m.p. 199–201°, $[\alpha]^{20}D$ −82°, kindly furnished by Dr. Sannié, was converted to the $\Delta^{1,4}$-dien-3-one in 20% yield of crystalline material. An infrared spectrum of the mother liquors indicated that they contained approximately another 30% of this material. In contrast to the results obtained by Burn, *et al.*,[3] our substance proved to be identical with the dienone obtained, by selenium dioxide dehydrogenation,[6] from diosgenone.[7] We believe the yield of dienone obtained in our sequence was high enough to preclude its chance origin from an impurity.

Oxidative degradation of the side chain of ruscogenin diacetate,[8] followed by saponification, furnished 1ξ,3β-dihydroxy-$\Delta^{5,16}$-pregnadiene-20-one, m.p. 228–235°, ϵ_{240}^{MeOH} 9,100; λ_{max}^{Nujol} at 2.93, 6.06 and 6.28 μ. Upon hydrogenation in pyridine over palladium-charcoal, one mole of hydrogen was absorbed and there was obtained 1ξ,3β-dihydroxy-Δ^{5}-pregnene-20-one, m.p. 195–198°, which showed no selective absorption in the ultraviolet between 220 and 300 mμ; λ_{max}^{Nujol} at 2.96, 3.03 and 5.83 μ, $[\alpha]^{23}D$ +22.4° (1% in $CHCl_3$). When this substance was subjected to the action of *Flavobacterium dehydrogenans*[9] there was obtained the hydroxylated progesterone, 1ξ-hydroxy-Δ^{4}-pregnene-3,20-dione, m.p. 155–157°, λ_{max}^{Nujol} at 2.98, 5.84, 6.01, 6.19 μ; C, 76.58; H, 9.05; ϵ_{241}^{MeOH} 15,800. In methanolic alkali at 60°,[10] a shift from 241 to 244.5 mμ (characteristic of a dienone) was observed. The absence of any selective absorption near 370 mμ[10] under these conditions establishes the formation of a dienone which should result from the dehydration of the proposed structure.

Further 1-hydroxylated hormone analogs are being synthesized, and we are investigating the stereochemistry of this hydroxyl group as well as the relationship of these substances to other 1-hydroxylated steroids.[11,12]

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ELECTRIC PROPERTIES OF MACROMOLECULES. II. EVIDENCE FOR A PERMANENT DIPOLE MOMENT IN THE RIB GRASS STRAIN OF TOBACCO MOSAIC VIRUS

Sir:

A previous investigation of the common strain of the tobacco mosaic virus by means of electric birefringence showed that the large Kerr effect in dilute aqueous solutions is due primarily to an induced polarization, and that the permanent dipole moment contribution is negligible.[1] One indication of this was the fact that reversal of a square polarizing pulse after a steady-state orientation was achieved did not produce a transient in the birefringence. In further studies of various strains, similar behavior has been observed with the Masked and Green Aucuba strains, but a large transient was obtained with the Holmes Rib Grass strain (HR). All experiments were carried out in solutions sufficiently dilute to eliminate complications arising from macromolecular interactions. Figure 1 contains oscillograms illustrating the wave form of the polarizing field and the transient electric birefringence signal of HR.

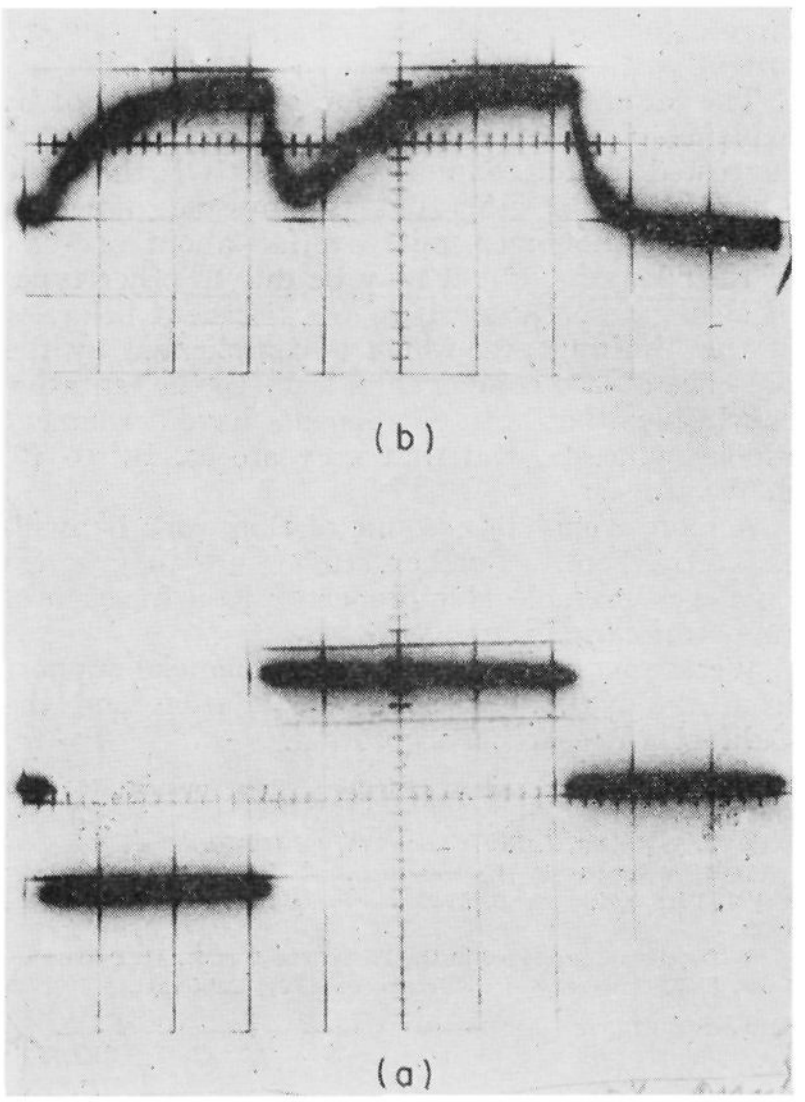

Fig. 1.—Nature of the birefringence transient upon reversal of the field for the Holmes Rib Grass strain of tobacco mosaic virus: (a) oscillogram of the polarizing field; (b) transient response. The central minimum does not occur with the common strain.[1]

The transient upon field reversal was observed with three separate preparations of the Rib Grass strain.[2] Analysis of the field-free decay of the birefringence revealed that each preparation had two relaxation times, corresponding to rotational diffusion constants of 4.8×10^2 and 2×10^2 sec.$^{-1}$. The larger value was assigned to the HR monomer. It is 1.4 times the corresponding value for the common strain, and corresponds to a rod length

(1) C. T. O'Konski and A. J. Haltner (Paper I), THIS JOURNAL, **79**, in press (1957).
(2) Kindly furnished by C. A. Knight of the Virus Laboratory. Prepared by the general method of W. M. Stanley, *ibid.*, **64**, 1804 (1942).

around 10% less than that reported for the monomer of the common strain.[3]

The Kerr constant increased a factor of about 20 as the *p*H was increased from 5.6 to 8.3. The variation resembled a titration curve with an inflection around *p*H 7. Experiments with the square reversing pulse as a function of *p*H indicated that the permanent moment contribution also increased with *p*H. The amino acid analyses, which have been reported[4] for all four strains mentioned above, show that histidine is present only in the Rib Grass strain. Titration of the imidazole group of histidine occurs around *p*H 7, and there is no other known constituent which is titrated in this region.[4,5] This leads to the suggestion that an asymmetric arrangement of histidine residues in the macromolecular structure might be the origin of the large changes of dipole moment and Kerr constant around *p*H 7.

The lower rotation diffusion constant cannot be explained by rigid end-to-end dimers, which were suggested for the common strain.[3] It is the value expected for a staggered side-by-side dimer in which the monomer units overlap about one-half of their lengths, but it may be due to other types of aggregates. Analysis of the transient behavior of the birefringence, which is complicated by the presence of two components, leads to the tentative conclusions that both components have permanent dipole moments, and that they are *ca.* 10^4 to 10^5 debye units.

A more complete account of this work is available elsewhere.[6] Further studies are in progress on the macromolecular properties as a function of preparation procedure.

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(4) C. A. Knight, *J. Biol. Chem.*, **171**, 297 (1947).

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(6) Details are presented in the Ph.D. Thesis of R. M. Pytkowicz, June, 1957, University of California, Berkeley, California.

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THE *p*H OPTIMUM OF THE HILL REACTION

Sir:

One of the contradictions in the Hill reaction literature is that concerning the *p*H optimum at high light intensity. The "optimal" values previously cited range from *p*H 5.9 to 8.3 (Table I). As will be shown in this paper, this discrepancy is largely due to the fact that the *p*H optimum is dependent on the activity of the chloroplast material. A high *p*H optimum is characteristic of high activity preparations, and the optimum decreases as the chloroplasts lose activity.

In the experiment from which the following data were taken, washed, turgid leaves of field grown chard (*Beta vulgaris*) were used as the source of chloroplasts. The leaves were ground with a mortar and pestle in the presence of 0.03 *M* phosphate buffer, *p*H 7.0, containing 0.01 *M* KCl. The suspension was filtered through glass wool, then centrifuged for 90 seconds at 100 *g* to remove whole cells and other debris. It was then decanted and recentrifuged for five minutes at 900 *g* to bring down "whole chloroplasts." The supernatant was discarded, the centrifuge tube and the surface of the chloroplast pellet were rinsed with fresh, cold buffer, and the inside of the tube was wiped dry. The chloroplasts were resuspended by gentle stirring during slow addition of fresh buffer of the same composition as the grinding medium. All operations were carried out as close to 0° as possible, and no effort was made to avoid exposing the preparation to either light or air.

TABLE I

*p*H OPTIMA FOR THE HILL REACTION AS REPORTED BY DIFFERENT WORKERS

Holt, Smith and French[1]	5.9
Warburg and Lüttgens[2]	6.5
Wessels[3]	6.5
Arnon and Whatley[4]	6.9
Spikes, Lumry, Eyring and Wayrynen[5]	6.9
Punnett and Fabiyi[6]	6.9
Clendenning and Gorham[7]	7.15
Holt and French[8]	7.6–7.9
Punnett[9]	7.9
Hill and Scarisbrick[10]	8.0
Davenport, Hill and Whatley[11]	8.3

Reaction rates were measured by following the rate of reduction of purified 2,6-dichlorophenolindophenol (molar extinction coefficient 22,400).[9,12] The reaction vessel contained 0.03 *M* phosphate or Tris buffer, 0.01 *M* KCl, 1.5×10^{-5} *M* dye, and chloroplasts giving a final concentration of chlorophylls *a* plus *b* of 3.5 μM./l (3.2 μg./ml.). In the dark the rate of reduction of the dye by the chloroplasts corresponded to a $Q_{O_2}^{ch}$ of 30, which is roughly the same as the error in the determination of the rate in high intensity red light.

When freshly prepared chloroplasts were tested for *p*H response within one to two hours after grinding the leaves, the optimum was found to be equal to or greater than *p*H 8.7. Following storage of the preparation at 0°, the *p*H optimum decreased half a *p*H unit during the first nine hours of storage, and another unit and a half during the next 43 hours (Table II).

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