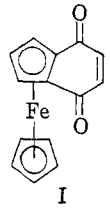
m.p. 119–120°, identical with an authentic sample from an independent route.[5a,b]

Similar oxidation of ethylferrocene[6] gave acetylferrocene[7] in 52% yield (15% conversion), while the only product obtained (as judged by paper chromatographic analysis)[8] from 1,1′-dimethylferrocene[1d] was 1′-methyl-1-ferrocenecarboxaldehyde, (15% yield, 7% conversion) m.p. 79.5–80.5° [*Anal.* Found: C, 63.07; H, 5.22]. More easily oxidizable alkylferrocenes gave better yields under milder conditions (chloroform solution, room temperature four to six hours). Thus, diferrocenylmethane (prepared in 83% yield by lithium aluminum hydride–aluminum chloride reduction[9] of diferrocenylketone[10]), m.p. 124–125° [*Anal.* Found: C, 65.27; H, 5.36] was reoxidized by manganese dioxide to the ketone in 72% yield (and conversion). Deoxyferrocoin[11] similarly gave in 86% yield (66% conversion) the purple ferrocil, m.p. 193.5–195.5° [*Anal.* Found: C, 61.84; H, 4.19].

A novel use of the reagent is in the preparation (11% yield) of a ferroçobenzoquinone (I) from 1,2-(α-ketotetramethylene)-ferrocene.[12] The deep violet quinone (I), purified by sublimation at 135–145° (atm.), m.p. 146–147° (*Anal.* Found: C, 62.96; H, 4.14], has an infrared carbonyl band



I

at 1653 cm.$^{-1}$ and electronic absorption maxima at 520 mμ (ϵ_{max} 2260), 320 mμ (ϵ_{max} 3200) and 248 mμ (shoulder, ε 8400). For comparison, naphthoquinone is reported to have a carbonyl band at 1682 cm.$^{-1}$,[13] and electronic absorption maxima at 338 mμ (ϵ_{max} 3160) and 246–251 mμ (ϵ_{max} 21,900).[14] I may be reduced either chemically (sodium hydrosulfite) or polarographically to the unstable pale yellow hydroquinone (λ_{max} 320 mμ, ϵ_{max} 5000; naphthalenediol[14] λ_{max} 327–334, ϵ_{max} 5240, λ_{max} 244 mμ, ϵ_{max} 15,100), which is reoxidized rapidly to the quinone.

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PROPERTIES OF A PURIFIED SIALIDASE AND ITS ACTION ON BRAIN MUCOLIPID

Sir:

The various biological high polymers containing sialic acid, *e.g.*, the mucoproteins and mucolipids, command increasing interest. Our original observations[1] on the enzymic release of sialic acid from normal brain mucolipid,[2] but not from ganglioside[1,3,4] prompted the use of sialidase for the elucidation of the mode of linkage of glycolipid-bound sialic acid. As crude filtrates of cultures of *Vibrio cholerae* were inadequate for this purpose, a relatively simple procedure for the isolation from such filtrates of highly purified enzyme preparations in satisfactory yield was elaborated. It differs from recent adaptations,[5,6] published while this work was in progress, of the ingenious method employing adsorption on erythrocytes as proposed by the discoverers of the enzyme.[7] Since the properties of sialidase are largely unknown, we present preliminary information on this enzyme and its action on mucolipids.

The essential features of the isolation of sialidase are listed in the table.

TABLE I

ISOLATION OF *V. Cholerae* SIALIDASE

Stage	Procedure	Activity Recovery of total (%)[a]	Specific[b]
1	Original culture filtrate	100	10
2	Pptd. 26% ammonium sulfate, *p*H 6.2	95	
3	Pptd. 30% ammonium sulfate, *p*H 6.6	105	100
4	Pptd. 26% ammonium sulfate, *p*H 5.3	100	
5	H_2O extraction and lyophilization	22	1,300
6	DEAE cellulose, 0 to 0.6 *M* NaCl, *p*H 6.65	22	12,600
7	Pptd. 15–25% ammonium sulfate, *p*H 5.3	22	303,000

[a] The original culture filtrate contained 180,000 units per liter. [b] Units per μg. of protein.

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The enzyme unit is defined as liberating 1 μg. of sialic acid[8] in 1 hr. at 37° from 3 mg. of ox brain mucolipid[2] in 1 ml. of 0.01 *M* Tris-acetate buffer of *p*H 6.6. At Stage 5, the protein sedimented with a single boundary having an s_{20} value near 1 *S* and could be crystallized as needles by being kept in concd. aqueous solution around 0° overnight; both criteria are not indicative of purity as shown by the table. Provisional determinations showed the final product (Stage 7) to have almost the same s_{20} value and a diffusion constant D_{20} well above 15×10^{-7}. It would seem that sialidase is an unusually small enzyme, conceivably with a molecular weight of below 10,000. The preparations are relatively stable at all stages except 7 following which they must be either lyophilized immediately or kept in frozen solution in order to preserve activity.

Treated with neutral Versene and subjected to exhaustive dialysis, the enzyme lost activity completely. The latter was restored by Ca^{++} ion[9] which was optimal near a 4 m*M* concentration. Mn^{++} or Co^{++} was equimolarly almost as effective, Cd^{++} or Mg^{++} restored 29% of the activity, Ba^{++} was ineffective. The activation pattern is, perhaps, in agreement with the assumption that the cation functions with the carboxylate groups of the enzyme and those of glycosidically bound sialic acid as the ligands.[10]

Loss of activity occasionally encountered during purification could be reversed entirely by treatment with Versene, dialysis and addition of Ca^{++}. The treatment of 70 μg. of enzyme with 5 micromoles of either iodoacetate or arsenite resulted in the almost complete loss of activity. Full inactivation was produced by Fe^{+++} or Hg^{++}, a 50% inhibition by Pb^{++}. Cysteine, thioglycolate, glutathione or cyanide did not restore enzymic activity.

In its action on mucolipid[2] the *p*H optimum of sialidase was found between *p*H 6.6 and 6.9; a lower secondary maximum was observed between *p*H 5.4 and 5.9. A Michaelis constant K_m of 4.1×10^{-2} *M* was calculated. Initial studies indicated that three out of four molecules of polymer-bound sialic acid are liberated by the enzyme. Two different experimental arrangements were employed for the study of the enzymic degradation of highly purified, homogeneous mucolipid preparations. When the release of free sialic acid[8] into a fixed volume of reaction mixture was followed, hydrolysis of the lipid polymer terminated with the liberation of 66% of the sialic acid within about 6 hr. When serial estimations of non-dialyzable sialic acid[11] were performed, the assay mixture being kept under conditions permitting the dialysis of liberated sialic acid, a 72% release was recorded, though more slowly.

We shall present later more detailed studies of this interesting enzyme which appears to be a protein of remarkably low molecular weight, possibly having sulfhydryl functions necessary for its action, and requiring Ca^{++}, Mn^{++} or Co^{++} ions for activity.

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ON THE MECHANISM OF THIAMINE ACTION

Sir:

A recent publication by Breslow and McNelis appearing under the above title,[1] which described the kinetic instability of 2-acetylthiazolium compounds, prompts us to report a similar observation we have made with 2-benzoylthiazolium salts.

The known 2-(α-hydroxybenzyl)-4-methylthiazole was prepared by a previous method.[2] Dichromic oxidation of this alcohol in acetic acid solution[3] gave 2-benzoyl-4-methylthiazole in 90% yield; m.p. 42–43°, calcd. for $C_{11}H_9NOS$: C, 65.00; H, 4.46; N, 6.89; S, 15.78. Found: C, 65.43; H, 5.05; N, 6.81; S, 15.75. The 2,4-dinitrophenylhydrazone, m.p. 218–220°, calcd. for $C_{17}H_{13}N_5O_4S$: C, 53.25; H, 3.41; N, 18.26; S, 8.36. Found: C, 53.21; H, 3.64; N, 18.53; S, 8.50. Quaternization of the 2-benzoyl-4-methylthiazole was found to be rather difficult in agreement with the findings of Breslow and McNelis for 2-acetyl-4-methylthiazole. However, some quaternization was accomplished when 0.85 g. of the ketone was refluxed with 15 ml. of methyl iodide and 10 ml. of dimethylformamide for 13 hours. Pouring the reaction mixture into 100 ml. of cold dry ether gave upon standing 0.21 g. (15% yield calculated as the methiodide) of crude crystals.

In order to test the methanolysis of this product, 50 mg. was dissolved in 1 ml. of methanol and immediately analyzed by gas chromatography. A peak was obtained which corresponded to a known sample of methyl benzoate. The volatile material was removed at 0.15 mm. from an 80° bath. Hydrolysis with base, and acidification with HCl gave 12 mg. of benzoic acid (theoretical 17.6 mg.), m.p. 122°.

These experiments show in agreement with those of Breslow and McNelis that 2-acylthiazolium salts can be solvolyzed very easily in methanol. The reaction of methanol with 2-acylthiazolium salts to give methyl benzoate is suggestive of a reaction of phosphate ion with 2-acetylthiamine to give acetyl phosphate. Thus 2-acetylthiamine is a possible in-

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