drostatic pressure difference which develops we are afforded a ready means of measuring the accumulated salt.

The cell is constructed of Pyrex glass, and consists of two vertical compartments connected through two horizontal tubes, one above the other. The upper of these is a piece of 2.5-mm. capillary tubing. The lower contains the membrane which is to separate the compartments. For this purpose we have found fritted glass disks of "ultrafine" porosity to be the most suitable. The compartments themselves are large tubes near the bottom, which taper rapidly up to the level of the capillary. Above this point each side consists of uniform tubing of 6-mm. inside diameter flared sharply at the top to permit easy introduction of material. Through the bottom of each compartment is sealed a piece of tungsten wire.

In operation the electrodes consist of pools of molten lead which cover the tungsten wires. Above this the cell is filled with molten lead chloride in such a way that an air bubble is trapped in the capillary. The difference in levels which develops on electrolysis is most readily equalized by flow through the capillary, so that the displacement of the bubble provides an accurate measure of the volume changes occurring. The cell is most conveniently used as a null instrument by adding a weighed quantity of powdered $PbCl_2$ to one compartment and measuring the number of coulombs required to return the bubble to its original position.

Assuming the only current carrying species to be Pb^{++} and Cl^-, it can be shown readily that the transport number of the negative ion is given by the equation

$$t_- = \frac{\pm\left(\frac{96{,}500 \text{ wt. salt}}{\text{coulombs passed}}\right) + \left(2 \text{ eq. wt. metal} \times \frac{\text{Density of salt}}{\text{Density of metal}}\right)}{2 \text{ eq. wt. salt}}$$

the positive sign being used in this case since the bubble moves toward the cathode during electrolysis. Using the data of Lorenz, *et al.*,[3] and that of Pascal and Jouniaux[4] for the densities of lead chloride and lead, we find the value of t_- at 565° to be 0.758 ± 0.014 (average deviation for 20 experiments). Substantially the same result was obtained using a plug of tightly packed asbestos as a membrane, but "fine" and "medium" porosity fritted disks permitted enough backflow to introduce appreciable errors.

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AMINOETHYL DIARYLBORINATES; ISOLATION OF A STABLE UNSYMMETRICAL ORGANOBORON COMPOUND

Sir:

The development of the chemistry of the diarylborinic acids has been hampered seriously by the lack of adequate methods for their isolation and characterization. Several arylborinic acids have been reported, but in no case was the yield specified and in some cases considerable doubt exists about the identity of the compounds described.[1-4]

We recently observed that these compounds readily could be isolated and characterized as their aminoethyl esters. The acids were prepared by the reaction of two mole equivalents of the appropriate Grignard reagent with one mole equivalent of butylethylene borate or butyl borate, followed by hydrolysis with dilute hydrochloric acid. Diphenylborinic acid in the form of the butyl ester was separated from triphenylborine, another reaction product, by distillation and from butyl benzeneboronate by precipitation from ether with ammonia. The relatively unstable ammonia complex (m.p. 64–67°), isolated in 48% yield (based on the phenylmagnesium bromide), was then converted (80–90%) to aminoethyl diphenylborinate (m.p. 189–190°. Calcd. for $C_{14}H_{16}ONB$: B, 4.807; C, 74.70; H, 7.16; N, 6.23; neut. eq., 225. Found: B, 4.795, 4.807; C, 74.02; H, 7.11; N, 6.47; neut. eq., 225) by reaction with ethanolamine in an alcohol–water solution. In the case of aminoethyl di-α-naphthylborinate, it was convenient to precipitate the ester directly from a toluene solution of the reaction products without prior distillation and ammonia precipitation. Purification was accomplished in this case by reprecipitating the aminoethyl ester from alcohol and water, followed by recrystallization (yield based on butyl borate, 45%; m.p. 192–193.5°; calcd. for $C_{22}H_{20}ON_{13}$: B, 3.33; neut. eq., 325. Found: B, 3.30; 3.20; neut. eq., 328). Acid hydrolysis of these esters yielded the free diphenylborinic acid as an oil which did not crystallize, and the α-naphthylborinic acid as a solid, m.p. 105–106°; neut. eq., 290 (calcd. neut. eq., 282).

These techniques made possible the isolation of a stable, unsymmetrical diarylborinate. Of the previous attempts to isolate organoboron compounds with two different groups attached to the boron atom[5-8] success has been had only with the alkenylmethylborines.[8] Even these substances disproportionated slowly at room temperature. We isolated from a reaction of 0.049 mole of naphthylmagnesium bromide and 0.05 mole of butyl benzeneboronate in ether at −60°, 10.1 g. (75%) of aminoethyl phenyl-α-naphthylborinate (m.p. 228–229°; calcd. for $C_{18}H_{18}ONB$: B, 3.93; neut. eq., 275. Found: B, 3.93, 3.74; neut. eq., 275). This compound is stable and shows no sign of undergoing disproportionation under normal conditions. From degradation experiments with hydrogen peroxide, zinc chloride and bromine were isolated naphthol, naphthalene and bromobenzene (characterized by conversion to benzoic acid) and bromonaphthalene, respectively.

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The B analyses were made by J. Thoburn; the C, H and N by H. Beck and V. Stryker.

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RECEIVED JUNE 23, 1954

THE DEGRADATION OF CHOLESTEROL BY MAMMALIAN TISSUE EXTRACTS

Sir:

As part of a study concerned with the metabolic degradation of cholesterol and its conversion to bile acids, we have successfully prepared aqueous particle-free extracts of mammalian tissue which are capable of degrading the side-chain of cholesterol. The major product, obtained in yields up to 5%, has been isolated and identified as isocaproic acid. Active enzyme preparations have been obtained from beef adrenals, ovary, testis and rat liver. Extracts of the first three tissues were obtained by homogenization in 0.3 *M* sucrose followed by high speed centrifugation (85,000 *G*) at 0° for 30 minutes to remove the particulate fraction. The sedimented material was essentially inactive. The aqueous phase required only adenosine triphosphate and diphosphopyridine nucleotide for activity. This was demonstrated by precipitation of the active enzymes by half saturation with $(NH_4)_2SO_4$, followed by dialysis of the precipitate for 24 hours against cold water. The resulting solution, which was rendered inactive by stirring for a few minutes with charcoal, was restored to activity by the addition of the above cofactors. In most instances the enzyme preparations were incubated for three hours at 37° under oxygen or air at a *p*H of 8.3 (0.07 *M* tris-(hydroxymethyl)-aminomethane buffer). The substrate was an albumin stabilized emulsion[1] of cholesterol-26-C^{14}.[2]

The radioactive isocaproic acid was isolated by steam distillation of the acidified extracts followed by paper chromatography[3] (diethylamine–butanol; R_f 0.67). After elution from the papers and subsequent addition of carrier isocaproic acid, the anilide (m.p. 110°) and the *p*-bromophenacyl ester (m.p. 76–77°) were prepared. The specific activity of these derivatives remained constant despite repeated recrystallizations. Although small amounts of radioactivity could be detected in other acids, the isocaproic acid invariably contained the greatest radioactivity.

Following incubation of the adrenal extracts with cholesterol-4-C^{14} [4] several radioactive steroids have been isolated by standard chromatographic procedures.[5] Surprisingly, neither progesterone nor pregnenolone were found to be radioactive; negative results were obtained regardless of whether these two steroids were added as carrier prior to or following incubation.

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The active system in rat liver was found to be localized in the particulate fraction which could be extracted by stirring with water to obtain a soluble preparation. In this tissue the original cytoplasmic supernatant fluid was found to be inhibitory unless the proteins were denatured by boiling and subsequently removed by centrifugation.

It is hoped that a more extensive report of this study will be published shortly.

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RECEIVED JUNE 24, 1954

THE ACONITE ALKALOIDS. XXVI. OXONITINE AND OXOACONITINE

Sir:

Permanganate oxidation of aconitine,[1] $C_{34}H_{47}NO_{11}$, furnishes two neutral products, oxonitine[2,3,4] and oxoaconitine.[3,4] The nature and formulation of the former has long been in doubt.[2,4] However, the formula $C_{32}H_{41}NO_{12}$ based in part on an oxidative scission of the N-ethyl group is still accepted by some.[1,5] Because of the important relationship these substances bear to a correct interpretation of the structure of aconitine, we wish to present evidence showing that oxidation of aconitine to oxonitine as well as to oxoaconitine *proceeds without the loss of carbon atoms.*

It appears that the use of acetic acid or chloroform to recrystallize oxonitine has led to incorrect formulations due to retention of solvent. When recrystallized from ethanol, benzene or acetone, the data obtained clearly support a $C_{34}H_{45}NO_{12}$ formulation. Calcd. for $C_{34}H_{45}NO_{12}$: C, 61.90; H, 6.87. Found: (EtOH) C, 61.73, 61.66; H, 6.65, 6.86; (benzene) C, 61.83, 61.81; H, 6.74, 6.90; (acetone) C, 61.88, 62.10; H, 6.76, 6.73; (pyridine–acetone) C, 62.08; H, 6.64. Oxonitine from $CHCl_3$ or CH_2Cl_2 gave consistently low carbon values and showed the presence of several per cent. chlorine. Our oxonitine melted at 279–284° though occasionally 288–293° was noted apparently due to dimorphism. $[\alpha]^{27}D$ −49° (*c* 0.25 in chf.). Oxonitine in contrast to oxoaconitine did not form an oxime.

The formulation of oxoaconitine has now been revised to $C_{34}H_{43}NO_{12}$; m.p. 266–272.5°, $[\alpha]^{27}D$ −100° (*c* 0.3 in chf.). Calcd. for $C_{34}H_{43}NO_{12}$: C, 62.09; H, 6.59. Found: C, 61.90; H, 6.57. Oxime, m.p. 282–285.5°. Calcd. for $C_{34}H_{44}N_2O_{12}$; C, 60.70; H, 6.59, N, 4.17. Found: C, 60.59; H, 6.67; N, 4.37.

The formation of oxonitine and oxoaconitine from aconitine appears to involve oxidation of an N-ethyl group to N-acetyl. Their behavior toward

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