bically on a minimal salt medium containing D-galacturonic acid as the sole carbon source. After 24 hours at 37°, the cells were harvested, disrupted by sonic vibration and the extract partially purified by isoelectric precipitation.

A reaction mixture containing 50 μmoles of either I or III was incubated separately with the extract for one hour at 37° and the reaction products isolated by chromatography on Dowex-1-formate[5] or by paper chromatography with ethyl acetate:acetic acid:water (3:1:3) as the solvent system. In either case, there was a clear separation of unreacted starting material and of a more rapidly moving component which was isolated and identified as II or IV, respectively.

Both of the unknown products gave color reactions characteristic of uronic acids[6,7] but which were stable to bromine water under conditions where the alduronic acids were completely oxidized. Upon treatment with HIO_4 under acidic conditions,[8] formaldehyde was not formed and the presence of a 2-keto acid therefore presumed unlikely. Quantitative measurement at pH 6.2[9] showed that 3.1 and 2.6 moles of HIO_4 were taken up per mole of II and IV, respectively. The glycolic acid formed[10] was 1.0 and 1.1 moles per mole of II and IV, a result in close accord with the theoretical values for 5-keto acids.

Further evidence that the enzymatic reaction products were II and IV was provided by reduction with KBH_4. The resulting aldonic acids were lactonized and chromatographed on paper in three different solvent systems. Upon visualization of the lactones with hydroxylamine and $FeCl_3$,[11] it was seen that II yielded galactono- and altronolactones while IV yielded gulono- and mannonolactones. These results are definitive for the structures as written.

The crystalline brucine salt of D-fructuronic acid was prepared by a modification of the procedure used to synthesize crystalline D-tagaturonic acid.[12] Paper chromatography in several solvents revealed that II invariably co-chromatographed with authentic D-tagaturonic acid and that IV acted similarly with pure D-fructuronic acid. Upon incubation of the synthetic keturonic acids with the enzyme, the formation of the corresponding alduronic acids (I or III) was readily demonstrable. Finally, the crystalline brucine derivatives of II and IV were prepared and checked by mixed melting points with the authentic derivatives.

NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES
NATIONAL INSTITUTES OF HEALTH
UNITED STATES PUBLIC HEALTH SERVICE
BETHESDA, MARYLAND

ALBERT J. WAHBA[13]
JEAN W. HICKMAN
GILBERT ASHWELL

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(13) Fellow of the Damon Runyon Memorial Fund for Cancer Research.

INHIBITION OF CRYSTALLIZATION IN POLYETHYLENE SUBSEQUENT TO GAMMA IRRADIATION

Sir:

Recent results[1] suggest that irradiation of polyethylene at room temperature and doses below 29×10^{20} e.v.g.$^{-1}$ causes no change[2] in crystallinity, but that on fusion and solidification subsequent to the irradiation, the crystallinity decreased.[2] We have confirmed these results by a direct measurement at room temperature of the amorphous content of polyethylene utilizing the infrared absorption bands[3] at 1080 and 1303 cm.$^{-1}$.

The open circles of Fig. 1 demonstrate the constancy of the crystallinity of 85% crystalline Marlex-50 during irradiation at room temperature with Co-60 γ-rays while the vertical arrows indicate the growth of relative amorphous content, ϕ_A, on post-irradiation fusion and solidification. The four solid circles represent data obtained at room temperature subsequent to irradiation at 140°. The post-irradiation fusion also causes additional vinyl decay and vinylene growth.[4]

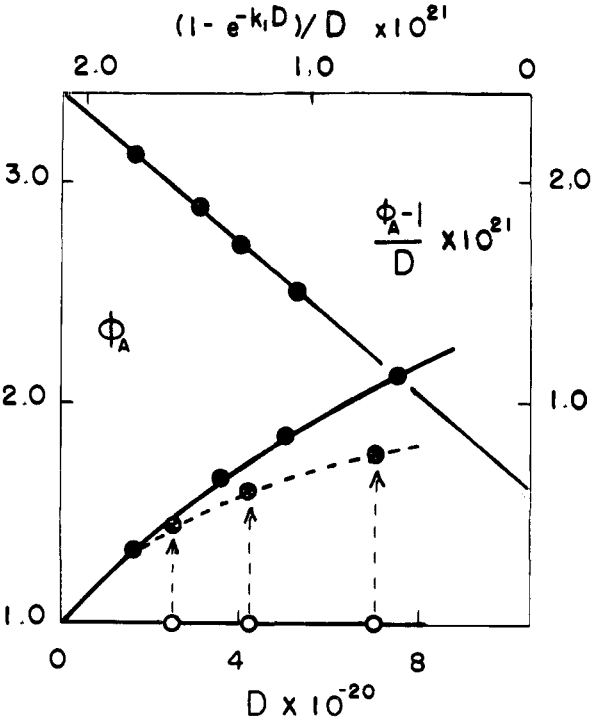

Fig. 1.—Open circles, are relative amorphous content (ϕ_A) of Marlex-50 polyethylene as a function of room temperature irradiation; solid circles, ϕ_A for irradiation at 140°. The dotted lines indicate the growth in amorphous content during post-irradiation fusion and solidification. Upper straight line is a test of Eq. (2).

Hitherto, only the effect of cross linking on crystallinity has been considered, but the following

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demonstrates that vinyl decay, presumably to form end links,[5] must be taken into account.

Let A represent CH_2 groups per gram in the amorphous regions, D the dose in e.v.g.$^{-1}$, $G[X]$ and $G[Vl]$ the number of cross links and vinylene groups produced per 100 e.v. absorbed, [Vi] the vinyl concentration in moles g.$^{-1}$, N Avogadro's number, k_1 the first order vinyl decay constant, and α, β and γ the number of methylene groups added to the amorphous regions per cross link, vinylene group and end link formed by the 140° irradiation, respectively. Then

$$\frac{dA}{dD} = \frac{\alpha G[X]}{100} + \frac{\beta G[Vl]}{100} + \gamma N k_1 [Vi] \qquad (1)$$

Below doses of 8×10^{20} e.v.g.$^{-1}$, $G[X]$ and $G[Vl]$ are constant. Letting M represent the first two terms on the right-hand side of (1), replacing [Vi] by $[Vi]_0 \exp(-k_1 D)$, integrating, setting A/A_0 equal to ϕ_A and rearranging, Eq. (2) results

$$\frac{\phi_A - 1}{D} = \frac{M}{A_0} + \frac{\gamma N [Vi]_0 [1 - e^{-k_1 D}]}{A_0 D} \qquad (2)$$

The upper curve of Fig. 1 is a linear plot of $(\phi_A - 1)/D$ *versus* $[1 - \exp.(-k_1 D)]/D$. Equation 2 is seen to be accurately verified for γ and $(\alpha + \beta)$ equal to 79 and 131, respectively. Four long branches extend into the polyethylene from a cross link and three from an end link. Dividing 131 by 4 and 79 by 3, the numbers 33 and 26, respectively, are obtained. These numbers represent the number of methylene groups per chain immobilized by a branch point with respect to the ability to crystallize. Inasmuch as 33 includes the contribution of vinylene groups (which is not expected to be as large as the contribution of a cross link) the agreement is satisfactory. Evidently long branches and gel structure are far more important in inhibiting crystallization than previously imagined[6] for short branches.

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(7) On leave from A. E. R. E., Harwell, England.

(8) On leave from the Nippon Telegraph and Telephone Public Corporation, Tokyo, Japan.

DEPARTMENT OF CHEMISTRY
NORTHWESTERN UNIVERSITY
EVANSTON, ILLINOIS

T. F. WILLIAMS[7]
H. MATSUO[8]
M. DOLE

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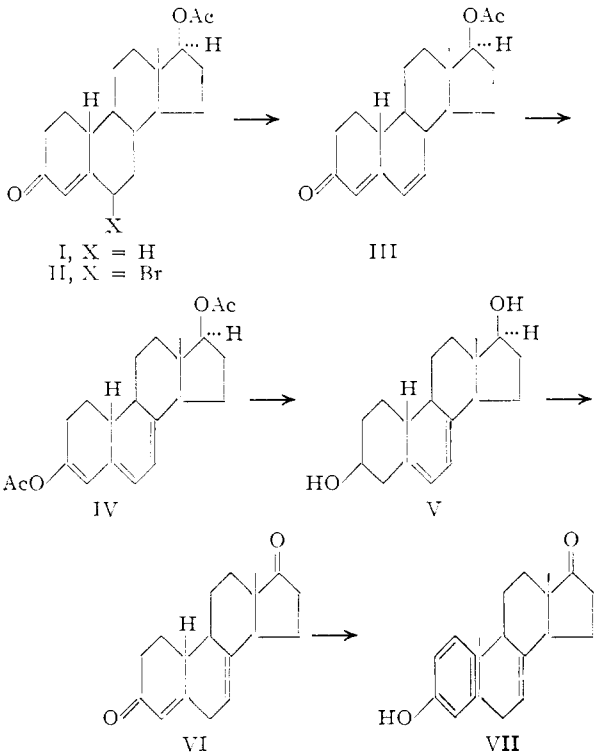
STEROIDS. XCVI.[1] THE SYNTHESIS OF EQUILIN

Sir:

Equilin (VII)[2] is the only known naturally occurring, physiologically active steroid hormone which until now has resisted all attempts at partial or total synthesis. This powerful estrogen is currently only available from mare's urine and it was clearly desirable to develop a synthesis of the hormone in order to permit more extensive clinical work as well as to prepare certain derivatives for biological investigation. The unusual feature of equilin (VII) is the non-conjugated double bond in ring B which is responsible for its ready isomerization with acid[3] or its aromatization to equilenin with palladium in the presence of hydrogen.[4] Consequently conditions had to be developed which did not affect this center of unsaturation and we should now like to record a successful synthesis of equilin.

Dehydrobromination of crude 6-bromo-19-nortestosterone acetate (II) derived from 19-nortestosterone acetate (I)[5] afforded 6-dehydro-19-nortestosterone acetate (III) (m.p. 113–114°, $[\alpha]_D$ −38°,[6] λ_{max}^{EtOH} 282–284 mμ, log ε 4.29; *Anal.* found for $C_{20}H_{26}O_3$: C, 76.62; H, 8.63). This key intermediate was transformed by the procedure employed by Velluz and co-workers[7] in the 19-norcholesterol series into the enol diacetate IV (m.p. 163–165° (dec.), $[\alpha]_D$ −29°, λ_{max}^{EtOH} 300, 312 and 328 mμ, log ε 4.32, 4.41 and 4.27, λ_{max}^{KBr} 5.71, 5.77, 6.05 (w), 6.13 (w) and 8.03 μ)[8] and reduced with sodium borohydride to $\Delta^{5,7}$-19-norandrostadiene-3β,17β-diol (V) (m.p. 192–195°, $[\alpha]_D$ +256°, λ_{max}^{EtOH} 272, 282–284 and 296 mμ, log ε 4.05, 4.06 and 3.80; *Anal.* found for $C_{18}H_{26}O_2$: C, 78.39; H, 9.82). Oppenauer oxidation of V provided 19-nor-$\Delta^{4,7}$-androstadiene-3,17-dione (VI) (m.p. 148–



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