Whatman No. 1 paper[3] demonstrated that two of the radioactive metabolites migrated identically with 6β-hydroxyestradiol (I) and 6-ketoestradiol (II),[4] respectively.

For absolute identification of these metabolites twenty-four reaction flasks, each containing 20 μg. of estradiol (7,500 c.p.m./μg.) in 1.0 ml. of 0.05 *M* potassium phosphate buffer *p*H 7.4, 0.3 ml. of TPN H (2.5 mg.), 0.15 ml. of 1.0 *M* nicotinamide, 0.35 ml. of 1.0 *M* KCl, 0.1 ml. of 1.0 *M* $MgSO_4$ and 0.4 ml. of mouse liver microsome suspension (1.0 ml. = microsomes from 1.25 g. of liver) were incubated for 15 minutes at 37°. The reactions were stopped with 0.3 ml. 1 *N* HCl, 25 mg. each of (I) and (II) were added, and extraction with acetone–benzene and then with chloroform–ethylene dichloride (1:1) carried out. The residue from the organic phase was chromatographed on a 100 × 1.8 cm. column of Florisil with increasing concentrations of methanol in benzene (Fig. 1). Radioactive peaks T and X_2 contained the carrier (I) and (II), respectively.

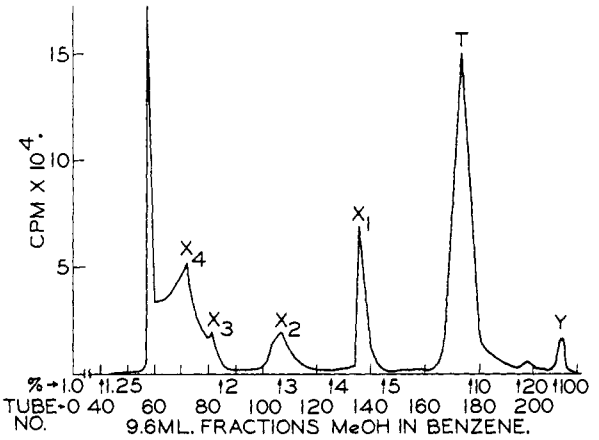

Fig. 1.

Rechromatography of the X_2 peak on Florisil with increasing concentration of methanol in ethylene dichloride (1–4%) showed that the elution of carrier mass coincided with elution of radioactivity. Crystallization to constant specific activity gave 4,860 c.p.m./mg. Conversion to the 2,4-dinitrophenylhydrazone[5] and chromatography on aluminum oxide (Activity = Brockmann II, elution with ethanol–$CHCl_3$ mixtures) gave a single peak in which the specific activity of the two halves of peak were 3040 and 3130 c.p.m./mg. (Calculated specific activity for a mono phenylhydrazone derivative = 2990 c.p.m./mg.) Therefore the identity of X_2 is established as 6-ketoestradiol (yield 3.4%).

The "T" peak was rechromatographed on Florisil using gradually increasing concentrations of methanol (1.25–4%) in ethylene dichloride. The elution of radioactivity coincided with the elution of the carrier I; the two halves of the peak had specific activities of 39,070 and 39,570 c.p.m./mg., respectively. Partition chromatography on Celite with ethylene dichloride-methanol[6] yielded a similar result: the two halves of the peak gave a specific activity of 36,300 and 35,890 c.p.m./mg., respectively. Conversion of the carrier to the triacetate yielded a derivative (m.p. 140–143°) with specific activity of 24,000 c.p.m./mg. Expected specific activity = 25,080 c.p.m./mg. Thus the identity of "T" is established as 6β-hydroxyestradiol (yield = 25%).

Preliminary results on the reduction X_1 to (I) and the oxidation of both X_1 and (II) to the same new product, presumed to be the 6-ketoestrone, indicated that the identity of X_1 is 6β-hydroxyestrone. More complete evidence for this conclusion will be reported later.

Accordingly it has been demonstrated that mouse liver microsomes in the presence of $TPN{\cdot}H^+$ hydroxylate estradiol primarily to form 6β-hydroxyestradiol which is converted[2] to 6-ketoestradiol and 6β-hydroxyestrone. This family of compounds appears to constitute a major metabolic pathway for estradiol in mouse and rat liver preparations.[7,8]

(3) Chromatograms were developed by descension of benzene in a paper lined jar saturated from a mixture of 500 ml. of methanol and 250 ml. of water placed in bottom of jar: G. Rumney, Ph.D. Thesis, Hebrew Univ., Jerusalem, 1954.

(4) We are extremely grateful to Dr. O. Wintersteiner for supplying us with authentic samples of I and II. Additional II has been prepared in this laboratory according to B. Longwell and O. Wintersteiner: *J. Biol. Chem.*, **133**, 219 (1940), and reduction of this product to I with $NaBH_4$.

(5) H. Reich, K. F. Crane and S. J. Sanfilippo, *J. Org. Chem.*, **18**, 822 (1953).

(6) W. S. Bauld, *Biochem. J.*, **59**, 294 (1955).

(7) This work was supported by a grant from the Alexander and Margaret Stewart Trust Fund, grant No. C-1897 from the United States Public Health Service and an Institutional Grant from the American Cancer Society.

(8) Authors wish to thank Miss Elba I. Porrato for valuable technical assistance.

McArdle Memorial Laboratory
University of Wisconsin
Medical School — Gerald C. Mueller
Madison, Wisconsin — Gideon Rumney

Received January 17, 1957

CONFIGURATIONAL EFFECTS IN THE PROTON MAGNETIC RESONANCE SPECTRA OF ACETYLATED CARBOHYDRATES[1]

Sir:

We have observed configurational effects in the proton magnetic resonance (NMR) spectra[2] of acetylated carbohydrates which promise to be of value in configurational and conformational analyses and which raise rather important questions regarding the interpretation of chemical shifts in general.

(a) There is a shift of 5 to 10 cycles per second (c.p.s.) between the signals for the methyl hydrogens of equatorial and axial acetoxy groups.

(b) There is a shift of approximately 8 c.p.s. between the signals for axial and equatorial hydrogens.

(c) The carbon-1 hydrogens of anomeric acetylated aldopyranoses produce signals which are separated by 10 to 26 c.p.s. when these hydrogens are axial in one of the anomeric forms and equato-

(1) Presented in part at the 130th Meeting of the American Chemical Society, Atlantic City, N. J., Sept. 16–21, 1956, Abstract of Papers, p. 10-D. N.R.C. Contribution No. 4257.

(2) The spectra were determined in chloroform solution at room temperature with a Varian High Resolution Spectrometer at 40 megacycles per second. The positions of the signals were measured in cycles per second from the chloroform signal.

rial in the other. The greater shift (than in (b)) appears related to the ring-oxygen since it has been detected for the axial and equatorial hydrogens of the methylene group of β-D-xylopyranose tetraacetate.

Because of spin coupling between the hydrogens on neighboring carbons, the shifts mentioned in (b) often are obscured by inadequate resolution of the complex spectrum. The configurational effects on the signals produced by the acetoxy groups are not obscured by spin coupling and will perhaps prove the most generally useful in configurational and conformational studies. These facts are illustrated by the spectra for *myo*- and *levo*-inositol hexaacetates[3] shown in Fig. 1. It is seen that the

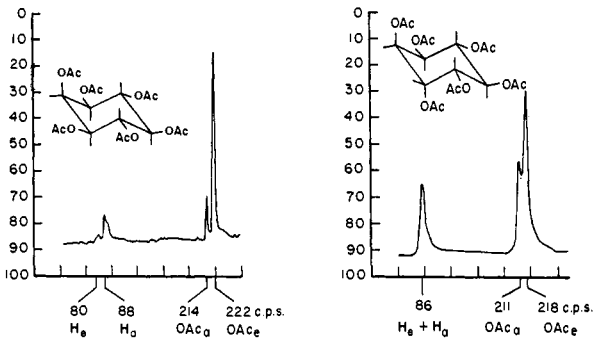

Fig. 1.—Proton magnetic resonance spectra of the *myo*- and *levo*-inositol hexaacetates, respectively.

equatorial hydrogen in the *myo*-isomer is identified readily from its relative intensity. The signals for the equatorial and axial hydrogens are blended into one broad band in the case of the *levo*-isomer. The relative intensities of the signals for the acetoxy groups clearly demonstrate the ratio of axial to equatorial acetoxy groups in both cases. It must be noted, however, that the spectra in the acetoxy group region are not always simple and readily interpreted. This is seen in the spectra D, E and F of Fig. 2. In the case of β-L-arabinopyranose tetraacetate we were not able to resolve the signal for the axial acetoxy group.

The assignments of the signals were made through a comparison of the NMR spectra of a variety of acetylated aldoses, ketoses, glucosides and sugar alcohols and appear unequivocal. The spectra of the anomeric xylopyranose and arabinopyranose tetraacetates provided evidence in support of the conformations shown in Figs. 1 and 2.

It is of considerable interest to note that the NMR spectra have yielded a convincing confirmation of the configurations at the anomeric center of sugar acetates which previously in most cases were assigned[4] entirely on the basis of Hudson's rule of isorotation.[5]

It was also observed that configuration has an effect on spin-coupling constants as well as on chemical shift. Analysis of the spectra for the acetyl derivatives of glucose, galactose, xylose and arabinose showed that the spin coupling between

(3) We are indebted to Dr. E. C. Horswill for providing us with the levo compound.

(4) R. U. Lemieux, *Can. J. Chem.*, **29**, 1079 (1951).

(5) C. S. Hudson, *Advances in Carbohydrate Chem.*, **3**, 15 (1948).

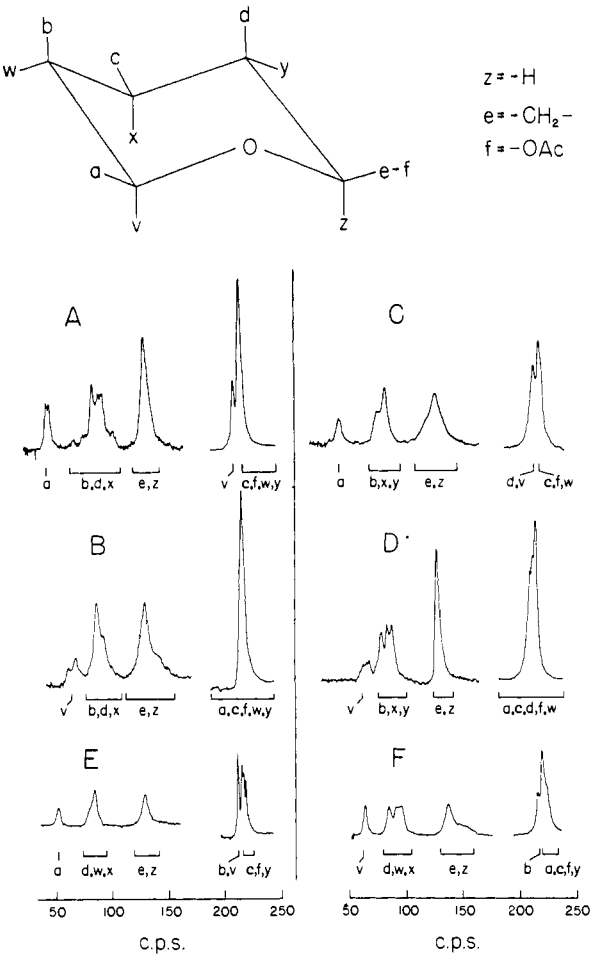

Fig. 2.—Proton magnetic resonance spectra of acetylated sugars: A, α-D-glucopyranose pentaacetate (a, b, x, d = — H, v, w, c, y = — OAc); B, β-D-glucopyranose pentaacetate (v, b, x, d = — H, a, w, c, y = — OAc); C, α-D-galactopyranose pentaacetate (a, b, x, y = — H, v, w, c, d = — OAc); D, β-D-galactopyranose pentaacetate (v, b, x, y = — H, a, w, c, d = — OAc); E, α-D-mannopyranose pentaacetate (a, w, x, d = — H, v, b, c, y = — OAc); F, β-D-mannopyranose pentaacetate (v, w, x, d = — H, a, b, c, y = — OAc).

hydrogens on neighboring carbon atoms is 2 to 3 times greater when both the hydrogens are in axial orientation than when one or both of the hydrogens are in equatorial orientation.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF OTTAWA — R. U. LEMIEUX
OTTAWA, ONTARIO — R. K. KULLNIG
DIVISION OF PURE CHEMISTRY
NATIONAL RESEARCH COUNCIL — H. J. BERNSTEIN
OTTAWA, ONTARIO — W. G. SCHNEIDER

RECEIVED NOVEMBER 23, 1956

A NEW TYPE OF SUBSTITUTED BORANE

Sir:

We wish to report the discovery of a new class of borane derivatives formed by the reaction of a nitrile with a boron hydride. When a decaborane solution in acetonitrile is heated to reflux, the solution slowly becomes light yellow and evolves hydrogen. On cooling after one hour at reflux, a