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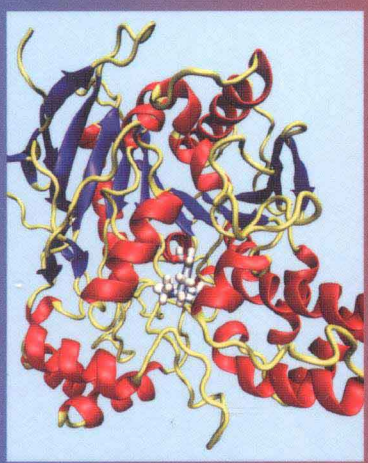
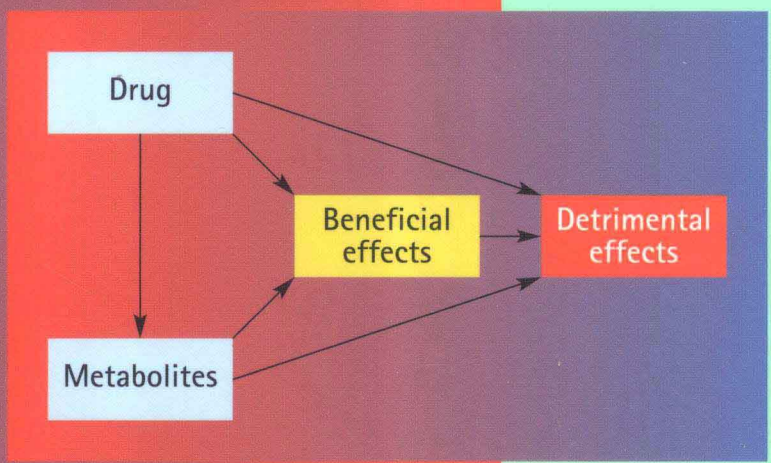
The Biochemistry of Drug Metabolism: Principles, Redox Reactions, Hydrolyses





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Prof. Dr. Bernard Testa
Pharmacy Department
University Hospital Centre
CHUV - BH 04
46 rue du Bugnon
CH-1011 Lausanne
Switzerland

PD Dr. Stefanie D. Krämer
Institute of Pharmaceutical Sciences
Department of Chemistry and Applied Biosciences
ETH Hönggerberg, HCI G494
CH-8093 Zürich
Switzerland

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The Biochemistry of Drug Metabolism: Principles, Redox Reactions, Hydrolyses

We dedicate this Work to all scientists and students in the pharmaceutical and toxicological sciences who believe that better knowledge leads to better health protection.

Foreword

In the late 1960s, a group of Swedish pharmacologists compared the half-lives of a number of anxiolytic drugs in rats both *in vitro* (microsomal) and *in vivo*, pre- and post-induction of enzymes by phenobarbital. *In vitro* half-lives decreased for all compounds following phenobarbital induction. However, *in vivo*, a significant portion of the anxiolytics showed no obvious change, while the others showed a comparable half-life decrease to that seen *in vitro*. The investigators concluded that *in vitro* studies could not be trusted to predict *in vivo* drug metabolism. In 1983, I spent a sabbatical in the Department of Pharmacology and Toxicology of the University of Tübingen with Professor *Herbert Remer*, a pioneer in cytochrome P450 (CYP) metabolism. Every Friday afternoon, all members of the Institute met for 'tea' – which translates to 'beer' in German. During these Friday afternoon sessions, we discussed philosophical scientific issues related to drugs. However, as I recall, about one half of those sessions in 1983 were devoted to a discussion as to whether there was '*...one CYP or two?*'

The two scenarios about highlight how far we have come in the last three to four decades in our understanding and application of the biochemistry of drug metabolism. *Bernard Testa* and *Stefanie D. Krämer* here document that progress and the major advances in our understanding of drug metabolism in an encyclopedic, but very readable format. The figures in this volume represent a *Powerpoint*™ presentation to simulate a well-organized, comprehensive, and readily understandable series of slides as would be given in an oral presentation. The text provides the reader with the 'sound bites' that the lecturers present to explicate the slides. But as an added bonus, the text contains all the thoroughly referenced citations to the discussion for further examination by the reader. I find the presentation technique to be delightful and expect that other readers will have the same experience.

The two anecdotes above spurred my interest in the development of the principles of intrinsic clearance to explain the *in vivo vs. in vitro* differences, to investigate the multitude of CYP enzymes both in animals and man, and then my recent work to try to explain further discontinuities between *in vitro* and *in vivo* metabolism by examining transporter–enzyme interplay in drug metabolism. I envy today's readers of this volume by *Testa* and *Krämer* since the present work provides an exemplary scientific basis for the biochemistry of drug metabolism upon which both experienced and newly initiated drug-metabolism scientists may depend, as they move the field forward. Understanding the biochemistry of drug metabolism is today one of the most critical aspects of new drug development to assure safe and efficacious medicines of the future. *Testa* and *Krämer*'s contribution to this development is significant.

November 2007

Leslie Z. Benet
University of California San Francisco

Preface

Drug metabolism is a fascinating discipline at the crossroads of numerous sciences such as Chemistry (physical, organic, inorganic, biological, analytical), Biopharmacy (including absorption, distribution, accumulation, excretion, and pharmacokinetics), Pharmacology and Toxicology, Genetics (including genomics, proteomics, and population genetics), and Environmental Sciences (see xenobiotics). As graduates in Pharmaceutical Sciences with a broad education in several of the above fields, we have always tried to combine the broader and the deeper view in our studies and research. But above all, we have it as our objective to convey our fascination to our students. The present Work has emerged from our experience as teachers at the M.Sc. and Ph.D. levels, but it also owes much to our desire to address a wider audience of students and research scientists. Modern computer technology now allows for lively and attractive teaching support, and we have attempted to transpose (and markedly expand) an entire course in *Powerpoint*™ format into a printed format. This was achieved by structuring it into seven Parts consisting mainly of colored figures each with an extensive caption, plus a short introductory text and an extensive bibliography. As a further original feature, the various Parts of the Work are first published as separate review papers before appearing in book form. We hope readers will enjoy these features as much as we enjoyed delivering our lectures and preparing this Work.

When Parts 1, 2, and 3 were published, it was realized that they did fill over 300 pages. With Parts 4 – 7 also estimated at >300 pages, it made sense to publish the Work in two volumes. The first Volume is now in your hand, and we are actively engaged in completing Volume 2.

November 2007

Bernard Testa
Stefanie D. Krämer

Contents

Part 1

Principles and Overview

Drug metabolism as a multidisciplinary science was born in the first half of the 19th century, when hippuric acid (the glycine conjugate of benzoic acid) was discovered in horse urine (hence its name). In 1841, it was discovered in the urine of a human after ingestion of 2 g of benzoic acid, an experiment that marked the beginning of human drug-metabolism studies [1][2]. Subsequent progress was impressive, but it remained restricted to a narrow circle of biochemists. It was only in the 1950s that drug metabolism really took off due to a convergence of factors including *a*) the progressive awareness among pharmaceutical scientists of the variety and significance of metabolic reactions, and the involvement of metabolites in unwanted drug effects; *b*) the groundbreaking studies of distinguished pioneers; *c*) the explosive development of analytic instrumentation; and *d*) the acknowledged scientific and didactic impact of a few books [3–6].

Since then, many books have appeared, most of them being edited ones offering expertly written reviews; some such books are listed in the *References* [7–20]. Other books were written by one or two authors, their import and tone being more unitarian and didactic (*e.g.*, [21–28]).

Before embarking on a systematic review of biotransformation reactions and their enzymes (*Parts 2–4*), of their pharmacological and toxicological consequences (*Part 5*) and of the factors affecting drug metabolism (*Parts 6* and *7*), it seems appropriate, if not necessary, to take a bird's-eye view and look at the extent and diversity of this multifaceted discipline. In doing so, a number of concepts will come to light and be explained. This will allow the readers to create a mental scaffold allowing the organization and classification of the many data and mechanisms to be presented in *Parts 2–7*.

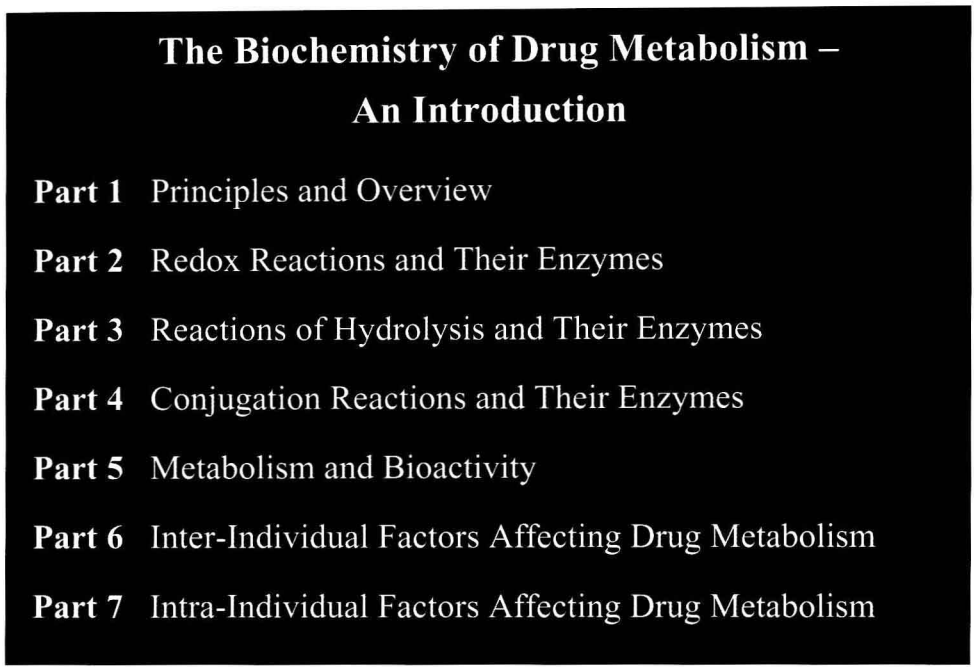

Fig. 1.1. The *Figure* presents the seven Parts of the work. These are being published first as seven separate reviews, and then together as a textbook. The construction of the entire work and the sequence of its Parts obeys a logic we found best adapted to our didactic mission and objectives. *Part 1* brings an overview and explains some basic principles. The core of drug metabolism, *i.e.*, its actors (the enzymes) and their actions (the metabolic reactions), are presented in *Parts 2*, *3*, and *4*. This is done by considering first oxidoreductases and their redox reactions (oxidations and reductions; *Part 2*), then hydrolases and reactions of hydrolysis (*Part 3*), and finally the vast diversity of conjugating enzymes (transferases) and their reactions of conjugation (*Part 4*). The pharmacological and toxicological consequences of drug and xenobiotic metabolism are explained in *Part 5*. The work ends with *Parts 6* and *7* which present in systematic form the many biological factors that influence (modulate) the metabolism of foreign compounds, namely inter-individual factors (which are 'written' in the genome of the organism; *Part 6*) and intra-individual factors (which change over time in a given organism; *Part 7*).

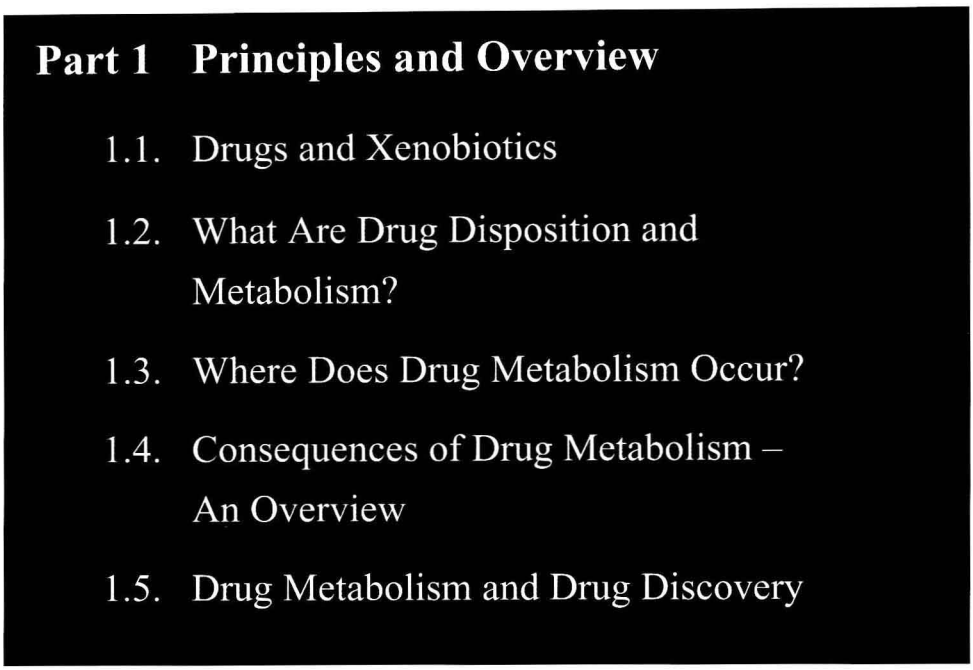

Fig. 1.2. The content of *Part 1* is summarized in this *Figure*. *Chapt. 1.1* defines xenobiotics and shows that drugs are but one class thereof. In other words, toxicological issues resulting from biotransformation (toxification) are a problem that goes well beyond medicinal compounds to encompass all foreign compounds our organism is exposed to. *Chapt. 1.2* examines the components of drug disposition, thus placing metabolism (= biotransformation) in the broader context of a drug's fate in the organism. We then take a brief look at where metabolism does occur in the body (*Chapt. 1.3*). This is followed (*Chapt. 1.4*) by a systematic overview of the consequences of biotransformation in terms of bioactivity (pharmacological and toxicological effects), pharmacokinetic-toxicokinetic behavior [29], and clinical effects. Finally, *Chapt. 1.5* takes a look at drug research, showing how and why drug metabolism has become so important in discovery and development. This Chapter also summarizes the *in vitro* biological methods and *in silico* tools used to assess or predict biotransformation.

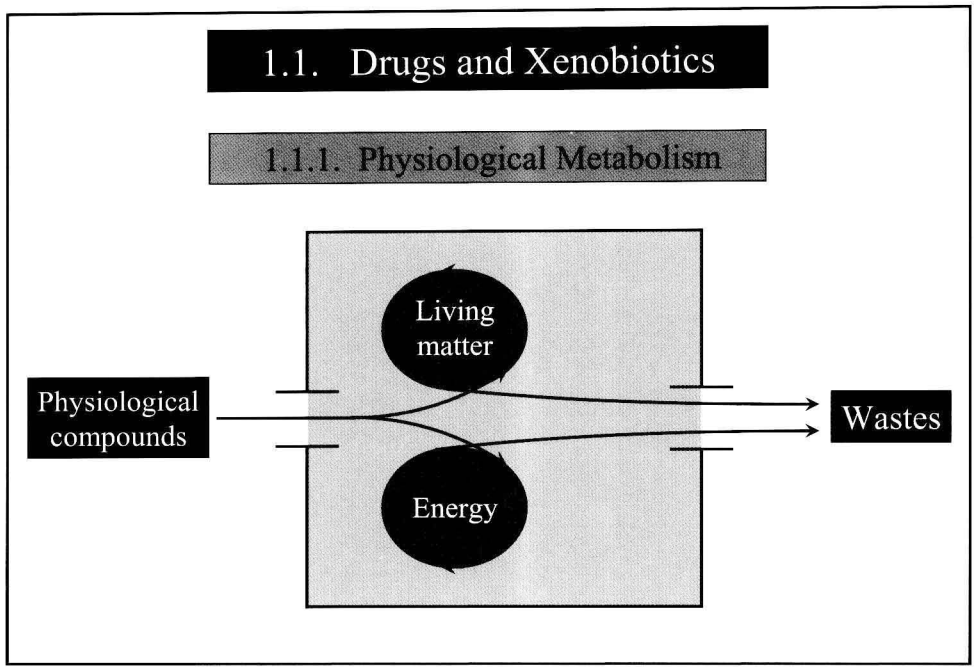

Fig. 1.3. This *Figure* opens *Chapt. 1.1*, whose aim is to define xenobiotics [21–23][25][26][30–32]. The definition is best approached by beginning with the *physiological metabolism*. Indeed, all organisms are open systems, *i.e.*, complex adaptive systems which maintain their low entropy content by extracting energy and 'building material' from a permanent flux of matter that enters them as physiological compounds, and exits as wastes and heat (plants obtain their energy directly from photons). After entering the organism, these physiological compounds (see *Fig. 1.4*) undergo catabolic and/or anabolic reactions. *Catabolic* (*degradation*) *reactions* liberate part of the energy content of these compounds and/or break them down to small building blocks (*e.g.*, amino acids). *Anabolic* (*synthetic*) *reactions* incorporate physiological compounds or some smaller components into living matter. The waste products resulting from physiological metabolism have a higher entropy content than the entering physiological compounds; they are, thus, of low or no value to the organism and are excreted mainly in the urine and feces.

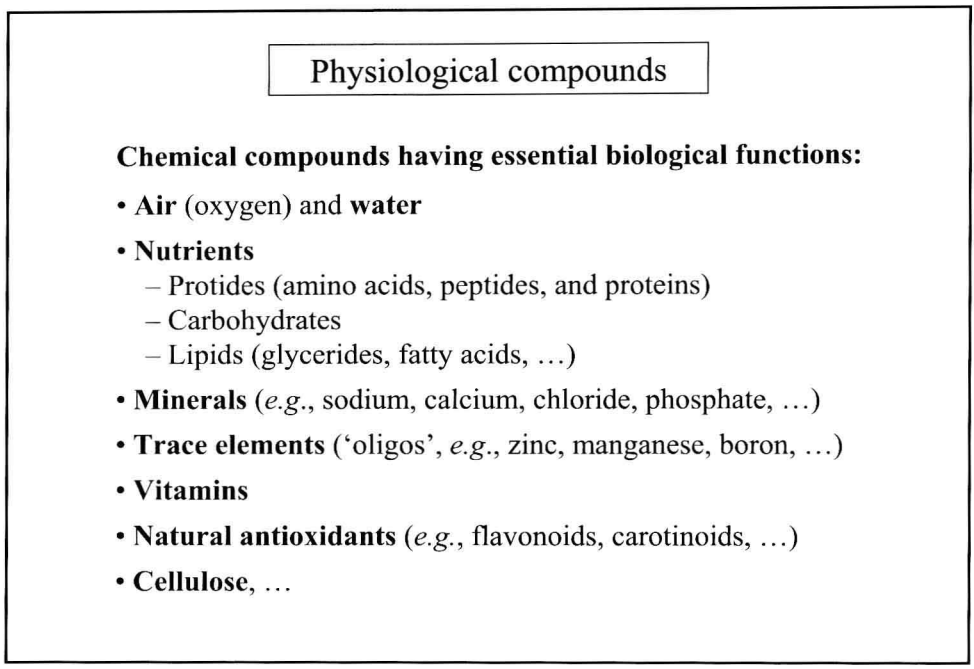

Fig. 1.4. The *Figure* defines *physiological compounds* as chemicals having essential biological functions, namely, which are indispensable to the survival of our body. Most of these compounds are listed here, beginning with the air we breathe and the water we take in. Note that differences exist between species, since oxygen, for example, is toxic to anaerobic microorganisms. Nutrients are conveniently classified into protides, carbohydrates, and lipids, but again some prokaryotes may not need them all. The list continues with the 'micronutrients', namely, inorganic compounds needed in modest (minerals) or trace amounts (oligo-elements). The list also contains compounds whose vital role was uncovered rather recently. These include those natural antioxidants which are not included among vitamins, *e.g.*, flavonoids and lycopene. It may well be that some of them are not indispensable individually, but representatives from different chemical classes are necessary, *i.e.*, acting by different mechanisms and differing in their hydro- and liposolubility. And finally, 'inert' compounds such as cellulose are now recognized to be vitally important in the long term.

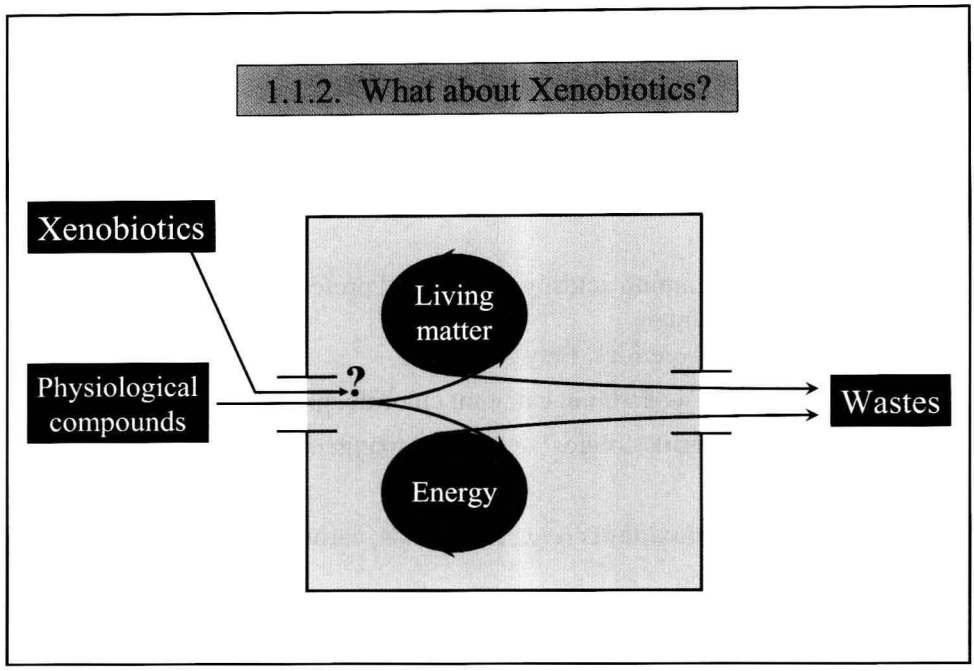

Fig. 1.5. This *Figure* completes *Fig. 1.3* by including xenobiotics and raising the question of their fate in the body. The word '*xenobiotic*' was coined in the early 1970s to indicate compounds that enter the body but have no physiological function and must, therefore, be eliminated [22]. This definition is correct but not complete, since there is also a tendency to view as xenobiotics endogenously produced compounds (endobiotics [32]) administered at relatively high doses, be it for medical or non-medical reasons. A proposed definition and a list of xenobiotics will be given in *Fig. 1.6*. To answer the question mark in the present *Figure* is the objective of the entire Work.

What are xenobiotics?

Foreign compounds that enter the body but are not normally present in it and have no physiological role, or are present at unphysiologically high concentrations after uptake from an external source:

- **Drugs** (therapeutic and diagnostic agents)
- **Food constituents** devoid of physiological function
- **Food additives** (preservatives, flavors, coloring agents, ...)
- **Cosmetics**
- **Doping agents** (EPO, anabolic steroids, growth hormone, ...)
- **Hallucinogens** (ecstasy, LSD, THC, cocaine, ...)
- So-called **'social stimulants'** (nicotine, alcohol, caffeine, ...)
- **Natural toxins** (animal venoms, plants, and bacterial toxins)
- Innumerable **technical and industrial compounds** (agrochemicals such as insecticides, herbicides, and fertilizers, plasticizers, fire-retardants, ...)
- Environmental **natural pollutants** produced by volcanos, fire, *etc.* (*e.g.*, radon, sulfur dioxide, and hydrocarbons)
- Environmental **synthetic pollutants** (heavy metals, insecticides, ...)

Fig. 1.6. Our proposed definition is shown here, although it may not satisfy everybody. As a result, the best way to grasp the meaning of the concept is to list all classes of chemicals viewed as xenobiotics [25]. The first, and for us central, class is obviously that of *drugs*, with the reminder that drugs are chemicals administered for preventive, therapeutic (treatment), or diagnostic purposes, and the further note that some endobiotics administered to patients (*e.g.*, L-DOPA, hormones such as insulin) are also drugs. Two further groups are the *innumerable chemicals present in our foods*, and *articles for personal hygiene*, be they natural compounds or synthetic additives. For example, it is recognized that a cup of coffee contains several hundreds of compounds many of which contribute to its characteristic flavor and odor. There are then the damaging compounds that are usually taken deliberately, *e.g.*, *doping agents* (including endobiotics such as testosterone), *hallucinogens*, and so-called '*social stimulants*' (nicotine and ethanol are certainly toxic, but caffeine in reasonable amounts should not be considered as damaging). The last groups are the more or less *toxic chemicals to which we are exposed involuntarily*, *e.g.*, natural toxins, industrial compounds, and pollutants of various origins. Most classes of xenobiotics listed here contain synthetic compounds, but natural compounds are almost everywhere in the list.