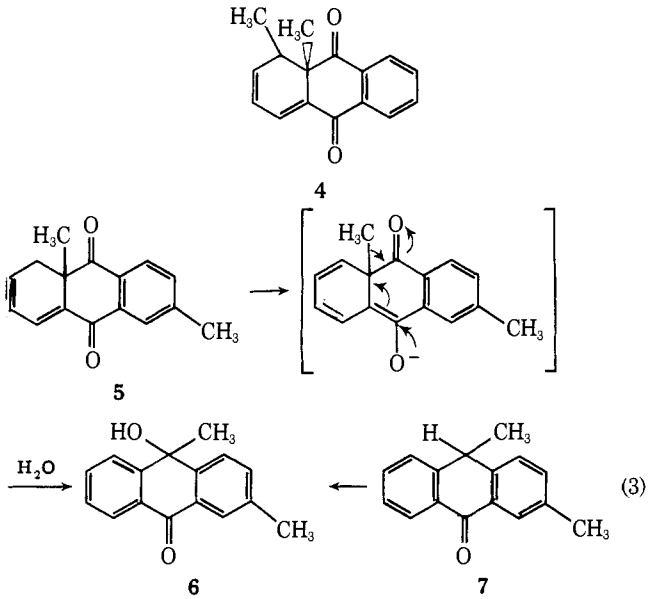
Formation of anthrone **3a** from reaction of **2** with base might proceed by either a [1,2] or a [1,3] shift of a methyl group to a carbonyl carbon.[10] To distinguish between these two mechanisms, it was necessary to carry out the rearrangement with a starting material which would yield a less symmetrical product. For this purpose diketone **4** was synthesized starting with a Diels–Alder reaction between 2-methyl-1,4-naphthoquinone and *trans*-piperylene. Surprisingly, however, no reaction was observed with **4** under conditions in which **2** was completely converted into **3a.** Use of stronger base or higher temperatures gave an inseparable mixture of products which did not appear to contain significant amounts of the desired analogue of **3a.**

Diketone **5** was therefore prepared in a manner similar to that used to synthesize **2**, starting with 2,6-dimethyl-1,4-naphthoquinone.[12] Rearrangement of **5**, catalyzed by potassium *tert*-butoxide in HMPT, yielded a single product, which was identified as **6** (eq 3). Hydroxyanthrone **6** was independently prepared by oxidation of anthrone **7**[13] with hydrogen peroxide in base.



Thus, rearrangement of **5** (and undoubtedly of **2**) proceeds by a [1,2] shift of the methyl group.[14] The occurrence of this novel type of rearrangement is clearly due to the fact that a new aromatic ring is formed in the reaction. The anions of **2** and **5** can thus be considered to be novel members of the class of "blocked aromatic molecules", which can become aromatic by migration of a single ring substituent.[16]

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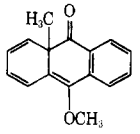

i

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Pyridine-Containing Polymers: New Matrices for Protein Immobilization

Sir:

Owing to the increased use of polymers in chemistry and biology, a considerable interest in immobilization of entities such as enzymes, cells and small ligands has developed.[1] Such preparations are used for practical applications such as purification of compounds by affinity chromatography[2] and for fundamental studies such as mechanisms of chemical[3] and enzymatic reactions.[1] The advantage of using immobilized species are numerous and include reuse, ease of separation from the reaction mixture and increased stability against heat, autolysis, and chemical effects.

The formation of a Schiff base between an aldehyde-containing matrix and free amino groups of proteins provides mild and simple reaction conditions for coupling. Polymeric aldehydes are usually prepared by reaction of an excess of glutaraldehyde with polymeric amines[4] or hydrazides[5] or by oxidation of polysaccharides with sodium periodate.[6] The excess of glutaraldehyde is used to block all the amino groups and to prevent cross-linking of the polymer.

The reaction of the nucleophilic pyridine nitrogen with strong electrophiles such as cyanogen bromide, fluorodinitrobenzene, and *p*-toluenesulfonyl chloride to yield glutaconic aldehyde is well known.[7] This reaction is the basis for a quantitative method for the determination of cyanides[8] and was recently used for determining the extent of activation of polysaccharides with cyanogen bromide.[9]

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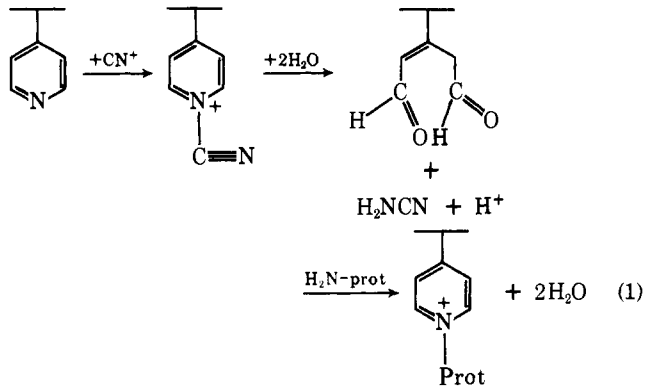
Polymers containing pyridine can be directly converted into polyaldehydes (glutaconic aldehyde) by the reaction with cyanogen bromide. The polyaldehyde formed can be used for the binding of protein and other ligands. The overall reaction is summarized in the eq 1. In these polymers no cross-linking can



occur, and the amount of aldehyde formed is controlled by the amount of cyanogen bromide added. Further advantages are that excess aldehyde groups can either be reduced with borohydrides or converted back to pyridine by reaction with ammonia. Also the products of the reaction with proteins are pyridinum salts and therefore they are completely stable and no leakage was observed on prolonged storage.

In order to show that polypyridines can be converted into the corresponding aldehydes, polyvinylpyridine, polysaccharides, glass beads, and polyacrylamide derivatives containing pyridine were prepared, and reacted with CNBr under anhydrous conditions. The polymers were filtered and treated with water followed by reagents which react with aldehyde. All the pyridine-containing polymers gave strong blue colors with barbituric acid and reacted strongly with *p*-nitrophenylhydrazine, indicating the presence of aldehydes. This was substantiated by infrared spectroscopy.

The aldehydes formed were also capable of binding amines. This was demonstrated by the binding of the copper complex of lysine through its ϵ-amino groups. The amount of lysine bound to polyvinylpyridine was 1.3 mmol/g of dry gel as determined by the amount of copper bound. All of the polymers described above were used to bind several proteins including trypsin and chymotrypsin and specific examples of enzyme binding are given below.

(1) Dry polythiol (Koch & Light), 1.0 g, was heated with 4-vinylpyridine in a well-stoppered flask on a shaker for 3 h at 37 °C. The suspension was then kept at room temperature overnight. The gel was filtered, washed carefully with ethanol, and dried. The gel was reacted at room temperature with 1-3 g of CNBr in dry dioxane (5 mL) for 5 min. Water was then added and the mixture stirred for 20 min at room temperature. The gel was filtered, washed with cold water, and stirred overnight with a solution of trypsin in phosphate buffer pH 5.0 at 4 °C. Using this procedure one can bind up to 117 mg of enzyme/g of dry gel. The specific activity of the immobilized enzyme was 70% that of soluble trypsin. The apparent K_M in the hydrolysis of (N,α)-benzoylarginine ethyl ester was close to that of soluble trypsin. The pH optimum for activity of the immobilized enzyme was about pH 9.5.

(2) Glass beads containing amino groups, 1.0 g,[10] were reacted with pyridine-4-carboxyaldehyde in phosphate buffer (pH 7.0 for 1 h at room temperature), washed with water, and dried carefully. The beads were suspended in dioxane (5 mL), degassed, and activated with 1-3 g of CNBr dissolved in absolute dioxane (3 mL) for 5 min at room temperature. Following the addition of 0.25 M carbonate buffer pH 9.0, the mixture was stirred at room temperature for 20 min, the pH being held constant by manual titration with 2 N NaOH. The activated glass beads were then washed with cold water and coupled to the enzyme in the same manner as described above. Trypsin, as well as α-chymotrypsin bound to glass matrices, had high specific activities. The pH optimum for activity of the immobilized trypsin was the same as with the other gel, whereas that of α-chymotrypsin was pH 9.0.

The polypyridines described are not the only polymers that can be used since the number of methods for preparing pyridine containing polymers and copolymers is almost unlimited judging from the numerous pyridine derivatives available. Pyridine is also a component of many synthetic membranes and ion-exchange resins. Thus, proteins as well as other molecules, can be bound to any of these polymers.

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Highly Selective Membrane Transport of Pb^{2+} from Aqueous Metal Ion Mixtures Using Macrocyclic Carriers[1]

Sir:

Incorporation of macrocyclic ligands into hydrophobic membranes to serve as cation carriers offers a method to exploit the high cation selectivity[2] demonstrated by these ligand molecules. Means have been developed[3,4] whereby cation transport of this type can be coupled to free-energy gradients which drive the flux of cations against the cation concentration gradient. Potential applications[5] of this technology include the separation and concentration of chemical species, the detection and measurement of chemical species, and the removal of undesirable chemical species from the environment or from biological systems.

In the present communication, we report the transport rates of Pb^{2+} and of several alkali, alkaline earth, and transition metal cations through liquid membranes containing one of several macrocyclic ligands. Our objective was to determine the effectiveness of membranes containing macrocycles in selectively transporting Pb^{2+} which is of interest in relation to the environment and human toxicity. Some membrane transport data have been published indicating rates of transport of individual cations.[3,4,6-10] In addition, Tl(I) and K^+ have been transported by macrocycles in the presence of a large excess of Na^+.[11] However, we believe that this is the first report of liquid membrane transport in which macrocyclic carriers are used to effect separation of divalent metal cations from cation mixtures. We have found that remarkably high transport selectivities for Pb^{2+} can be achieved even when the ratio of Pb^{2+} concentration to the concentration of another cation in the mixture is <1/100.

Liquid membrane experiments were performed using cells

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