phatic halides to aldehydes; thus, 1-chlorooctane gives *n*-octaldehyde in 71% yield. Furthermore, by the new procedure the tosylates of primary alcohols readily are converted into aldehydes. Table I summarizes our results.

The oxidation of benzylic tosylates is accomplished by treatment with a mixture of sodium bicarbonate and dimethyl sulfoxide; the reaction is complete in less than five minutes at 100°. With a saturated tosylate, *e.g.*, *n*-octyl tosylate, the reaction is conducted at 150° for three minutes. Halides are oxidized to aldehydes by converting to the tosylate with silver tosylate and then treating the crude tosylate with the sodium bicarbonate–dimethyl sulfoxide mixture.

As a typical example: 1-iodoheptane (7.0 g.) is added to a solution of 11 g. of silver tosylate[2] in 100 ml. of acetonitrile at 0 to 5° (protected from light). The mixture is allowed to come to room temperature overnight and the product then is added to ice-water and extracted with ethyl ether. The ether solution is dried and concentrated *in vacuo;* the oil that results is added to a freshly prepared mixture made by adding 20 g. of sodium bicarbonate to *ca.* 150 ml. of dimethyl sulfoxide through which nitrogen is bubbling and which has been heated to 150° (some foaming occurs). After three minutes at 150° the reaction mixture is cooled rapidly to room temperature and the product is isolated as the 2,4-dinitrophenylhydrazone; m.p. 106–107°, a mixed m.p. with an authentic sample is undepressed; yield, 6.9 g. (70%). The procedure is identical when benzyl halides are used except that the dimethyl sulfoxide is heated to 100° and the reaction time is five minutes.

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(2) Prepared quantitatively by mixing equivalent amounts of silver oxide and *p*-toluenesulfonic acid monohydrate in acetonitrile (protect from light). After one-half hour the silver tosylate is isolated by filtering, evaporating the acetonitrile and drying *in vacuo* at 65°.

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THE IDENTIFICATION OF 4α-METHYL-Δ⁸-CHOLESTEN-3β-OL, A NEW STEROL FROM A PREPUTIAL GLAND TUMOR[1]

Sir:

A transplantable preputial gland tumor in C57BL/6 mice which is maintained in this laboratory has been found to contain relatively large amounts of a wide variety of sterols.[2,3] In addition to cholesterol, Δ⁷-cholestenol, 7-dehydrocholesterol, lanosterol, and 24,25-dihydrolanosterol, a sterol (referred to as B_2), which had not been reported previously, was isolated by chromatography on silicic acid–Celite.[3] This new sterol has now been identified as 4α-methyl-Δ⁸-cholesten-3β-ol (I).

The free sterol melted[4] at 136.5–137.5°, $[\alpha]_D$ +55° (*anal.*[5] calcd. for $C_{28}H_{48}O$: C, 83.93; H, 12.08. Found: C, 83.85; H, 12.27). The acetate (II) melted at 106.5–108.5°, $[\alpha]_D$ +64°. The benzoate softened at 119° and melted at 123–124°, $[\alpha]_D$ +82°. I gave positive selenium dioxide[6] and Tortelli-Jaffe tests. Identification of the sterol as a mono-saturated derivative of 4α-methylcholestan-3β-ol was made by several reactions. Hydrogenation of II in acetic acid over Adams catalyst (no hydrogen uptake) resulted in a steryl acetate m.p. 78–78.5°, $[\alpha]_D$ +37°. The free alcohol, m.p. 158–160.5°, $[\alpha]_D$ +19°, had an infrared spectrum identical with that of 4α-methyl-Δ⁸⁽¹⁴⁾-cholesten-3β-ol,[7] m.p. 160–163°, $[\alpha]_D$ +19°, and did not depress the melting point of the authentic sterol. Compounds obtained by migrating the double bond to the 14 position and by saturating the sterol had constants that agreed well with those reported by Djerassi.[8]

Since the selenium dioxide test is specific for allo- or Δ⁵-steroids with a double bond adjacent to the hydrogen in the 14 position,[6] and only Δ⁷ or Δ⁸ double bonds migrate to the 8(14) position when the sterol is hydrogenated with platinum oxide in glacial acetic acid,[9,10] the unsaturation is limited to one of these two positions. This further evidence indicated unsaturation in the 8 rather than in the 7 position. The physical constants for I and its ester derivatives were unlike those reported for 4α-methyl-Δ⁷-cholesten-3β-ol (lophenol)[8] or (methostenol)[11] (III), while the effect of the double bond on the optical rotation of the parent compound, $[M]_{D(I)} - [M]_{D(stanol)} = +120$, was strongly positive as is characteristic for a double bond in the 8 position and in contrast to the negative effect of unsaturation in the 7 position.[8,12,13] The infrared spectrum of I differed from that recorded for III and the reaction with the Liebermann–Burchard reagent differed from that for III and for Δ⁷-cholestenol.[11]

I appears to be of general significance in sterol metabolism. There is suggestive evidence for its presence in liver[3] and labeled acetate is incorpo-

(1) Supported by a research grant (C-2758) from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

(2) A. A. Kandutsch, E. D. Murphy and M. E. Dreisbach, *Arch. Biochem. and Biophys.* **61**, 450 (1956).

(3) A. A. Kandutsch and A. E. Russell, *J. Biol. Chem.*, in press.

(4) Melting points are corrected. Optical rotations in chloroform.

(5) Elementary analyses were performed by the Schwartzkopf Microanalytical Laboratory, Woodside 77, N. Y.

(6) L. F. Fieser, THIS JOURNAL, **75**, 4395 (1953).

(7) Kindly supplied by Dr. C. Djerassi, Wayne State University, Detroit, Michigan.

(8) C. Djerassi, G. W. Krakower, A. J. Lemin, L. H. Liu, J. S. Mills and R. Villotti, THIS JOURNAL, **80**, 6284 (1958).

(9) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publ. Corp., New York, N. Y., 1949, pp. 240–242.

(10) D. H. R. Barton and J. D. Cox, *J. Chem. Soc.*, 214 (1949), noted that the frequently made assumption that a 9(11) double bond resists hydrogenation and migrates to the 8(14) position under these conditions is without foundation.

(11) D. H. Neiderhiser and W. W. Wells, *Arch. Biochem. Biophys.*, **81**, 300 (1959).

(12) W. Klyne in E. A. Braude and F. C. Nachod "Determination of Organic Structures by Physical Methods," Academic Press, Inc., New York, N. Y., 1955, p. 111.

(13) The contribution of a 9(11) double bond was found to be +15° by L. F. Fieser and W. Huang, THIS JOURNAL, **75**, 5356 (1953), who noted that the value of +109° cited by D. H. R. Barton and W. Klyne, *Chemistry and Industry*, 755 (1948), was based on zymosterol which was at that time thought to have a 9(11) double bond.

rated into I more rapidly than into cholesterol by preputial gland tumor slices incubated *in vitro* (unpublished). I is the probable precursor for the Δ^7-isomer (III), which has been found in rat feces[11] and in cactus,[8] but has not been identified in the tumor. The precursor of I is less obvious, since the pathway from lanosterol may lead through either dihydrolanosterol or through 14-norlanosterol.[14]

(14) F. Gautschi and K. Bloch, *J. Biol. Chem.*, **233**, 1343 (1958).

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PURIFICATION AND CHARACTERIZATION OF THE ANTIVIRAL AGENT HELENINE

Sir:

We wish to report a purification procedure for the antiviral agent helenine, and data which suggest that it is a ribonucleoprotein. It is of interest that this uniquely active agent is apparently of the same class of chemical compounds as are the viruses against which it acts.

Helenine is a product of the mold *Penicillium funiculosum*. It has been reported by Shope[1] to protect mice against Columbia SK encephalomyelitis and Semliki Forest viruses and to prevent development of poliomyelitis in monkeys.[2] The assay used to follow the fractionation was an *in vivo* test in mice, a modification by McClelland of that described by Shope.[1] To obtain more definitive assay values, a 24-hour pre-treatment dose was substituted for the 24-hour post-treatment dose described, the observation period was extended to 15 days, and survivors were evaluated statistically.

Helenine was extracted from the mycelium by homogenization in 0.005 *M* tris-(hydroxymethyl)-aminomethane or phosphate buffer, *p*H 7, containing 0.005 *M* Mg^{++}, precipitated by addition of one volume of acetone, and then taken up in more of the same buffer. About 20 g. of such material was obtained from an 80-gal. fermentation. All fractionation processes were performed near 0°.

The acetone-precipitated material was further purified by repeated ultracentrifugation for two hours at 110,000 × *g*. Helenine was sedimented completely. From every gram of acetone-precipitated material, 40 to 50 mg. of pellet was obtained, with a 25-fold increase in potency. The material was active at 50 to 100 μg. in mice. A well-defined 260-mμ absorption peak was noted with this fraction. It contained about 40% protein[3] and gave a pentose test with orcinol[4] and with sulfuric acid–cysteine.[5] No deoxyribose was detected. The perchloric acid hydrolysate[5] contained guanine, adenine, cytosine and uracil.[5] No hexose contamination was detected during the orcinol test, but approximately 1% non-pentose sugar was noted during the sulfuric acid–cysteine reaction.

The purified helenine was found to be unstable to lyophilization and to repeated freezing and thawing. Removal of Mg^{++} by dialysis caused a loss of activity. Helenine was more stable when stored in 0.25 *M* sucrose solution but, even under these conditions, inactivation occurred.

Our best preparations were heterogeneous when examined by electrophoresis and ultracentrifugation.[6] Three major components with sedimentation constants ranging from 43*S* to 100*S* usually were observed during ultracentrifugation. Electrophoresis showed one major peak (70%) and several minor ones. The mobility of the major component was -5.2×10^{-5} cm.^{2}/volt/sec. at *p*H 7. It seems likely that the inhomogeneity is caused by alteration of the native helenine and not by extraneous impurities in the usual sense of the word, since dissociation of ribonucleoproteins is well known.[7] Attempts at further purification were made using diethylaminoethyl-cellulose columns, but singly eluted peaks readily dissociated into smaller components. Because of this heterogeneity, final characterization of helenine cannot be made now. All the physical and chemical observations, however, including stability data, are consistent with the hypothesis that helenine is a ribonucleo-protein.

(1) R. E. Shope, *J. Exp. Med.*, **97**, 601 (1953).

(2) K. W. Cochran and T. Frances, Jr., *J. Pharmacol. Exp. Therap.*, **116**, 13 (1956).

(3) E. W. Sutherland C. F. Cori, R. Haynes and N. S. Olsen, *J. Biol. Chem.*, **180**, 825 (1949).

(4) Z. Dische in "The Nucleic Acids" edited by E. Chargaff and J. N. Davidson, Vol. I, Academic Press, Inc., New York, N. Y., 1955, p. 285.

(5) A. Bendich in "Methods in Enzymology" edited by S. P. Colowick and N. O. Kaplan, Vol III, Academic Press, Inc., New York, N. Y., 1957, p. 715.

(6) We wish to thank Dr. D. E. Williams for these studies.

(7) A. Tissieres and J. D. Watson, *Nature*, **182**, 778 (1958).

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ON THE EFFECT OF DOSE RATE ON THE RADIOLYSIS OF LIQUID HYDROCARBONS[1]

Sir:

Recent studies of the radiolysis of cyclopentane–cyclohexane mixtures with cobalt-60 γ-rays have indicated that secondary reactions between primary radicals and solvent play an important role in determining the path of this radiolysis.[2] Cyclohexyl radicals apparently preferentially abstract hydrogen atoms from cyclopentane and increase the relative yield of cyclopentyl radicals. From this one expects an effect of dose rate on the over-all radiolysis since the lifetime of the radicals and therefore the probability of abstraction decreases with increasing dose rate. Preliminary experiments were therefore undertaken to examine the dose rate dependence of the secondary reactions in this system and are briefly reported here.[3]

Degassed mixtures of equal volumes of Phillips research grade cyclopentane and cyclohexane were irradiated to a total dose of 5×10^6 rads. (3×10^{20} ev./g.) with cobalt-60 γ-rays at absorbed dose

(1) Supported in part by the U. S. Atomic Energy Commission.

(2) G. A. Muccini and R. H. Schuler, to be published.

(3) H. A. Dewhurst and E. H. Winslow, *J. Chem. Phys.*, **26**, 969 (1957), previously have compared the radiolysis of a simple hydrocarbon (*n*-hexane) by γ-rays and by fast electrons and have reported a difference in product ratios presumably due to the widely different dose rates involved in the comparison. *Cf.* also H. A. Dewhurst and R. H. Schuler, THIS JOURNAL, in press.