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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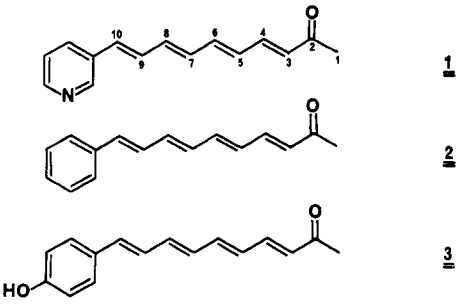
Navenones A–C: Trail-Breaking Alarm Pheromones from the Marine Opisthobranch *Navanax inermis*

Sir:

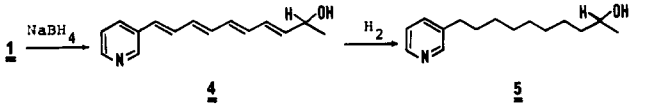
The carnivorous, hermaphroditic sea slug *Navanax inermis* Cooper[1-3] (syn. *Chelidonura inermis;* Opisthobranchia, Mollusca), possessing poor vision and thriving in the murky tidal mud flats along the California coast, follows and finds prey and potential mates by chemoreception of mucopolysaccharide 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

spectrum with stepwise addition of $Eu(fod)_3$ reagent. At various compound–reagent ratios, the complex bands between δ 6.5 and 8.3 became resolved into one-proton bands and were interrelated by spin-decoupling. Interpretation of these data allows the following assignments to be made for the unshifted spectrum: δ 2.16, 3 H, s (C-1); 6.02, 1 H, d, J = 15 Hz (C-3); 7.00, 1 H, dd, J = 15, 9 (C-4); ~6.3, 1 H, dd, J = 14, 9 (C-5); ~6.3, 1 H, dd, J = 14, 11 (C-6); ~6.3, 1 H, dd, J = 14, 11 (C-7); ~6.3, 1 H, dd, J = 14, 10 (C-8); 6.79, 1 H, dd, J = 14, 10 (C-9); 6.50, 1 H, d, J = 14 (C-10); 8.45, 1 H, s, (C-2′); 8.29, 1 H, d, J = 5 (C-6′); 7.09, 1 H, dd, J = 8, 5 (C-5′); 7.59, 1 H, d, J = 8 (C-4′). Since all olefin protons exhibit couplings of 14 or 15 Hz, the olefins of the conjugated tetraene system in **1** must all be *E*. Also, the multiplicities and coupling constants characteristic of an aromatic ABXY system rigorously show the side chain to be β-substituted (C-3′) on the pyridine ring.

Navenone B (**2**), mp 125–140 °C dec (CH_2Cl_2), analyzed for $C_{16}H_{16}O$ by mass spectrometry (obsd 224.1204; calcd 224.1201) and illustrated spectral features which related this metabolite to navenone A: UV $\lambda_{max}^{CH_3OH}$ 389 nm, ϵ 18 000; IR ($CHCl_3$) 1670, 1640–1550 cm^{-1}. The ^{13}C NMR spectrum was also in close accord with **1**, showing bands at 175.5, 143.1, 141.5, 137.5, 135.4, 132.3, 130.6, 129.9, 128.8, 128.6, 128.2, 127.9, 127.6, 126.7, 125.6, and 27.4 ppm. The proton NMR spectrum showed bands analogous to **1** which could be partially assigned by spin-decoupling experiments on the unshifted spectrum: δ 2.25, 3 H, s (C-1); 6.14, 1 H, d, J = 15 Hz (C-3); 7.17, 1 H, dd, J = 15, 11 Hz (C-4); 6.32–6.70, 4 H, m (C-5–C-8); 6.86, 1 H, dd, J = 15, 10 Hz (C-9); 6.66, 1 H, d, J = 15 (C-10); 7.18–7.45, 5 H, m (aromatics).

Navenone C (**3**), $C_{16}H_{16}O_2$, M^+ *m/e* 240, UV $\lambda_{max}^{CH_3OH}$ 412 nm, ϵ 11 400, base shift $\lambda_{max}^{CH_3OH}$ 432 nm, ϵ 12 500, was difficult to fully purify as the free phenol. Acetylation (Ac_2O/py) gave navenone C acetate, mp 135–137 °C (Bz), which was easily purified and gave the following spectral features: IR ($CHCl_3$) 1735 (OAc), 1630, 1620–1560 cm^{-1}, proton NMR (220 MHz, $CDCl_3$) δ 2.16, 3 H, s (OAc); 2.26, 3 H, s (C-1); 6.14, 1 H, d, J = 15 Hz (C-3); 7.27, 1 H, dd, J = 15, 11 Hz (C-4); 6.3–6.7 (m, C-5–C-8); 6.79, 1 H, dd, J = 15, 10 Hz (C-9); 6.61, 1 H, d, J = 15 Hz (C-10); 7.02, 2 H, d, J = 8 Hz (C-3′, C-5′); 7.32, 2 H, d, J = 8 Hz (C-2′, C-6′). Para disubstitution was clearly indicated for the acetate of **3** by the pair of two-proton doublets (a simplified AA′BB′ pattern) at 7.02 and 7.32 ppm, respectively.

For navenones B and C, olefin–proton coupling constants for C-5 through C-8 could not be measured, and hence the stereochemistries of olefins of the central diene cannot be rigorously assigned. However, the complex bands for these protons are virtually superimposable on those of **1** and indicate similar couplings from an all *E* configuration, as in navenone A.

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Biosynthesis of *Cephalotaxus* Alkaloids. 3. Specific Incorporation of Phenylalanine into Cephalotaxine[1]

Sir:

Conifers of the genus *Cephalotaxus* elaborate a group of novel alkaloids, the most abundant of which is cephalotaxine (**1**) (Scheme I).[2] We recently reported experiments indicating that two molecules of the amino acid tyrosine (**2**) are incorporated into **1** in an unusual manner.[3] We now describe the results of additional experiments which further clarify the mode of tyrosine incorporation into cephalotaxine and demonstrate that phenylalanine is also a specific precursor of this alkaloid.

It has been previously shown[3] that administration of side-chain-labeled tyrosine to *C. harringtonia* plants for an 8-week period yields radioactive cephalotaxine. Degradations of the labeled samples of **1** obtained from these experiments disclosed a labeling pattern consistent with the derivation of carbons 10–17[4] of cephalotaxine from one molecule of tyrosine. However, the labeling pattern also indicated that tyrosine was incorporated into the C/D ring system of the alkaloid in an unexpected manner. In order to account for these observations, it was hypothesized that a second molecule of tyrosine served as a precursor of the C/D ring system of the alkaloid via a route involving cleavage of the aromatic ring of the amino acid. This hypothesis predicted that carbon atoms 1–3 and 6–8 of **1** should be derived from the aromatic ring of tyrosine. This prediction has now been tested, and the results are summarized in Table I (expt 1, 2).

[*ring*-^{14}C]-L-Tyrosine[5] was administered to *C. harringtonia*[6] for 8 weeks and radioactive cephalotaxine was obtained. Permanganate oxidation[3] of the labeled alkaloid gave 4,5-methylenedioxyphthalic acid which was degraded[7] to the an-

Scheme I