dark at room temperature. The reaction mixture was processed as previously described[1b] to yield 20 mg. of XIII. The product was recrystallized from ethyl acetate, and showed a m.p. 225–228°.

B. A portion of VIa was oxidized with sodium bismuthate and the 17-ketosteroid was isolated in the usual manner.

The infrared spectra of both samples were identical with that of authentic XIII.[12]

Spectra of 3β,11β,17α,21-tetrahydroxyallopregnan-20-one (VIa), *of* VIa-*hemihydrate and of the diacetate* VIb *in sulfuric acid solution.* The solutions and the spectra were prepared as described by E. Caspi and M. M. Pechet.[19] The spectra were identical and changed in an identical manner with time.

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[CONTRIBUTION FROM THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY AND CHEMISTRY DEPARTMENT, CLARK UNIVERSITY]

Synthesis of Radioactive Dehydroepiandrosterone[1,2]

MARCEL GUT AND MILAN USKOKOVIĆ

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A method for the synthesis of dehydroepiandrosterone-4-C^{14} using testosterone-4-C^{14} as starting material and 17-yl sulfite as an intermediate is described. Also described is specifically tritiated dehydroepiandrosterone, obtained by the reduction of 7α-bromodehydroepiandrosterone acetate.

Endocrinological studies on the metabolism of dehydroepiandrosterone prompted us to study the preparation of radioactive dehydroepiandrosterone whereby highest specific activity might be obtained. We are reporting here the synthesis of dehydroepiandrosterone-C^{14} labelled in position 4 and of dehydroepiandrosterone-H^3 labelled mostly in position 7.

The only previously published method for the preparation of isotopically labelled dehydroepiandrosterone (dehydroepiandrosterone-16-C^{13}) by E. B. Hershberg *et al.*[3] was rather involved, requiring the preparation and use of diazomethane C^{14}. Furthermore a few reports[4] on the bio-oxidation of ring D justified the elaboration of a ring A, B, or C labelled dehydroepiandrosterone.

(1) Presented in part at the 133rd Meeting of the American Chemical Society, San Francisco, Calif., April 1958.

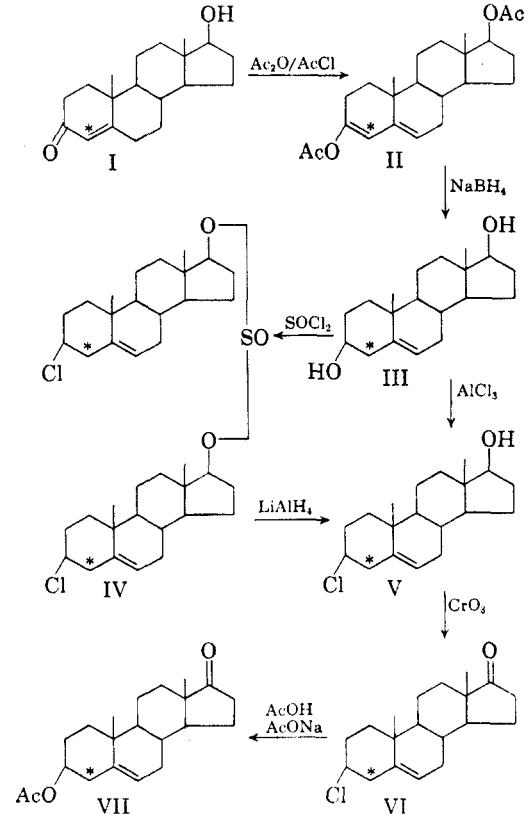
(2) This investigation was supported in part by research grants USPH C-231 and A-2672.

(3) E. B. Hershberg, E. Schwenk, and E. Stahl, *Arch. Biochem.* **19**, 300 (1948).

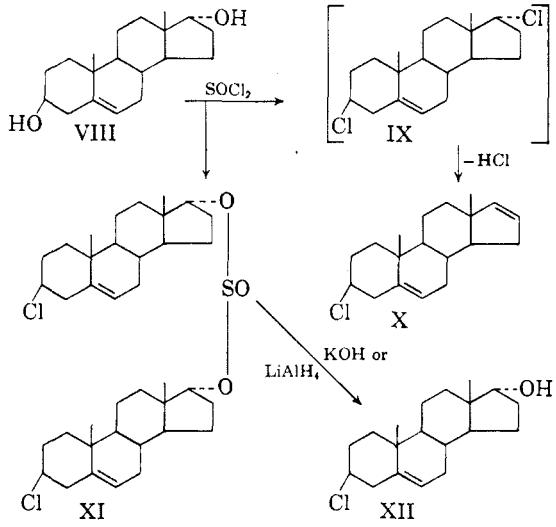
(4) R. D. H. Heard, R. Jacobs, V. O'Donnell, F. G. Peron, J. C. Saffran, S. S. Solomon, L. M. Thompson, H. Willoughby, and C. H. Yates, *Recent Progress in Hormone Research*, 9, 386, (1954).

Since the readily available testosterone-4-C^{14} (I) can easily be transformed to either androst-4-ene-3,17-dione or to androst-5-ene-3β,17β-diol, the main problem of this synthesis consists of either a selective reduction of the 3-ketone (with concomitant shift of the double bond to the 5,6-position), or of the selective oxidation of the 17-hydroxyl group. The latter was carried out as follows:

Testosterone-4-C^{14} (I) was converted to its enol diacetate (II) (with acetic anhydride and acetyl chloride) and the crude reaction product was reduced with sodium borohydride in methanol. The reduced product was refluxed with methanolic hydrochloric acid to hydrolyze residual 17-acetate and eliminate the allylic alcohols which are always obtained as by-products from the hydride-reduction of the enol acetate. The resulting mixture was then chromatographed on silica gel whereby androst-3,5-dien-17β-ol-4-C^{14} and androst-5-ene-3α,-17β-diol-4-C^{14} were separated from the desired androst-5-ene-3β,17β-diol-4-C^{14} (III). The latter product, on treatment with thionyl chloride, yielded bis(3β-chloroandrost-5-en-17β-yl)-sulfite-4,4'-C^{14} (IV). The chloroester was cleaved with lithium aluminum hydride, with retention of the homoallylic chlorine, and the resulting 3β-chloroandrost-5-en-17β-ol-4-C^{14} (V)[5] was oxidized with chromic oxide in acetic acid (the short duration of the oxidation made it unnecessary to protect the double bond by bromination) to 3β-chloroandrost-5-en-17-one-4-C^{14} (VI). This product gave the desired dehydroepiandrosterone-4-C^{14} as its acetate (VII) after refluxing with anhydrous sodium acetate in acetic acid.



In connection with the formation of the above mentioned sulfite ester, the reaction of androst-5-ene-3β,17α-diol with thionyl chloride was examined. As already described, the more[6] hindered 17β-hydroxyl (III) (pseudo equatorial conformation) reacts with thionyl chloride to give the 17β-sulfite ester, while the less[6] hindered 17α-hydroxyl (VIII) (pseudo axial) gives as main product the extremely unstable 17α-chloro compound (IX). The latter could not be isolated as such, but its Δ^{16}-analog (X) was obtained after spontaneous elimination of the elements of hydrochloric acid. In addition very little 17α-sulfite ester (XI) was also isolated. This ester could be hydrolyzed with lithium aluminum hydride or with a methanolic potassium hydroxide solution to the 17α-alcohol.



The striking difference in behavior of the 17α- and 17β-hydroxyl towards thionyl chloride is noteworthy and probably due to differences in steric factors.

Tritiated dehydroepiandrosterone was prepared by catalytic reduction of 7α-bromodehydroepiandrosterone acetate with tritium. We found, however, that more than the calculated amount of tritium had entered; we shall report at a later date on the position and amount of tritium introduced.

EXPERIMENTAL

Melting points were determined on a Fisher-Johns hot stage and are uncorrected. Analyses were performed by

(5) This compound could also be obtained though in lower yield, from the corresponding diol by the action of anhydrous aluminum chloride, causing selective replacement of the 3β-hydroxyl group: J. Broome, B. R. Brown, and G. H. R. Summers, *J. Chem. Soc.*, 2071 (1957).

(6) In this pentacyclic alcohol the pseudo equatorial hydroxyl is indeed more hindered (mainly from the 18-methyl group) than the pseudo axial 17α-hydroxyl as can readily be seen from inspection of a molecular model.

Schwarzkopf Microanalytical Laboratory, Woodside 77, N. Y. Ultraviolet absorption spectra were determined in methanol by means of a Cary Model 11 MS spectrophotometer. Optical rotations were determined in a 1-dm. semimicro tube. The infrared spectra were obtained from a pressed potassium bromide prism and taken on a Perkin-Elmer Model 12C spectrometer. All chromatographic separations were made on Davison Silica Gel mesh 100–200.

Testosterone-4-C^{14} (I). This material was prepared following exactly the procedure given by G. I. Fujimoto.[7]

Testosterone enol diacetate-4-C^{14} (II). This substance was prepared as described by U. Westphal.[8] The crude product was recrystallized once from methanol which contained a few drops of triethylamine and melted then at 145–151°, $[\alpha]_D^{23}$ − 147° (chloroform), ultraviolet maximum λ 239 mμ (ε 15100).

Androst-5-ene-3β,17β-diol-4-C^{14} (III). A solution of 1.5 g. of testosterone enol diacetate-4-C^{14} in 10 ml. methanol was added to a suspension of 1 g. of sodium borohydride in 30 ml. of methanol and allowed to stand with occasional shaking at 25° for one day. One additional gram of sodium borohydride was added in portions while the reaction mixture stood for an additional day. Then the mixture was evaporated to dryness, the residue taken up in chloroform, and the chloroform extract washed successively with 2*N* hydrochloric acid, water, 2*N* sodium carbonate solution and again with water. The residue which remained after evaporation of the chloroform was dissolved in 50 ml. of 95% ethanol, 1 ml. of concentrated hydrochloric acid was added, and the resulting solution refluxed under nitrogen for 2 hr. This solution was again evaporated to dryness, extracted with chloroform, the chloroform extract washed with water, 2*N* sodium carbonate solution and water. After drying and evaporation, the residue was dissolved in little benzene and chromatographed. The benzene fractions gave some crystallized material, consisting, most likely, of androsta-3,5-dien-17β-ol-4-C^{14}. The benzene-ethyl acetate fractions gave first a small amount of androst-5-ene-3α,17β-diol-4-C^{14}, melting at 199–205°, and then androst-5-ene-3β,17β-diol-4-C^{14}, melting at 175–178° (not depressed on admixture to authentic material). The yield was 35%.

Bis(3β-chloroandrost-5-en-17β-yl) sulfite-4,4'-C^{14} (IV). To 500 mg. of androst-5-ene-3β,17β-diol-4-C^{14} were added 10 ml. of thionyl chloride and the mixture was shaken for one minute with moisture excluded (until all solid material was dissolved). The solution was evaporated to dryness and the residue stored in a vacuum desiccator over solid sodium hydroxide for 2 days. The remaining crystals, which were now free from the last traces of thionyl chloride, were recrystallized from large amounts of acetone and melted at 231–232° with slow decomposition; yield 82%; $[\alpha]_D^{20}$ − 122° ± 3° (*c*, 0.895 in chloroform); ultraviolet maximum: none; infrared maxima: ν_{max} 3540, 1439, 767, 760 (—Cl), 1200 (sulfite), 822 (trisubstituted double bond), 894 (five ring), 802 (six ring) cm.$^{-1}$.

Anal. Calcd. for $C_{38}H_{56}O_3Cl_2S$: C, 68.75; H, 8.50; Cl, 10.68; S, 4.83. Found: C, 68.93; H, 8.50; Cl, 10.65; S, 4.62.

3β-Chloroandrost-5-en-17β-ol-4-C^{14} (V). A suspension of 305 mg. of bis(3β-chloro-androst-5-en-17β-yl) sulfite-4,4'-C^{14} in 150 ml. ether containing 900 mg. lithium aluminum hydride was refluxed overnight in a dry atmosphere. Then ethyl acetate was added dropwise to the reaction mixture until the excess hydride was consumed. Addition of saturated sodium sulfate solution and then of solid anhydrous sodium sulfate produced a dry ether solution, which was decanted. The residue was extracted several times with ethyl acetate, the extracts added to the ether solution and the combined solutions finally evaporated to dryness. Chromatography of the residue gave, with the benzene-ethyl acetate eluates, colorless prisms, melting 160–163°, $[\alpha]_D^{23}$ −41° ± 3° (*c*, 1.11 in chloroform); ultraviolet maximum: none; infrared maxima: ν_{max} 3350, 1465, 1440, 870, 821, 800, 764 cm.$^{-1}$. Kuwada and Miyasaki[9] give m.p. of 163°.

3β-Chloroandrost-5-en-17-one-4-C^{14} (VI). To the solution of 120 mg. of 3β-chloroandrost-5-en-17β-ol-4-C^{14} in 15 ml. acetic acid was added 1.5 equivalents chromium oxide in 5 ml. 90% aqueous acetic acid and the mixture was allowed to stand for 10 min. at room temperature. Then 5 drops of methanol were added, the solution evaporated to dryness and the residue extracted with benzene. The benzene extract was washed with water, dried and evaporated. Upon chromatography the benzene-ether eluates gave the desired chloro ketone in 81% yield; melting point 155–157° (same melting point on admixture with authentic material).

Dehydroepiandrosterone acetate-4-C^{14} (VII). Three g. of fused powdered sodium acetate was added to a solution of 80 mg. of 3β-chloroandrost-5-en-17-one-4-C^{14} in 30 ml. of acetic acid and the mixture was refluxed for 18 hr. The reaction mixture was extracted with benzene, and the benzene solution washed with 2*N* sodium carbonate solution. The acetate, obtained after evaporation of the benzene, was chromatographed and 76 mg. of dehydroepiandrosterone acetate-4-C^{14}, m.p. 169–171° (unchanged upon admixture with authentic material), was obtained from the benzene-ethyl acetate eluate.

Reaction of thionyl chloride with androst-5-ene-3β,17α-diol.[10] *3β-Chloroandrosta-5,16-diene* (X), *bis(3β-chloroandrost-5-en-17α-yl) sulfite* (XI), *and 3β-chloroandrost-5-en-17α-ol* (XII). To 550 mg. of finely powdered androst-5-ene-3β,17α-diol were added 5 ml. of thionyl chloride and the reaction mixture, with exclusion of atmospheric moisture, immediately cooled to −70°. After all crystals were dissolved (approx. 3 min.), the solution was evaporated to dryness *in vacuo* at −20° and stored for 2 weeks in a vacuum desiccator over solid sodium hydroxide. However, as soon as the crude semicrystalline product was exposed to atmospheric moisture, a pungent gas escaped. The product was chromatographed without any attempt at recrystallization. The hexane eluates furnished, in a yield of 16%, 3β-chloroandrosta-5,16-diene. The analytical sample which was sublimed at $35°_{0.01}$ had a m.p. of 74–76°; $[\alpha]_D^{21}$ −115° ± 3° (*c*, 1.385 in acetone); ultraviolet maximum: none; infrared maxima: ν_{max} 3500, 1440, 872, 839, 817, 805, 760, 725 cm.$^{-1}$.

Anal. Calcd. for $C_{19}H_{27}Cl$: C, 78.45; H, 9.36; Cl, 12.19. Found: C, 78.24; H, 9.62; Cl, 12.00.

From the benzene eluates were obtained, in a yield of 7%, bis(3β-chloroandrost-5-en-17α-yl) sulfite, m.p. 235–236°, with instantaneous decomposition; $[\alpha]_D^{20}$ −36° ± 4° (*c*, 1.00 in chloroform); ultraviolet maximum: none; infrared maxima: ν_{max} 1460, 1440, 1205, 877, 829, 764, 730 cm.$^{-1}$.

In another run the crude reaction product was treated with 5% methanolic potassium hydroxide solution for 2 hr. at 25°, the mixture then extracted with ether, washed with water, dried, evaporated, and chromatographed. The hexane fractions gave the above described 3β-chloroandrosta-5,16-diene and from the benzene-ether fractions a third compound was isolated in a yield of 11%, namely 3β-chloroandrost-5-en-17α-ol, m.p. 140–141°; $[\alpha]_D^{20}$ −82° ± 3° (*c*, 0.815 in chloroform); ultraviolet maximum: none; infrared maxima: ν_{max} 3450, 1460, 1435, 823, 760 cm.$^{-1}$.

Anal. Calcd. for $C_{19}H_{29}OCl$: C, 73.88; H, 9.47; Cl, 11.48. Found: C, 74.25; H, 9.49; Cl, 10.98.

The same compound was also obtained from the reduction of bis(3β-chloroandrost-5-en-17α-yl) sulfite with lithium aluminum hydride.

7α-Bromo-3β-ol-androst-5-en-17-one acetate. This material was prepared exactly as described by Antonucci *et al.*[11]

(7) G. I. Fujimoto, *J. Am. Chem. Soc.*, **73**, 1856 (1951).

(8) U. Westphal, *Chem. Ber.*, **70**, 2128 (1936).

(9) S. Kuwada and M. Miyasaki, *J. Pharm. Soc. Japan*, **57**, 234 (1937).

(10) Kindly donated by Dr. Horst Witzel, Schering A. G., Berlin.

(11) R. Antonucci, S. Bernstein, D. Giancola, and K. J. Sax, *J. Org. Chem.*, **16**, 1126 (1951).

*Dehydroepiandrosterone acetate-7α(?)-H*³.[12] The solution of 103 mg. of 7α-bromo-3β-hydroxyandrost-5-en-17-one acetate was added to the suspension of 1075 mg. of 5% palladium on calcium carbonate which was prereduced. The mixture was shaken with 5 ml. hydrogen to which 2 curies of tritium were added for 1 hr. The reduction mixture was filtered, the filtrate evaporated, and the residue hydrolyzed with methanolic *N* sodium hydroxide solution at room temperature overnight. The hydrolyzed product was worked up and reacetylated. Purification of the acetate by chromatography yielded 48 mg. (57%) of crystalline dehydroepiandrosterone acetate-7α-H³ with the benzene-ethyl acetate eluates. After recrystallization from methanol it melted at 168–171° and its infrared absorption spectrum was identical[13] with the spectrum of authentic material. The specific radioactivity of this sample was 3.5 mC per mg. On subsequent hydrolysis followed by reacetylation the specific activity remained unchanged.

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(12) Compare D. K. Fukushima, S. Lieberman and B. Praetz, *J. Am. Chem. Soc.*, **72**, 5205 (1950).

(13) The relatively low tritium concentration does not produce changes in the fingerprint region.

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF THE UNIVERSITY OF CALIFORNIA]

Utilization of Gas Phase Chromatography for Identification of Volatile Products from Alkaline Degradation of Herqueinone

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Low molecular weight carbonyl compounds may be conveniently identified by gas chromatography of their oximes. By use of di-2-ethylhexyl phthalate as partitioning agent, there may be identified in presence of each other the aldehydes with 2–4 carbons, acetone, butanone, and 3-methyl-2-butanone. From the alkaline degradation of herqueinone, the only volatile carbonyl component detected by this method was acetaldehyde. The volatile acids from alkaline degradation of herqueinone, also examined by gas chromatography, have been found to be a mixture of formic, acetic, and isobutyric acids.

In an earlier publication[1] from this laboratory, there was reported an examination of the volatile carbonyl component obtained by heating herqueinone, the red pigment from *Penicillium herquei*, with aqueous alkali. Although the 2,4-dinitrophenylhydrazone originally precipitated from the aqueous distillate agreed rather well in properties with the derivative of acetaldehyde, it was concluded from chromatography experiments that the derivative must be either a mixture, or some other substance than the derivative of acetaldehyde. In a simultaneous publication[2] by Raistrick and co-workers, it was reported that the volatile carbonyl component is acetaldehyde, on the basis of the properties of the 2,4-dinitrophenylhydrazone. Since our earlier results based on chromatography on silicic acid and on Bentonite were probably obscured[3] by decomposition or isomerization of the hydrazone, this carbonyl component has been reexamined by use of gas phase chromatography.

Since acetaldehyde is inconveniently volatile, certain derivatives were considered for use in gas chromatography, and the oxime was found to be well adapted for gas chromatography of low molecular weight carbonyl compounds. A procedure has been developed for formation of this derivative from an aqueous solution of 5–25 mg. of the carbonyl compounds. As shown in Table I, the several compounds examined have sufficiently different retention times to permit their detection.

TABLE I

RETENTION TIMES OF OXIMES IN GAS PHASE CHROMATOGRAPHY

Oxime	Retention Time[a] (min.: sec.)
Acetaldehyde	3:35
Acetone	4:45
Propionaldehyde	5:10
Isobutyraldehyde	6:45
Butanone	7:55
n-Butyraldehyde	9:10
3-Methyl-2-butanone	10:55

[a] Retention time, which is given in minutes and seconds, was taken as time elapsing between injection and maximum in peak. The column was 2 meters × 8 mm. o.d., packed with 30–60 mesh Celite firebrick impregnated with 3% di-2-ethylhexyl phthalate; temperature, 88°; helium flow rate, 35 ml./min.

When the volatile neutral material from alkaline degradation of herqueinone was converted to the oxime and subjected to gas chromatography, a single peak was observed with the retention time of acetaldoxime; no other peak was observed after lapse of 55 minutes. Thus, our observations based on gas chromatography are in accord with the report of the British investigators[2] that acetaldehyde is the only steam-volatile carbonyl component

(1) R. E. Harman, J. Cason, F. H. Stodola, and A. L. Adkins, *J. Org. Chem.*, **20**, 1260 (1955).

(2) J. A. Galarraga, K. G. Neill, and H. Raistrick, *Biochem. J.*, **61**, 456 (1955).

(3) Work to be published in *J. Am. Chem. Soc.* on the alterations of phenylhydrazones has been carried out by Professor H. Rapoport and R. J. O'Connor in this department.