sphere. After stirring 72 h, the benzene was removed under reduced pressure and the product was extracted from the crude using hot hexane. This method gave 12.32 g (10.8%) of product: mp 65.3–66.0 °C; IR, 1740 cm^{-1} (C=O); NMR δ 3.48 (s, 2 H, $COOCH_2COO$), 3.70 (s, 12 H, $COCH_2CH_2O$), 3.79 (t, 4 H, $COOCH_2CH_2O$), 4.36 (t, 4 H, $COOCH_2$).

Anal. Calcd for $C_{13}H_{22}O_8$: C, 50.98; H, 7.24; mol wt, 306.3. Found: C, 50.90; H, 7.40; mcl wt, 306.

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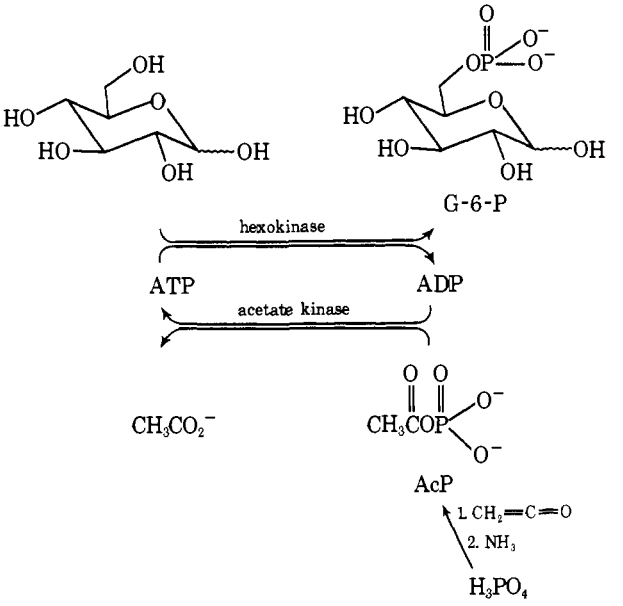
Large-Scale Enzymatic Synthesis with Cofactor Regeneration: Glucose 6-Phosphate[1]

Sir:

Many important reactions in enzyme-catalyzed biosynthesis consume cofactors in stoichiometric quantities. The cost[2] of the most commonly required cofactors has discouraged the use of these enzymatic reactions for the synthesis of organic compounds on any scale greater than a fraction of a mole.[3,4] We have previously proposed a scheme for the enzymatic regeneration of ATP from ADP or AMP, and outlined its possible use in large-scale cofactor-requiring synthesis.[5] Here we demonstrate the practicality of this scheme by the preparation of glucose 6-phosphate (G-6-P) from glucose on a mole scale.

A representative reaction was carried out in a 5-L flask modified to accept a pH electrode. The flask was charged with 1200 mL of solution (pH 6.6) containing glucose (1.4 mol), ATP (10 mmol), $MgCl_2$ (98 mmol), EDTA (4.8 mmol), and dithiothreitol (18 mmol). Polyacrylamide gel particles (20–50 μm in diameter) containing covalently immobilized hexokinase (ATP: D-hexose-6-phosphotransferase, E. C. 2.7.1.1, 1200 U.) and acetate kinase (ATP: acetate phosphotransferase, E. C. 2.7.2.1, 1100 U.) were suspended in this solution.[6] Diammonium acetyl phosphate (AcP, 0.7 M) was added continuously over 48 h at 40 mL/h to the magnetically stirred reaction mixture.[7] The solution was maintained between pH 6.6 and 6.9 by addition of 4 M potassium carbonate solution using an automatic pH controller.[8] The reaction was conducted at 25 °C, and the reaction mixture and reagent solutions were deoxygenated before use and maintained under argon. After



50 h of operation (1.36 mol of AcP added), enzymatic assay[9] indicated that 1.09 mol of G-6-P had been formed: its final concentration was 0.31 M. The polyacrylamide gel particles were allowed to settle, and the solution was decanted. Inorganic phosphate (0.27 mol, estimated by the difference between the AcP added and the G-6-P formed) was precipitated by addition of a stoichiometric quantity of $Ba(OH)_2$ and removed by filtration. G-6-P was then precipitated by addition of 1.2 mol of $Ba(OH)_2$: the resulting solid (502 g) contained 92% Ba G-6-P·$7H_2O$ (0.89 mol) by enzymatic assay.[9] This quantity corresponds to a 65% yield based on AcP added. The activities of hexokinase and acetate kinase were recovered in the gel in 93 and 75% yield, respectively. The turnover number for ATP during the reaction was >100; no effort was made to recover it.

Three points concerning experimental details deserve mention. First, the initial quantities of ATP and Mg(II) were chosen such that the concentration of MgATP and MgADP would be well above the Michaelis constants for the soluble enzymes,[10] even after dilution by the AcP solution. Second, the reaction proceeded satisfactorily with AcP having >80% purity. If the purity fell below 80%, complexation and precipitation of Mg(II) by the phosphate impurities made it difficult to maintain adequate concentrations of MgADP and MgATP in solution, and troublesome to isolate Ba G-6-P in high purity. Third, it was useful to carry out the reaction so that addition of AcP to the solution was overall rate-limiting and AcP was never present in the reaction mixture in high concentrations, to minimize spontaneous hydrolysis of AcP with concomitant release of phosphate.

Comparison of this preparation of G-6-P with existing chemical[11] or enzymatic[12] methods illustrates the potential of ATP-requiring enzymatic synthesis for the regioselective modification of unprotected, water-soluble, polyfunctional substrates. Since the hexokinases have broad substrate specificity,[13] this sequence should be directly applicable to the preparation of phosphates of a number of other sugars (e.g., fructose, mannose, deoxy-D-glucose, glucosamine). In broader terms, this conversion establishes that it is *practical* to couple enzymatic ATP regeneration with ATP-requiring enzymatic synthesis to achieve large-scale organic transformations. Reactions which require regeneration of ATP from AMP are also accessible using this reaction sequence, by adding adenylate kinase (AMP:ATP phosphotransferase, E. C. 2.7.4.3) to catalyze the conversion of AMP and ATP to ADP;[5] we will provide examples of this type of reaction sequence in the imme-

diate future. The good stability of the immobilized enzymes, and the ease of their recovery, suggests that these synthesis and regeneration schemes should have broad applicability in preparative organic chemistry.[12]

References and Notes

(1) Supported by the National Science Foundation (RANN), Grant No. GI 34284.
(2) Estimated costs, $/mole: ATP, 2,000; NAD^+, 2,500; NADH, 18,000; $NADP^+$, 60,000; NADPH, 250,000.
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(6) Enzymes were immobilized by addition to a solution containing a polymerizing mixture of acrylamide, *N,N'*-methylenebisacrylamide, and the *N*-hydroxysuccinimide active ester of methacrylic acid, 10 s before gel formation. The procedure used is a modification of that described (G. M. Whitesides et al., *Methods Enzymol.*, in press). Immobilization yields were 35% for hexokinase, and 40% for acetate kinase. The enzymes were commercial preparations (Sigma), and were used without purification: their specific activities (μmol min^{-1} mg^{-1}) were: hexokinase (from yeast), 420; acetate kinase (from *E. coli*) following activation with dithiothreitol, 300.
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(8) Preliminary experiments indicated that, under simulated reactor conditions, the rate of G-6-P formation was faster at pH 6.7 than at higher pH where the soluble enzymes would be expected to be more active (A. Sols, G. deLaFuente, C. D. Villar-Palasi, and C. Ascensio, *Biochem. Biophys. Acta*, **30**, 92 (1958); I. A. Rose, M. Grunberg-Manago, S. T. Korey, and S. Ochoa, *J. Biol. Chem.*, **211**, 737 (1954)).
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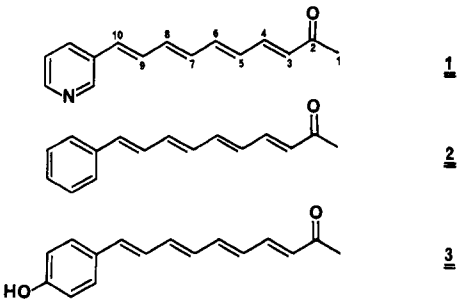
Navenones A–C: Trail-Breaking Alarm Pheromones from the Marine Opisthobranch *Navanax inermis*

Sir:

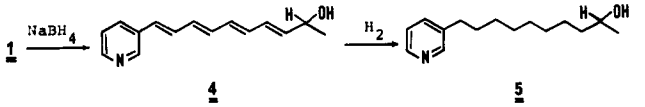
The carnivorous, hermaphroditic sea slug *Navanax inermis* Cooper[1-3] (syn. *Chelidonura inermis;* Opisthobranchia, Mollusca), possessing poor vision and thriving in the murky tidal mud flats along the California coast, follows and finds prey and potential mates by chemoreception of mucopolysaccharide slime trails produced by numerous opisthobranchs and by *Navanax* itself.[4-6] As an apparent mechanism of species preservation, *Navanax*, when greatly molested, secretes a yellow hydrophobic substance directly into its slime trail. This secretion emanates from a small specialized gland located beneath and near the anus of the animal. When this is encountered by a trail-following *Navanax*, an immediate alarm and avoidance response is induced, which terminates trail-following behavior and generates a deviation in direction of greater than 90°. By this mechanism a trail-following animal avoids entrance into habitats of potential danger. This pheromone response[7] is produced by three bright-yellow conjugated methyl ketones, **1–3**, the structures of which are reported here.[8]

The crude yellow pheromone secretions from *Navanax* (animals collected near Mission Bay, San Diego, Calif., in June, 1976) were conveniently obtained by irritating the highly sensory anterior end of the animal and rinsing the subsequent secretion into seawater. The combined secretions of 50 individuals, which generated a brightly colored solution, were extracted into chloroform, and the yellow chloroform extract was reduced, yielding a semisolid residue (500 mg). Thin layer



chromatography (1:1, Et_2O:Bz) of the residue on silica gel indicated that three bright-yellow compounds, R_F 0.2, 0.4, and 0.7, composed ca. 70% of the extract. Column chromatography under similar conditions gave semipurified samples of each of these substances as fluorescent-yellow solids which were subsequently purified by HPLC (μ-porasil) and identified as 10-(3′-pyridyl)-deca-3*E*,5*E*,7*E*,9*E*-tetraene-2-one (**1**, R_F 0.2, 40% extract), 10-phenyldeca-3*E*,5*E*,7*E*,9*E*-tetraene-2-one (**2**, R_F 0.7, 20% extract), and 10-(4′-hydroxyphenyl)-deca-3*E*,5*E*,7*E*,9*E*-tetraene-2-one (**3**, R_F 0.4, 10% extract). We suggest the trivial names navenones A–C for these unsaturated ketones.

Navenone A (**1**), mp 144–145 °C (Bz), analyzed for $C_{15}H_{15}NO$ by high resolution mass spectrometry (obsd 225.1152; calcd 225.1154) and showed intense loss of C_2H_3O (*m/e* 182), assigned to methyl ketone α-cleavage fragmentation. The UV-visible absorption of **1**, λ_{max}^{MeOH} 399 nm, ϵ 14 400, indicated a highly conjugated molecule, and IR absorptions ($CHCl_3$) at 1670 cm^{-1} ($\gamma_{C=O}$) and 1550–1640 cm^{-1} ($\gamma_{C=C}$) confirmed that an extensively conjugated methyl ketone constellation was present. ^{13}C NMR data (20 MHz, $CDCl_3$) illustrated the existence of 1 high field carbon atom at 27.4 ppm, 1 low field carbonyl carbon at 173.6 ppm, and 13 carbon atoms of an olefinic or aromatic nature at 149.0, 148.5, 142.8, 141.2, 138.0, 133.9, 132.7, 132.5, 131.6, 131.4, 130.6, 129.5, and 123.6 ppm. In the proton NMR spectrum (220 MHz, $CDCl_3$), a sharp methyl ketone singlet at δ 2.16 and multiple bands from δ 6.02 to 8.29 accounted for the total of 15 hydrogen atoms. Navenone A was extractable into dilute HCl but failed to acetylate under mild conditions, indicating that the nitrogen atom in **1** is in the form of a tertiary amine. Treatment of navenone A with $NaBH_4$ in methanol cleanly produced the corresponding slightly yellow alcohol (**4**), M^+ *m/e* 227 ($C_{15}H_{17}NO$). The proton NMR and infrared spectra of **4** illustrated that a methyl-substituted allylic alcohol was produced: NMR δ 5.8–8.27 (12 H), 4.40 (m, 1 H), and 1.32 (d, J = 6.5 Hz, 3 H), IR (ν_{O-H}) 3450 cm^{-1}. Hydrogenation of **4** (H_2/Pt) resulted in the uptake of 4 mol of hydrogen, to yield the expected octahydro alcohol (**5**), M^+ *m/e* 235 ($C_{15}H_{25}NO$), the UV spectrum of which was superimposable on that of pyridine ($\lambda_{max}^{CH_3OH}$ 213, 249, 255, 261, 268 nm).[9]



Final confirmation of the gross structure and stereochemistry of **1** was obtained by interpretation of the proton NMR