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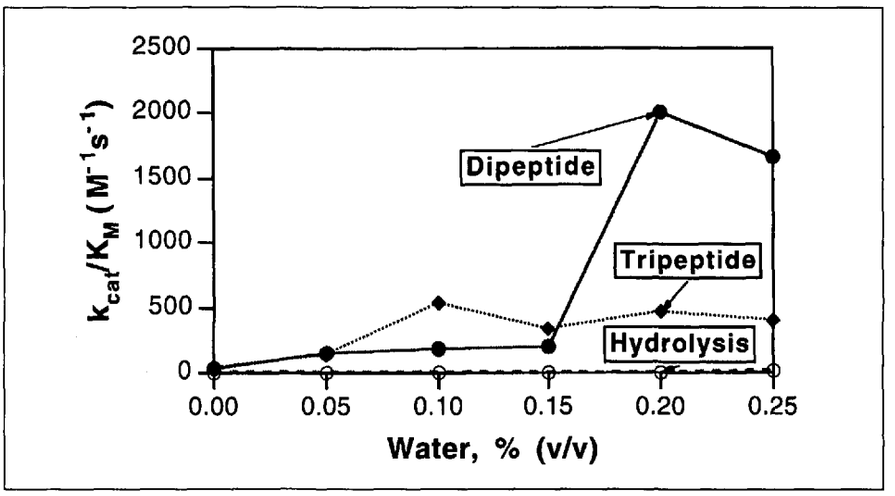

Figure. *Effect of water on α-chymotrypsin-catalyzed peptide synthesis in isooctane/THF and AcOEt*

2. Preparation of 'Plastic enzymes'

The solubility of enzymes in organic solvents led us to develop an approach for the incorporation of enzymes into vinyl-based polymers. These polymers form the basis of a wide range of plastics such as poly(methyl methacrylate) (PMMA) and poly(styrene). Activity of the enzymes embedded the plastic matrix in hexane are up to 30-fold higher than the native chymotrypsin suspended in the solvent. The results for peptide synthesis using the PMMA-entrapped chymotrypsin are particularly striking. The condensation between *N*-Bz-Tyr-OEt and Leu-NH_2 proceeds 500-times faster than with the suspended enzyme in isooctane containing 30% (*v/v*) THF.

[1] V.M. Paradkar, J.S. Dordick, *J. Am. Chem. Soc.* **1994**, *116*, 5009.
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Importance of Natural Chemical Resources in Drug Discovery

Louis J. Nisbet*

'The most important consequence of the structural change in the worldwide pharmaceutical markets is the reduction in R & D returns ... the industry will not realize a satisfactory return on its R & D investment unless research productivity increases substantially'.
(*Lehman Brothers, PharmaPipelines*, 1994)

It is a widely held view in the drug industry today that good margins will only be achieved with innovative drugs that produce significant therapeutic advantage. Also, new markets will be dominated by the first 2–3 drugs approved and, thus, speed in identifying high-quality drug leads provides a competitive edge in pharmaceuticals R & D.

In pursuing new drug leads, the industry has focused on chemical diversity and screening intensity, at times with a focus on vastness of numbers rather than the quality of outputs. Chemical libraries must be biologically relevant and diversity of pharmacophore is much more critical than hundreds of thousands of simple and similar chemicals. Combinatorial chemistry is an exciting and promising new tool but is appropriately gaining a more realistic perspective contrary to its earlier hype.

It has been estimated, that combinatorial chemistry could have provided possibly 10% of the drugs under development today and that methods development might only double this. Therefore, 80% of new drugs will come from other approaches including medicinal chemistry, computational chemistry and natural-products chemistries.

Another important element of success in drug discovery is the use of genomic information to define processes that drive disease and to convert these processes into high throughput screens. The number of screens in large companies are growing from 10 targets per year in the late 1980s to 30 per year in 1995 and over 100 targets per year by the late 1990s. Also, there is a need to extend screening to incorporate *in vivo* factors, including absorption, distribution, biological half-life, metabolism and toxicity.

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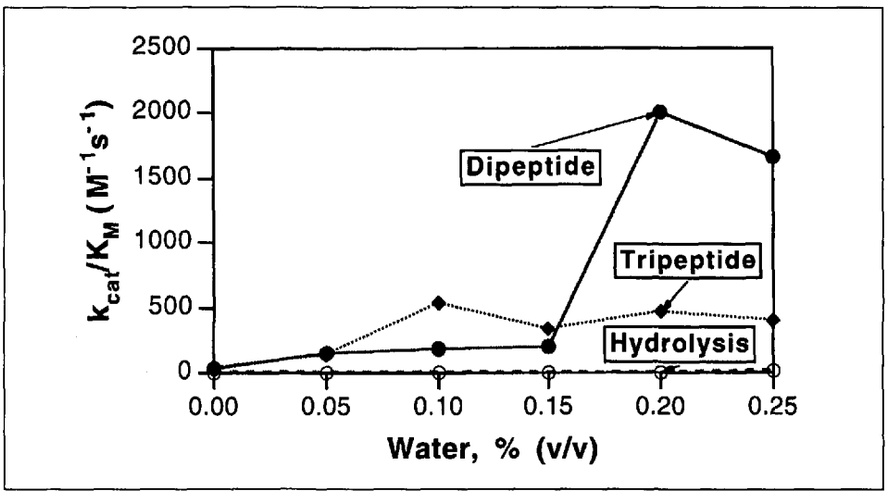

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So, where do natural products fit in this brave new world? Two words are all that are needed to answer this, quality and utility. We should remember that natural-products chemistries represent the greatest sources of scaffold diversity and that these scaffolds are pharmacophore rich, *i.e.*, the molecules are biologically relevant and have been conserved through an evolutionary process on the basis that they confer competitive advantage on the producing organism. The biological relevance of these molecules is manifest in growth and differentiation modulation, in enzyme inhibition or regulation, in receptor agonism or antagonism – biological events common to human disease processes. The ultimate test of quality and utility is market sales, at the very pinnacle of which is the presence of 8 natural-products drugs in the top 25 drugs of 1994.

However, the '*Achilles* heel' of natural-products drug discovery is the time and cost on a per target basis. *Xenova* has revolutionised its approach to natural products to create chemical libraries that enable natural-products drug discovery to be undertaken quickly and cost-effectively and on a level-playing field with synthetic chemicals. The three most important elements of this are technological innovation (including informatics), process engineering and process management techniques.

Xenova has harnessed natural-products chemistries across many therapeutic targets to create a group of preclinical programmes with its corporate partners and to establish a proprietary pipeline of synthetic and semi-synthetic compounds from preclinical research through to clinical evaluation. The chemical output is over 300 bioactive compounds, one-third of which are novel including numerous new chemical scaffolds.

New techniques, including combinatorial biology and biotransformations, will expand access to novel and challenging chemistries based on natural scaffolds and combinatorial-chemistry technologies will be applied increasing to expand the structural diversity around pharmacophores of natural origins. The author believes that natural products will continue to provide valuable drugs and will continue to represent one-third of the top 25 drugs for decades to come.

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Rational Enzyme Design: Computer Modeling and Site-directed Mutagenesis for the Modification of Catalytic Specificity in Organophosphorus Hydrolase

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Organophosphorus neurotoxins are widely used as insecticides in crop production, municipal hygiene, and disease vector control as well as providing the major classes of chemical warfare neurotoxins (V-agents and G-agents). Organophosphorus hydrolase (OPH) is a bacterial metalloenzyme which performs a hydrolytic cleavage of a variety of organophosphorus neurotoxins including common insecticides and chemical nerve agents. The enzyme is capable of hydrolyzing P–O, P–F, P–S, and P–CN bonds of toxic inhibitors of acetyl- and/or butyryl-cholinesterases (AChEs and BChEs) as well as neurotoxic esterases (NTEs). While there are numerous 'OP Anhydrolases' (E.C. 3.1.8.1) in many different organisms, most of them have limited substrate specificities, and there are dramatic differences in the hydrolytic capacity between classes of substrates: phosphotriesters (P–O bonds), fluorophosphonates (P–F bonds), and phosphothioates (P–S bonds).

The enzyme has extremely high efficiency in hydrolysis of many different phosphotriester and phosphothiolester pesticides (P–O bond) such as paraoxon (k_{cat} > 5,000 s^{-1}) and coumaphos (k_{cat} = 800 s^{-1}) or fluorophosphonate (P–F) neurotoxins such as DFP (k_{cat} = 350 s^{-1}) and the chemical warfare agent Sarin (k_{cat} = 350 s^{-1}). In contrast, the enzyme has poor specificities for phosphorothioate insecticides such as acephate (k_{cat} = 5 s^{-1}) and the nerve agent VX (*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate) (k_{cat} = 0.3 s^{-1}) and its analogues as reflected by the specificity constants (k_{cat}/K_m values for VX ~ 0.75 x 10^3 $M^{-1}s^{-1}$ as compared to 5.5 x 10^7 $M^{-1}s^{-1}$ for paraoxon. Different metal-associated forms of the enzyme with Co or Zn at the binuclear metal-active center demonstrated significantly different hydrolytic capabilities for VX and its analogues; the activity of OPH (Co) was consistently greater than that of OPH (Zn) by five- to ten-fold. Significant improvement of the catalytic activity (k_{cat}) and substrate specificity (k_{cat}/K_m) of this stable, quite flexible enzyme (OPH) has been achieved through site-directed mutagenesis of histidinyl residues affecting the metal content of the enzyme and apparently modifying the boundaries of the active site. Individual mutants have been developed which have demonstrated 20-fold improvement in activity against analogues of VX and 30-fold improvement in activity against Soman. Many of these mutants retain excellent catalytic activity and specificity for the native enzyme's preferred phosphotriester substrates such as paraoxon, despite the loss of one of the two molecules of metal present in each native enzyme. X-Ray crystallographic coordi-

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