100-ml. portions of 5% acetic acid. The acetic acid extract was adjusted to *p*H 7.5, and 8 g. of precipitated amorphous alkaloids were separated by filtration. This product was dissolved in 60 ml. of ethanol, and 4 g. of oxalic acid in 20 ml. of hot ethanol was added. After standing overnight, a colorless crop of fine needles precipitated. These crude crystals were digested in 130 ml. of hot ethanol for 20 minutes to yield larger crystals, Wt. 4.0 g., m.p. 265–266° (dec.). Recrystallization from hot ethanol did not change the melting point. An 0.8-g. portion of this material was converted to the free base and was crystallized twice from benzene to yield 0.48 g. of colorless crystals, m.p. 213–214° (d.) $[\alpha]_D^{25}$ +5.6° (c, 1, $CHCl_3$). The infrared spectrum of this material was identical to that of serpine from *R. serpentina* (m.p. 213°) $[\alpha]_D^{25}$ +70° (pyridine),[1] and like serpine, it showed four spots on chromatography on formamide-impregnated paper, with benzene-chloroform as the developing solvent. Two major spots, R_f = 0.40 and 0.62, were identical with yohimbine and rauwolscine respectively, while two minor spots, of slower R_f appeared to be due to traces of other alkaloids. Four recrystallizations of this product from benzene raised the melting point somewhat to 217–219° (dec.), without significantly changing the paper chromatographic picture.

Separation of serpine from R. heterophylla into yohimbine and rauwolscine. A 0.2-g. portion of serpine, from *R. heterophylla*, m.p. 217–219°, was dissolved in 0.5 ml. of hot methanol, and mixed with 0.17 g. of picric acid in 2 ml. of hot methanol. Water, 0.25 ml. was added to incipient turbidity. The crystalline picrate which separated (0.15 g.) melted at 191–195° (dec.). The picrate was converted to the free base, and crystallized from benzene, and then from aqueous ethanol to yield about 100 mg. of the characteristic twinned prisms of rauwolscine m.p. 233–235° (dec.) mixture m.p. with an authentic sample of rauwolscine, m.p. 233–235°, not depressed. The infrared spectrum and R_f on paper chromatography (0.62) were also identical to those of pure rauwolscine. The hydrochloride salt, which separated from aqueous acid as long needles, had m.p. 268–270° (dec.) $[\alpha]_D^{25}$ +70° (0.5 in water).

The mother liquors from the preparation of the picrate were converted to the base, and crystallized from benzene-ligroin, then from aqueous methanol to yield about 20 mg. of yohimbine, m.p. 220–222°, mixture m.p. with an authentic sample, m.p. 221–223°, not depressed. The R_f on paper chromatography, 0.40 and the infrared spectrum were identical to those of authentic yohimbine.

Separation of serpine from R. serpentina into yohimbine and rauwolscine. A 10-mg. portion of serpine[1] (obtained from Dr. A. Chatterjee) m.p. 213–214° dissolved in 0.1 ml. of methanol was mixed with 9 mg. of picric acid in 0.2 ml. of methanol; then 0.05 ml. of water was added, and the solution was left overnight at room temperature. The crystalline precipitate was separated by filtration, suspended in 0.5 ml. of chloroform, and washed with 0.5 ml. of 5% ammonium hydroxide to convert it to the base. The chloroform solution was concentrated to dryness, and the amorphous residue was crystallized from aqueous methanol to yield about 2 mg. of colorless crystals of rauwolscine, m.p. 230–233° (Kofler hot stage) mixture m.p. with an authentic sample, m.p. 236–238° not depressed. The infrared spectrum and paper chromatographic behaviour of this material were identical to those of pure rauwolscine.

The mother liquors from the isolation of the rauwolscine picrate were converted to the base, and the amorphous base was crystallized from aqueous methanol to yield about 1 mg. of yohimbine, m.p. 210–218° (Kofler hot stage); mixture m.p. with an authentic sample, m.p. 223–224°, not depressed. The infrared absorption spectrum and behavior on paper chromatography were identical to those of authentic yohimbine, though the melting point suggests that the preparation was not pure.

Acknowledgment. We are greatly indebted to Dr. A. Chatterjee, who made this study possible by providing authentic rauwolscine and serpine. We also wish to acknowledge the interest and suggestions of Dr. C. Djerassi. We are indebted to Mrs. A. Paradies, for her very capable help with this study, and to Mr. W. Boegemann for the paper chromatographic studies.

RESEARCH LABORATORIES
CHAS. PFIZER AND CO., INC.
BROOKLYN 6, NEW YORK

The Oxidation of Cholesterol by Periodic Acid

R. P. GRABER, C. S. SNODDY, JR.,
H. B. ARNOLD, AND N. L. WENDLER

Received July 20, 1956

The recent appearance of an article by Chatterjee and Majumdar,[1] describing the cleavage oxidation of certain isolated and terminal double bonds by periodic acid, prompts us to report findings of a related nature arising from a study of the effect of this reagent on steroidal olefins. Our observations revealed that the Δ^5 double bond of the steroid nucleus may respond more or less specifically to the oxidizing action of periodic acid and this reagent, therefore, may be of diagnostic value for this grouping.[2]

Periodic acid has generally been considered to be a specific reagent for the cleavage oxidation of glycol and ketol systems. However, we have found that cholesterol (I) is oxidized at room temperature by periodic acid in aqueous tetrahydrofuran to give cholestane-3β,5α,6β-triol (III) in 60% isolated yield. Moreover, by greatly increasing the reagent concentration and extending the time interval of reaction the concomitant formation of 3β,5α-dihydroxycholestane-6-one (V) also occurred.

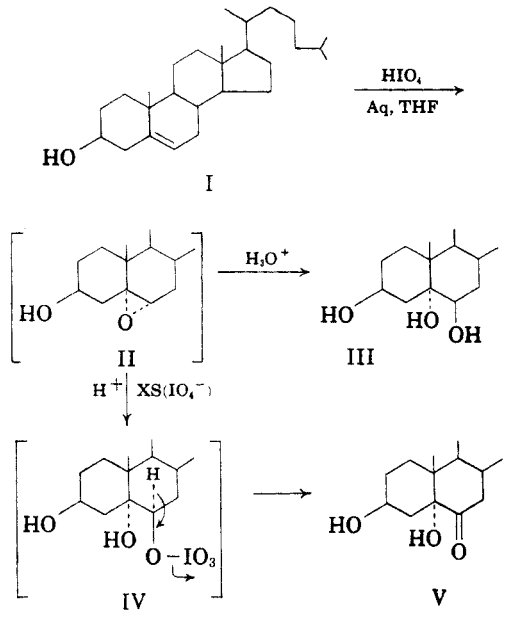
A rate study run under the optimum conditions found for producing the triol indicated that one mole of periodate was consumed per mole of steroid and that the reaction was essentially complete in 48 hours. Heating accelerated the oxidation reaction but gave an inferior melting product.

The periodate oxidation of cholesterol probably proceeds by way of a 5,6-oxido derivative (II) as suggested by the Indian authors to explain their particular findings. This route further would be compatible with the known conversion in high yield of cholesterol oxide to III by periodic acid

(1) Chatterjee and Majumdar, *Anal. Chem.*, **28**, 878 (1956).

(2) For example $\Delta^{9(11)}$-dehydrotigogenin acetate and $\Delta^{9(11)}$-anhydrohydrocortisone acetate were not functionally affected by this reagent.

instead of cleavage in the normal manner.[3] It is suggested that at higher periodate concentrations, periodate ion competes favorably in the oxide



scission to give an intermediate periodate ester (IV) which collapses by α-elimination to the 6-ketone (V) and iodate. This interpretation is suggested since the triol (III) itself does not give any isolable 6-ketone when independently treated under the conditions effecting the conversion I → V.

EXPERIMENTAL

3β,5α,6β-Trihydroxycholestane (III). A solution of 1.16 g. (0.003 mole) of cholesterol in 50 ml. of purified tetrahydrofuran was treated with a solution of 2.05 g. (0.009 mole) of periodic acid dihydrate in 15 ml. of water and the clear colorless mixture was stored at room temperature for 94 hours. At the end of this time, the deep red solution was treated with excess 10% aqueous sodium thiosulfate which discharged the color. The resulting colorless solution was concentrated *in vacuo* to remove the tetrahydrofuran and the colorless oil which separated was extracted with three portions of ethyl acetate. The combined ethyl acetate extracts were washed successively with water, 2% aqueous sodium thiosulfate, 5% aqueous sodium bicarbonate, water, and finally with saturated brine and filtered through magnesium sulfate. The colorless crystalline residue remaining after removal of the solvent *in vacuo* weighed 1.20 g. One recrystallization from aqueous ethanol afforded 0.785 g. (62.2%) of the *trans*-triol, m.p. (cap.) 226–229°. Two additional recrystallizations, once from aqueous ethanol and once from ethyl acetate gave material m.p. (cap.) 235–240°. This material was identical, by mixture m.p. determination and by infrared spectra, with an authentic sample of the *trans*-triol,[4] m.p. (cap.) 233–238°; conversely, a sample of 3β,5α,6α-trihydroxycholestane[5] (*cis*-triol), m.p. (cap.) 236–240°, depressed the m.p. of the periodic acid product and exhibited a distinctly different infrared spectrum. In addition, the periodic acid product gave on acetylation a substance, m.p. (micro) 168–170°, identical with an authentic specimen of *trans*-triol 3,6-diacetate.[6] Oxidation of the periodic acid product with chromium trioxide in acetic acid afforded a substance, m.p. (micro) 237–241.5°, identical with an authentic specimen of 3,6-diketo-5α-hydroxycholestane[7] obtained by oxidation of authentic *trans*-triol.

It is of interest to note the effect of several variations in the above procedure: (1) addition of a quantity of aqueous formaldehyde *ca.* equivalent to the periodic acid completely inhibited the oxidation reaction and cholesterol was quantitatively recovered; (2) use of sodium metaperiodate instead of periodic acid again gave essentially quantitative recovery of cholesterol; (3) the use of aqueous methanol instead of aqueous tetrahydrofuran apparently greatly decreased the rate of oxidation, since after five days only a small quantity of the *trans*-triol could be isolated by chromatography; (4) heating the aqueous tetrahydrofuran reaction mixture to reflux accelerated the reaction, *i.e.* after 3½ hours a 50% yield of material of m.p. 223–233° could be isolated, but that after 24 hours under reflux, the isolated material had a considerably lower m.p. A rate study run exactly as described above indicated that at room temperature one mole of periodic acid was consumed per mole of cholesterol and that the reaction was essentially complete in 48 hours.

3β-Acetoxy-5α,6β-dihydroxycholestane. To a solution of 4.286 g. (0.010 mole) of cholesterol acetate in 150 ml. of purified tetrahydrofuran was added a solution of 6.84 g. (0.030 mole) of periodic acid dihydrate in 45 ml. of water and the colorless solution was stored at room temperature for 95 hours. The resulting deep red solution was worked up as described above to give 5.00 g. of amorphous residue. Two recrystallizations from methanol afforded 1.305 g. of needles, m.p. (micro) 204–206.5° (reported m.p. 206–207°).[4a]

Anal. Calc'd for $C_{29}H_{50}O_4$: C, 75.28; H, 10.89. Found: C, 74.98; H, 10.82.

3β-Acetoxy-5α-hydroxy-6-ketocholestane (V). A 3.866-g. (0.010 mole) sample of cholesterol in 150 ml. of purified tetrahydrofuran was treated with a solution of 22.75 g. (0.10 mole) of periodic acid dihydrate in 55 ml. of water. After seven days at room temperature, the reaction mixture was worked up as described above to give 4.25 g. of a colorless crystalline solid. A 4.15-g. portion of this material was treated with 10 ml. of pyridine and 10 ml. of acetic anhydride overnight at room temperature. After removal of the excess reagents *in vacuo*, the solid residue was dissolved in ethyl acetate and the solution was washed with water, dilute hydrochloric acid, water, dilute sodium bicarbonate, water, and with a saturated salt solution. The solution was filtered through magnesium sulfate and the solvent was removed to give a buff-colored crystalline residue, weight 4.48 g., which was chromatographed on 218 g. of neutral alumina. The fractions eluted with 5%, 10%, 20%, and 50% ether-benzene and with ether were combined, weight 2.49 g., and were recrystallized twice from ether to afford 0.71 g. of the diolone 3-acetate, m.p. (micro) 235–239°. An authentic specimen of this substance prepared by N-bromosuccinimide oxidation of the *trans*-triol followed by acetylation showed no depression of the m.p. of the sample above and the infrared spectra were identical. Oxidation of a sample of the *trans*-triol 3-monoacetate with N-bromosuccinimide also afforded the diolone 3-acetate.[3]

MERCK SHARP & DOHME RESEARCH LABORATORIES
RAHWAY, NEW JERSEY

(3) Fieser and Rajagopalan, *J. Am. Chem. Soc.*, **71**, 3938 (1949).

(4) (a) Ruzicka & Bosshard, *Helv. Chim. Acta*, **20**, 244 (1937); (b) Fürst and Koller, *Helv. Chim. Acta*, **30**, 1454 (1947).

(5) Ushakov and Lutenberg, *Nature*, **140**, 466 (1937).

(6) Pickard and Yates, *J. Chem. Soc.*, **93**, 1678 (1908).

(7) Prelog and Tagmann, *Helv. Chim. Acta*, **27**, 1867 (1944).