5.30μ (bridge) as well as the bands commonly associated with the triphenylmethyl phosphonium ion. Acidification of III with dilute hydrochloric acid produced immediate decolorization and decaborane was recovered in 50% yield. Air oxidation and hydrolysis of III was not apparent after three months at ambient temperature.

It appears that this material is the triphenylmethylphosphonium salt of decaborane and is the first such material to be isolated as a pure stable

$$(C_6H_5)_3P^+CH_2^- + B_{10}H_{14} \longrightarrow (C_6H_5)_3P^+CH_3B_{10}H_{13}^-$$

substance.

The reactions of other phosphine methylenes with decaborane are under investigation.

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RECEIVED MAY 23, 1958

ISOLATION OF GUANOSINE DIPHOSPHATE FUCOSE FROM AEROBACTER AEROGENES

Sir:

We wish to report the isolation of a new sugar nucleotide, guanosine diphosphate fucose, from a strain of Aerobacter aerogenes[1] that produces a polysaccharide containing L-fucose.[2]

The nucleotides from 150 g. wet weight of bacteria were extracted with boiling 70% ethanol, precipitated with mercuric ion, and chromatographed on a Dowex 1-Cl^- column.[3] Elution with 0.01 *N* HCl and increasing concentrations of NaCl yielded an ultraviolet absorbing peak which was determined to be 75% uridine diphosphate glucose and uridine diphosphate galactose by enzymatic analysis.[4] This peak contained a total of 10 μM. of nucleotides calculated as uridine from spectral data. A minor component of this fraction amounting to 10% of its optical density at 260 mμ could be isolated by paper electrophoresis or paper chromatography. The ultraviolet absorption spectrum of this component was typical of a guanosine derivative. Its mobility during electrophoresis in sodium formate buffer, *p*H 3.5, was less than guanosine diphosphate, but greater than guanosine monophosphate while it had a higher R_f than guanosine monophosphate when chromatographed with ethanol-neutral ammonium acetate solution.[5]

The isolated component was analyzed colorimetrically[6] and found to contain approximately 0.8 μM. of 6-deoxyhexose per μM. guanosine. The absorption peak at 400 mμ given by the guanosine derivative in this test was identical in shape with that given by authentic fucose and also disappeared at the same rate upon dilution with water.[7]

Hydrolysis of the guanosine derivative in 0.01 *N* HCl at 100° for 10 minutes liberated a compound having an R_f identical with that of fucose when chromatographed with butanol–acetic acid–water,[8] phenol–water,[8] or pyridine–ethyl acetate–water.[9] In addition, an ultraviolet absorbing compound was formed which exhibited the same chromatographic and spectral properties as guanosine diphosphate. Longer hydrolysis led to the formation of a second ultraviolet absorbing compound which exhibited the same properties as guanosine monophosphate.

In view of the well-established role of the uridine sugar nucleotides as glycosyl donors in the biosynthesis of many complex saccharides, it is an interesting variation to find fucose occurring in a guanosine nucleotide. The only other guanosine sugar nucleotide known at present is guanosine diphosphate mannose.[10,11]

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(12) U. S. Public Health Service Postdoctoral Fellow.

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RECEIVED APRIL 5, 1958

ORGANOBORON COMPOUNDS. X. MIXED TRIALKYLBORANES DISTILLABLE WITHOUT DISPROPORTIONATION[1,2]

Sir:

It was suggested recently[1] that mixed trialkylboranes characterized by the presence of a *t*-butyl group may manifest unusual stability to disproportionation. One such substance, diisobutyl-*t*-butylborane, was described previously and its stability to disproportionation was attributed to steric interference with the disproportionation mechanism.[1]

We wish to describe now the first distillable trialkylborane containing *three dissimilar alkyl groups*, namely, *t*-butyl-isobutyl-*n*-amylborane. This substance, b.p. 43.5–44.0° at 0.5 mm., $n^{25}D$ 1.4296, d^{25} 0.7506, was fractionally distilled twice *in vacuo* without decomposition, rearrangement or disproportionation. *Anal.* Calcd. for $C_{13}H_{29}B$: B, 5.52. Found: B, 5.56. MR_D: calcd.,[3] 67.30; obsd., 67.48. Oxidation with alkaline hydrogen peroxide[1] produced equimolar quantities of *t*-butyl, isobutyl and *n*-amyl alcohols in high yield.

t-Butyl-isobutyl-*n*-amylborane was prepared in two ways: (a) in 50% yield by the alkylation of *n*-amyldifluoroborane with *t*-butylmagnesium chloride in anhydrous ether; (b) in 30% yield by the reaction of *t*-butyl-di-*n*-amylborane with isobutylmagnesium bromide.[4] The physical constants and the infrared spectra of the two samples were practically identical. In connection with method (a), it is noteworthy that one *t*-butyl group derived from the Grignard reagent rearranges to isobutyl during the alkylation reaction. Concerning

(1) Previous paper, G. F. Hennion, P. A. McCusker and A. J. Rutkowski, THIS JOURNAL, **80**, 617 (1958).
(2) Contribution from the Radiation Project operated by the University of Notre Dame and supported in part under Atomic Energy Commission Contract AT-(11)-38.
(3) The B–C bond refraction was taken as 1.93.
(4) S. L. Clark and J. R. Jones, Abstracts, 133rd Meeting, American Chemical Society, San Francisco, April, 1958, p. 34-L.

method (b), it should be noted that *t*-butyl-di-*n*-amylborane (b.p. 47.5° at 0.14 mm., $n^{25}D$ 1.4333, d^{25} 0.7585) is also a new mixed trialkylborane stable to disproportionation when distilled *in vacuo* and was prepared by the alkylation of *t*-butyldichloroborane with *n*-amylmagnesium bromide in ether. *Anal.* Calcd. for $C_{14}H_{31}B$: B, 5.15. Found: B, 5.26; MR_D calcd.,[3] 71.93; obsd., 72.06.

A number of other mixed trialkylboranes stable to distillation have been prepared in This Laboratory. Details regarding their properties and methods of preparation will be reported at a later date.

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RECEIVED JUNE 2, 1958

A MICROMETHOD OF ELECTRODIALYSIS AND ITS APPLICATION TO THYROTROPIC HORMONE

Sir:

Electrodialysis has been used successfully with corticotropin[1] and the posterior pituitary hormones[2] to demonstrate that their respective biological activities do not reside in relatively large proteins but in smaller peptides. We wish to report a simple micromethod of electrodialysis utilizing membranes implanted in starch gels which has allowed us to study this question with regard to pituitary thyrotropin.[3]

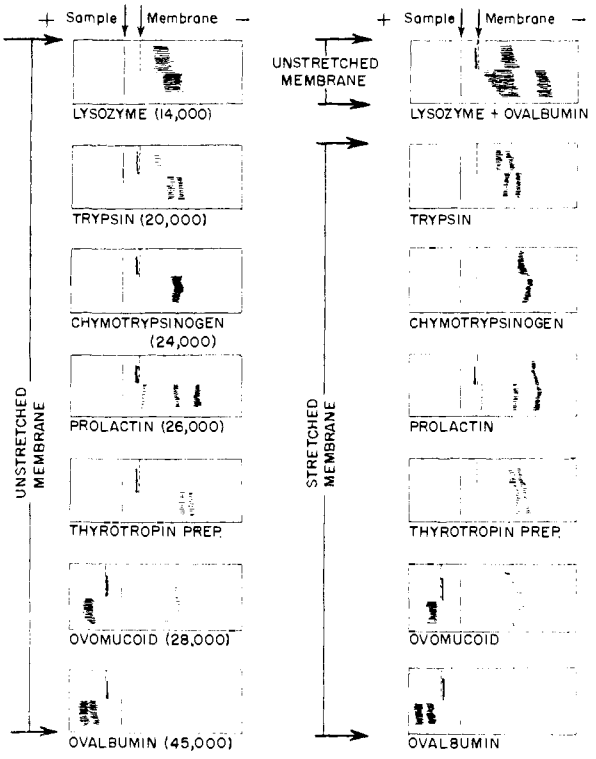

Fig. 1.—Diagram of starch gel electrodialysis with a series of proteins of increasing molecular weight (mol. wts. from ref. 5) in acetate buffer, *p*H 5.0, $\Gamma/2 = 0.012$: lysozyme + ovalbumin was run at *p*H 3.5; duration of runs 2–3 hr.; 260–300 volts; 14 ma. Proteins were commercial preparations except prolactin.[6] Those proteins not passing the membrane were detected as heavily stained areas just behind the membrane. With *p*H 9.5 glycine buffer, analogous results were obtained with trypsin, prolactin, ovomucoid and ovalbumin.

Based on the passage or non-passage of thyrotropic activity through cellophane membranes whose permeabilities were calibrated with proteins of known molecular weight, we have tentatively assigned a molecular weight in the range of 26,000–30,000 to the active principle.

The method makes use of the starch gel electrophoresis of Smithies[4] and is carried out in a trough, 2.3 by 30 cm., 1 cm. deep. The membrane is placed in the gel in either of two ways. One way is to insert the membrane across half the width of the gel, parallel to and about 1 cm. away from the filter paper[4] containing the sample. The effect of the membrane can thus be seen in direct comparison with the electrophoretic pattern of the sample. If the membrane, however, is impermeable to the protein, the protein tends to travel around it. This can be prevented by placing the membrane in a semicircle held in place by masking tape and then pouring the hot starch solution in the apparatus. The latter method was used when bioassay experiments were carried out.

Visking 20/32 dialysis tubing, which has been used by Craig and co-workers in studying dialysis of proteins by diffusion,[5] was found to be most suitable for the studies with thyrotropin (30 U.S.P. units per mg.[3]). With this membrane it was found that neither the biological activity nor any stainable material would pass through at *p*H 5.0 or at *p*H 9.5. If stretching by hydrostatic pressure is carried out,[5] the membrane allows both the activity and the staining components to pass at *p*H 5.0. These results afford strong evidence that the thyrotropic activity does not reside in a small peptide bound solely by electrostatic forces to a larger protein and thus separable by electrodialysis. The results together with those on known proteins, upon which the tentative molecular weight is based, are shown in Fig. 1. The resolving power of the starch gel electrophoresis and the selectivity of the membranes[5] are clearly illustrated in the case of the prolactin preparation, which separates into three components. None of these will pass the unstretched membrane but one component passes through the stretched membrane at both *p*H 9.5 and 5.0.

With respect to thyrotropin it should be remembered that other methods may still demonstrate that the activity resides in a small molecule. A full report of this work together with other attempts to dissociate the thyrotropic activity from

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