methallyl chloride was established with the m.ps. and mixed m.ps. of samples of II prepared from each sample of I.

The rearrangement has been found to occur only in chloroform solution as yet. The allylic halide is required for the rearrangement; in its absence NBS, chloroform, and benzoyl peroxide did not react (except to form a small amount of free bromine) when refluxed for periods four times as long as those required for the rearrangement to go to 70% completion. Since the reaction was inhibited by picric acid or trinitrobenzene, it appears to be free radical in nature and, indeed, to be a free radical analog of the Hofmann hypohalite reaction of amides.[3] Work on this reaction is continuing.[4]

(3) E. S. Wallis and J. F. Lane, "Organic Reactions," Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1946, p. 267.

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2-METHOXYESTRONE, A METABOLITE OF ESTRADIOL-17β IN THE HUMAN[1]

Sir:

After the administration of small or large doses of estradiol-17β-16-C^{14} or estrone-16-C^{14} to humans, the phenolic fraction of urine contained considerable radioactivity other than that associated with estrone, estradiol or any of the isomers of estriol. Counter-current distribution in the system 70% aqueous methanol as the upper phase and carbon tetrachloride as the lower phase showed a peak of radioactivity with a partition coefficient[2] = 0.30 (estrone = 1.3). The same peak of radioactivity was observed after either hot acid or β-glucuronidase[3] hydrolysis of the urinary conjugates; the amount of material present was greater in the first and second days' urine than thereafter. The new metabolite was a ketone as evidenced by the essentially quantitative reaction with Girard Reagent T; counter-current distribution studies indicated that it was not identical with 3-hydroxy-$\Delta^{1,3,5(10)}$-estratriene-16-one.

A pure sample of the new metabolite was isolated from urine after the administration of 1 g. of estradiol-17β-16-C^{14} (specific activity = 191 counts per minute per milligram (c.p.m./mg.)) over a period of ten days. The product melted 187–189.5°; $[\alpha]^{28}$D +179° (ethanol); λmax. 284.5–288.5 mμ (ϵ = 4000), λmin. 254 mμ (ϵ = 420); specific activity = 172 c.p.m./mg.; the infrared spectrum in carbon tetrachloride solution exhibited bands at 3560 (hydroxyl group), 1743 (17-ketone), 1508, 1503 (C=C stretching in aromatic ring), 1409 (unsubstituted CH_2 at C-16) and 1374 (C-18 methyl group) cm.$^{-1}$. A prominent band at 874 cm.$^{-1}$ in carbon disulfide was interpreted as the out of plane C–H deformation of isolated hydrogens in ring A. Comparable bands were noted in the spectrum of a dispersion in potassium bromide.

Anal. Calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05. Found: C, 75.82; H, 8.40.

The compound formed a monoacetate, m.p. 152–153.5°; *Anal.* Calcd. for $C_{21}H_{26}O_4$: C, 73.66; H, 7.65. Found: C, 73.29; H, 7.93.

With this information it seemed that the compound might be a methoxy derivative of estrone and in view of the lack of polarity of the phenolic hydroxyl as evidenced by the partition coefficient relative to that of estrone, a monomethyl ether of 2- or 4-hydroxyestrone was considered probable. The four possible monomethyl ether isomers of 2- and 4-hydroxyestrone were synthesized by methods based upon the work of Mueller and Mills,[4] and Horner and Stöhr.[5] The synthetic route to the metabolite involved the following reactions: 2-nitroestrone, 2-aminoestrone diazonium salt, photodecomposition in methanol. The details of the synthetic work will be reported in the near future. The new metabolite proved identical with 2-methoxyestrone (synthetic product, m.p. 184.5–188.5°; $[\alpha]^{24}$D +178°) as judged by identity of the infrared spectrum[6] in both carbon disulfide solution and potassium bromide dispersion as well as the m.p. and rotation; there was no depression of the melting point on admixture of the natural and synthetic samples.

2-Methoxyestrone failed to exhibit fluorescence[6] with sulfuric acid in a test commonly employed for estrogens and their metabolites.[7] Biologically[6] the compound is a very weak estrogen with activity less than 1/20,000 that of estradiol-17β as judged by the intravaginal assay of Emmens.[8]

Quite apart from the interest attached to the identification of another metabolite of the estrogenic hormone, the biochemical introduction of a methoxyl group is a novel reaction in the steroid field. The significance of this new type of transformation is under further investigation.

(1) This investigation was supported in part by a grant from the American Cancer Society and a research grant (C-2271) from the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

(2) B. Williamson and L. C. Craig, *J. Biol. Chem.*, **168**, 687 (1947).

(3) Ketodase, obtained from the Warner Chilcott Laboratories, a division of Warner-Lambert Pharmaceutical Co., New York.

(4) G. C. Mueller and M. E. Mills, personal communication; *cf.* Mueller, *Nature*, **176**, 127 (1955). The work of these authors related to the proof of structure of 2-nitro and 4-nitroestrone. Independent evidence for the correctness of these assignments has been obtained in these laboratories from infrared and ultraviolet spectrometry; Werbin (*Fed. Proc.*, **15**, 382 (1956)) has also reached similar conclusions.

(5) L. Horner and H. Stöhr, *Chem. Ber.*, **85**, 993 (1952).

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(7) L. L. Engel, *Rec. Prog. in Hormone Res.*, **5**, 335 (1950).

(8) C. W. Emmens, Med. Res. Council, Spec. Rept. Series 234, H. M. Stat. Office (1939).

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