uenesulfonyl chloride, 4-dimethylaminopyridine, and triethylamine in $CHCl_3$ at 23 °C.

Treatment of keto tosylate **16** with base leads to internal α alkylation at either C-12 or C-15 depending on reaction conditions, the use of kinetically controlled enolate formation with a highly hindered base at low temperatures favoring the desired alkylation at C-12. Thus addition of **16** in 2-methyl-tetrahydrofuran to an excess of lithium di-*tert*-butylamide[26] in the same solvent at −120 to −130 °C, followed by gradual warming, produced the tetracarbocyclic ketone **17** in 90% yield.[27] On the other hand reaction of **16** with sodium methoxide in methanol at 0 °C led exclusively to the product of internal alkylation at C-15, probably the consequence of fast reversible enolate formation and relatively slow internal alkylation at C-12. Synthetic (±)-**17** obtained as described above was indistinguishable from an authentic sample (prepared by the acetalization of pivalaldehyde with keto diol **18** derived from 1,2-glycol cleavage of aphidicolin of natural origin[28]) by chromatographic, ^1H NMR, IR, and mass spectral comparison. Hydrolysis of (±)-**17** (70% aqueous perchloric acid in methanol at 80 °C for 5 days) afforded synthetic (±)-**16** which was spectroscopically and chromatographically identical with a naturally derived reference sample.[28] Reaction of (±)-**17** with 1-ethoxyethoxymethyllithium[29] afforded, after hydrolysis of the resulting C-16 carbonyl adduct with 2:2:1 acetic acid–methanol–water, a 1:1 mixture of (±)-aphidicolin and the epimer at C-16 which was not readily separable by chromatography.[30] The corresponding mixture of bisacetonides (prepared from tetraol, 10 equiv of 2-methoxypropene, and pyridinium tosylate at 23 °C for 10 min) could be separated into **1** bisacetonide and the C-16 epimer, R_f 0.22 and 0.16, respectively, on silica gel plates using three developments with 5.5% ethyl acetate in hexane. The bisacetonide of synthetic (±)-**1** was chromatographically and spectroscopically identical with the bisacetonide of natural aphidicolin.[28] Finally, acid-catalyzed hydrolysis of the synthetic bisacetonide as described previously[1] afforded (±)-aphidicolin, indistinguishable chromatographically and spectroscopically from naturally obtained aphidicolin.[28,31] The synthesis of (±)-aphidicolin described here raised a number of interesting and unexpected problems which have now been successfully overcome.[32]

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(30) The conversion of the ketone **18** into aphidicolin via two other routes was also found to be nonstereoselective. One of these involved methylenation by the Wittig reaction and hydroxylation with osmium tetroxide (giving desired and undesired epimers in a 1.3:1 ratio) and the other addition of 2-lithio-1,3-dithiane, dithiane cleavage, and reduction of formyl to primary alcohol (giving desired and undesired epimers in a ratio of ~1:3.5).
(31) To our knowledge this is the first conversion of (±)-**18** into (±)-aphidicolin, although this step should have been a required process in previously announced syntheses of (±)-aphidicolin.[2,3]

(32) This research was assisted financially by a grant from the National Science Foundation. We are indebted to Dr. Larry C. Blaszczak for much valuable help and for providing a quantity of 5,9-dimethyl-5(10)-octalin-1,6-dione. Mr. Jay W. Ponder made helpful contributions to the experimental work.

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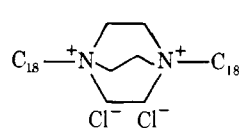
Carrier-Mediated Selective Transport of Nucleotides through a Liquid Membrane

Sir:

Bioenergetics is based on the interconversions among various nucleoside phosphates and other so-called high-energy phosphate compounds. This process vital to a variety of functions of living organisms requires a transmembrane movement specific for the particular phosphate involved. These phosphates should be encapsulated in an intrinsic carrier molecule such as an ionophoric protein in mitochondria[1] to facilitate the entry of otherwise highly hydrophilic phosphate anions into a lipophilic biological membrane. Although cationic transport is known to be mediated by several antibiotics and synthetic polyethers,[2] very few carrier models have been reported for the selective membrane transport of anionic species.[3]

Here we report the first successful selective transport of nucleoside phosphates through a chloroform liquid membrane. The carrier used was a lipophilic diammonium salt of diazabicyclooctane, such as **1**,[4] which bound a given nucleotide se-



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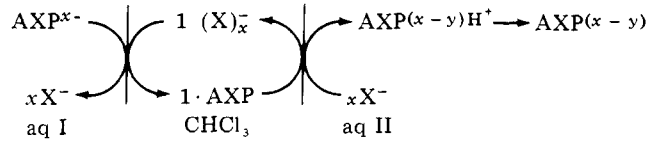

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Table I. Carrier-Mediated Transport of AMP and ADP through a Chloroform Liquid Membrane

run	aqueous I nucleotide	aqueous I concn, M	aqueous I pH	$CHCl_3$, **1**, M	aqueous II pH	aqueous II salt, M	transport rate, $\mu M/h \cdot cm^2$
1	ADP	1.0×10^{-2}	8.0	2.5×10^{-4}	0.5		2.62
2	ADP	1.0×10^{-2}	8.0	2.5×10^{-4}	1.0		1.05
3	AMP	1.0×10^{-2}	8.0	2.5×10^{-4}	1.0		0.22
4	ADP	1.0×10^{-2}	5.0	2.5×10^{-4}	0.5		0.238
5	ADP	1.0×10^{-2}	5.0	1.0×10^{-3}	0.5		1.8
6	AMP	1.0×10^{-2}	5.0	2.5×10^{-4}	0.5		0.006
7	ADP	1.0×10^{-3}	5.0	2.5×10^{-4}	5.0	NaBr (1.0×10^{-2})	0.267
8	ADP	5.0×10^{-4}	5.0	2.5×10^{-4}	5.0	[NaBr (1.0×10^{-2})-ADP (5.0×10^{-4})]	0.267

lectively at one interphase of water-chloroform and transported it through a chloroform liquid membrane and released the nucleotide into the other aqueous phase.[5] Proton and salt concentration gradients successfully drove the passive as well as the active transport of AMP and ADP.

Thus, distearyl-Dabco dichloride **1**[4] was dissolved in chloroform (2.5×10^{-4} M, 10 mL) and the solution was stirred gently (rpm, 50) in contact with two aqueous phases, one (aq I) containing adenosine mono- or diphosphate (1.0×10^{-2} M, 5 mL) and the other (aq II) filled with water (5 mL). The pH of each aqueous phase was adjusted by the addition of a small amount of concentrated NaOH or HCl solution to the specified value listed in Table I. The nucleotide concentration in aq II, monitored spectroscopically (λ_{max} 260 nm), increased following zeroth-order kinetics.[6] Transport rates obtained from their slopes are listed in Table I. When comparison was made with rates obtained at the same pH gradients, ADP was transported more effectively than AMP. The transport rate ratio, k_{ADP}/k_{AMP}, was estimated to be 4.8 at pH 8.0 (runs 2 vs. 3) and amounted to 40 at pH 5.0 (runs 4 vs. 6). The higher rate ratio at pH 5 is assignable to the higher selectivity observed for the binding of ADP compared with that ofAMP at pH 5.0.[4] The higher the pH of aq I, the larger was the overall transport rate (runs 1 vs. 4), as expected from the more effective uptake of nucleotide from the aqueous to the organic phase at higher pH. It is worth mentioning here that the transport rate reported here is comparable with that reported by Lehn et al.[3a] for the transport of amino acid by means of phase-transfer reagent tricaprylmethylammonium chloride. Our value is, e.g., 1.05×10^{-2} M/(h·cm²·M carrier) for run 1 and Lehn's ranges from 2.0×10^{-4} for serine to 1.25×10^{-2} for phenylalanine. Note that, in the amino acid transport, much more drastic condition was applied to promote the membrane potential difference, i.e., 0.1 N KOH and 0.1 N HCl and the initial substrate concentration was 5-fold larger than ours.

To suppress the concomitant hydrolysis of ADP in aq II observed during the above slow transport,[7] the transport rate should be accelerated. For the purpose, we examined the relative rate of release of the nucleotide by the exchange with an anion dissolved in aq II. The relative rates of the liberation of ADP bound to **1** were in the following decreasing order: $ClO_4^- > SCN^- > Br^- > NO_3^- > PPi > SO_4^{2-} > Cl^- > HCO_3^- > F^- > CH_3CO_2^- > Pi$. Perchlorate and thiocyanate anions released all of bound ADP very readily but they formed stable complexes with diammonium salt **1** inhibiting any further transport of the nucleotide. Thus, bromide anion was chosen for the exchange reagent in aq II. As shown in run 7, the ADP transport was successfully driven by use of the salt gradient of NaBr without proton gradient. ADP transported did not suffer undesirable hydrolysis to an observable extent.

Active transport of ADP was attempted by using the triphase system where the initial concentrations of ADP were the same (5×10^{-4} M) for both aqueous phases (run 8). The ADP concentration in aq II increased at the same rate as observed for run 7, consistent with the identical decrease rate of ADP in aq I. The result clearly indicates the first successful active transport of nucleotide by means of synthetic carrier molecule. A direct application of this transport device may involve an accumulation of nucleotides which formed in low concentrations via e.g., oxidative or photophosphorylation.

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(6) When aq I was adjusted to pH 8, an induction period (~50 h) was observed during which the nucleotide concentration in the chloroform phase increased gradually to a steady-state value (1.27×10^{-5} M). The transport rates listed in Table I are values obtained at the steady state. When pH was set to 5.0 in aq I, no appreciable induction period was observed.

(7) Analysis found that 85% of ADP transported was hydrolyzed to AMP after 68 h.

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Resolution and Assignment of the 270-MHz Proton Spectrum of Cellobiose by Homo- and Heteronuclear Two-Dimensional NMR

Sir:

The growing interest in complex naturally occurring carbohydrates encourages the development of methods for the determination of their primary structure and solution geometry. Although conventional ^1H NMR methods have sufficient resolving power to provide detailed structural information for derivatized oligosaccharides which are soluble in organic solvents, studies of oligosaccharides in aqueous solution are largely confined to the anomeric resonances. However, as we now demonstrate for cellobiose (**1**, D-glucopyranosyl-(β1→4)-D-glucopyranose), the combined use of homo- and heteronuclear two-dimensional (2D) NMR[1,2] makes possible a complete resolution and assignment of the proton spectrum.

Standard experimental methods were used to obtain the proton 2D *J* spectrum[3-5] and the carbon-13-proton chemical shift correlation 2D spectrum[2,6] of a 0.3 M solution of cellobiose in D_2O. The 2D *J* spectrum of Figure 1 was obtained by applying[7] a 45° tilt[3,8] to the experimental 2D spectrum, so that the horizontal and vertical frequency axes display pure

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