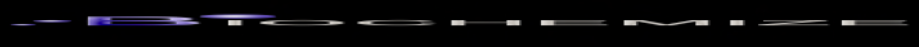

BIOCHEMIZE

BACK TO THE BEGINNING (WITH SOMME DIFFERENCES): THE USE OF ENZYMES AND MICROORGANISMS IN CHEMISTRY

HotDrops/
Agrobiotech Innovación/
Spring waters as a source of new enzymes for the industry/
26.11.15.

BIOTECHNOLOGY IS THE USE OF LIVING ORGANISMS OR THE USE OF ITS PRODUCTS FOR THE OBTENTION OF PRODUCTS OR SERVICES.

THIS IS THE OLDEST TECHNOLOGY DEVELOPED BY HUMANS, TOGETHER WITH METALLURGY.

Vine, beer, dairy products
Penicillins

Products naturally produced by microorganisms

BIOTECHNOLOGY IS THE USE OF LIVING ORGANISMS OR THE USE OF ITS PRODUCTS FOR THE OBTENTION OF PRODUCTS OR SERVICES.

Some highlights:

First quarter of eighteen century: ORGANIC CHEMISTRY IS BORN

Wöhler synthesizes urea

Mid eighteen century: THE GOLDEN MICROBIOLOGY ERA

Pasteur, Koch and other stablish the basis for the chemistry of life, that is the same that organic chemistry

BIOTECHNOLOGY IS THE USE OF LIVING ORGANISMS OR THE USE OF ITS PRODUCTS FOR THE OBTENTION OF PRODUCTS OR SERVICES.

Some highlights:

1906. THE CELL FACTORY CONCEPT.

Johnson postulates that some day all chemicals and sustances will be produced using microorganisms.

Mid twenty century: some products are launched that are biotechnologically produced:

VERY REMARCABLE, BUT MANY FEW

ONLY NATURALLY OCCURRING PRODUCTS

BIOTECHNOLOGY IS THE USE OF LIVING ORGANISMS OR THE USE OF ITS PRODUCTS FOR THE OBTENTION OF PRODUCTS OR SERVICES.

Last highlights:

Last quarter of twenty century:

NEW TECHNOLOGIES AND SCIENTIFIC PLATFORMS START TO WORK:

GENETIC ENGINEERING
COMPUTATIONAL CALCULATION
MOLECULAR BIOLOGY
ADVANCED MICROBIOLOGY
COMBINATORIAL CHEMISTRY
CHEMICAL ANALYSIS INSTRUMENTATION

BIOTECHNOLOGY IS THE USE OF LIVING ORGANISMS OR THE USE OF ITS PRODUCTS FOR THE OBTENTION OF PRODUCTS OR SERVICES.

THE CELL FACTORY PARADIGM

Microorganisms have been used for decades as sources of antibiotics, vitamins and enzymes and for the production of fermented foods and chemicals.

In the 21st century microorganisms **will** play a vital role in addressing some of the problems faced by mankind.

BIOTECHNOLOGY IS THE USE OF LIVING ORGANISMS OR THE USE OF ITS PRODUCTS FOR THE OBTENTION OF PRODUCTS OR SERVICES.

THE CELL FACTORY PARADIGM

In the 21st century microorganisms **will** play a vital role in addressing some of the problems faced by mankind.

REALLY?

Some figures:

-at the beginning of 21st century, enzyme market is around 5% of the world chemical market. GROWING, BUT NOT SO MUCH WITH NEW ENZYMES AVAILABLE FOR NEW CHEMICAL REACTIONS

BIOTECHNOLOGY IS THE USE OF LIVING ORGANISMS OR THE USE OF ITS PRODUCTS FOR THE OBTENTION OF PRODUCTS OR SERVICES.

THE CELL FACTORY PARADIGM

In the 21st century microorganisms **will** play a vital role in addressing some of the problems faced by mankind.

REALLY?

Some figures:

-at the beginning of 21st century, only 2% of chemicals are produced using biotechnology (biosmilars and protein APIs excluded)

-the number of new naturally occurring or engineered microorganisms and/or enzymes (that work at lab level and are published) increases exponentially

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THE CELL FACTORY PARADIGM

In the 21st century microorganisms **will** play a vital role in addressing some of the problems faced by mankind.

REALLY?

Some figures:

- the number of industrially applied patents for chemicals to be biotechnology produced is only a few percent of the new enzymes/ microorganisms launched

BIOTECHNOLOGY IS THE USE OF LIVING ORGANISMS OR THE USE OF ITS PRODUCTS FOR THE OBTENTION OF PRODUCTS OR SERVICES.

THE CELL FACTORY PARADIGM

In the 21st century microorganisms **will** play a vital role in addressing some of the problems faced by mankind.

REASONS WHY:

-Nevertheless high chemical reaction yields are claimed, main of the basic science published results work at mM enzyme and substrate concentrations.

-This results are not enough for the industrial scale implementation at competitive operational costs.

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THE CELL FACTORY PARADIGM

In the 21st century microorganisms **will** play a vital role in addressing some of the problems faced by mankind.

REASONS WHY:

- Almost all enzymes and microorganisms need to be artificially modified before suitable for industrial purposes.

- Conditions for chemical reactions are usually different from those working for naturally occurring enzymes.

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THE CELL FACTORY PARADIGM

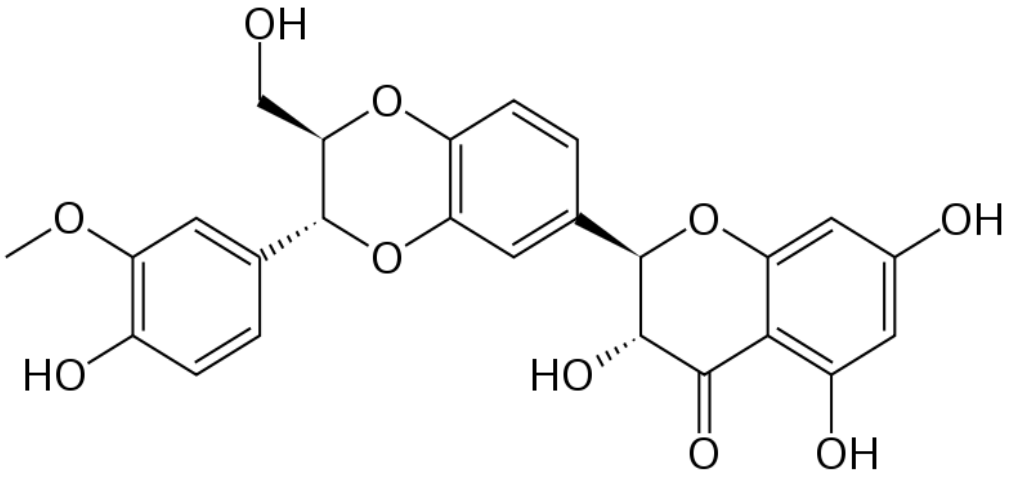
In the 21st century microorganisms **will** play a vital role in addressing some of the problems faced by mankind.

TECHNOLOGIES TO BE IMPLEMENTED:

-Cell and/or enzyme immobilization.
-Modification of the enzyme active catalytic center.
-Overexpression of enzyme genes in microorganisms.
-Use of alternative solvents, i. e. Ionic liquids.
-Overgeneration of new enzymes, usimg random techniques, i. e. metagenomic libraries, directed mutagenesis, highthroughput screening methods, computational models.

Biochemize developments, some examples

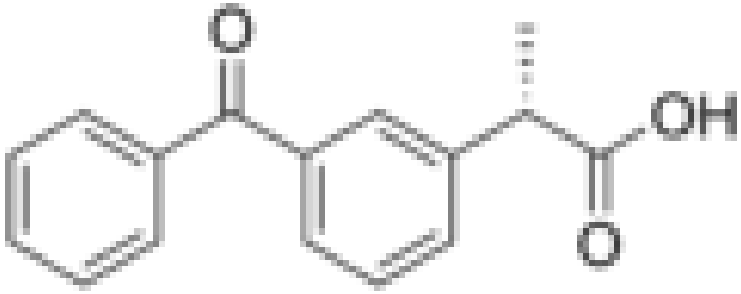
ISOLATION OF SIBILININE ENANTIOMER TROUGH ESTERIFICATION



Silibinin (), also known as **silybin**, is the major active constituent of **silymarin**, the mixture of extracted from (*Silybum marianum*) consisting of and , and , and . Both *in vitro* and animal research suggest that silibinin has (antihepatotoxic) properties that protect liver cells against toxins. Silibinin has also demonstrated anti-cancer effects against human prostate adenocarcinoma cells, estrogen-dependent and -independent human breast carcinoma cells, human ectocervical carcinoma cells, human colon cancer cells, and both small and nonsmall human lung carcinoma cells.

Biochemize developments, some examples

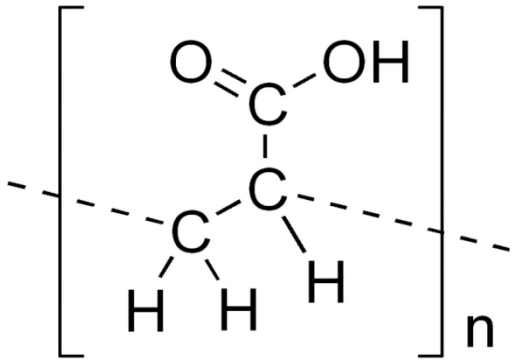
ISOLATION OF DEXKETOPROFEN ENANTIOMER THROUGH ESTERIFICATION



Dexketoprofen belongs to a class of medicines called non-steroidal anti-inflammatory drugs (NSAIDs). It works by blocking the action of a substance in the body called . is involved in the production of chemicals in the body called . Prostaglandins are produced in response to injury or certain diseases and would otherwise go on to cause swelling, inflammation and pain. By blocking cyclo-oxygenase, dexketoprofen prevents the production of and therefore reduces inflammation and pain. Along with Peripheral analgesic action it possesses central analgesic action.

Biochemize developments, some examples

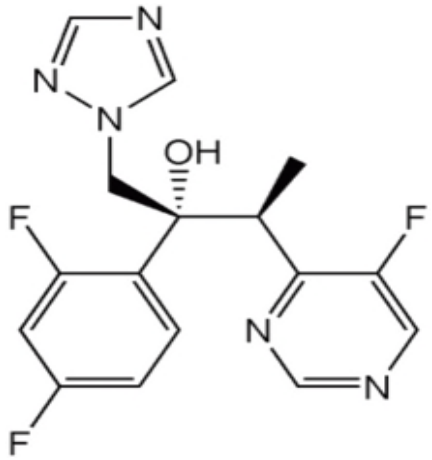
ESTERIFICATION OF POLYACRILIC ACID WITH ESTEARYL ALCOHOL



Poly(acrylic acid) or **PAA** or Carbomer is a type of . The monomer of poly(acrylic acid) is . In a water solution at neutral , many of the side chains of PAA will lose their protons and acquire a negative charge. This makes PAA a .
Dry PAA is a white solid. It is capable of adsorbing many times its weight in water, and hence is used in disposable diapers. It also is used as a thickening agent. It inactivates

Biochemize developments, some examples

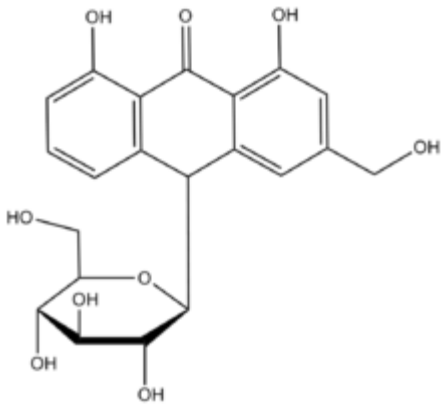
ALDOLIC CONDENSATION FOR THE PRODUCTION OF VORICONAZOLE

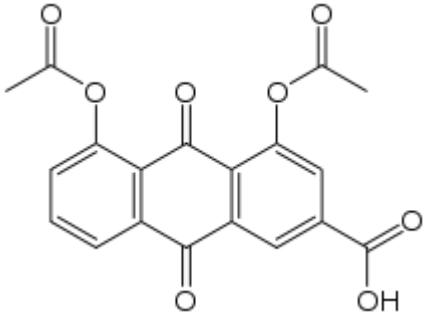


Voriconazole has become the new standard of care in the treatment of invasive , which may occur in patients, including allogeneic , other , and solid . This is based on the results of a large, randomized study in which voriconazole proved superior to with 53% complete or partial response, compared with 32% for amphotericin B. Importantly, voriconazole also offered a 22% greater survival benefit over amphotericin B, with 71% of voriconazole patients still alive at week 12. Only 13% of patients who received initial therapy with voriconazole died from invasive aspergillosis, compared with 29% of patients who initially received amphotericin B. Voriconazole was also better tolerated than amphotericin B, with significantly fewer serious adverse effects and a longer duration of therapy. Note that the design of these studies has been called into question, and some still consider (liposomal) amphotericin B as the drug of choice. For multiple site or CNS aspergillosis a combination therapy of voriconazole and caspofungin should be considered.

Biochemize developments, some examples

OXYDATION OF ALOIN FOR THE PRODUCTION OF DIACEREIN



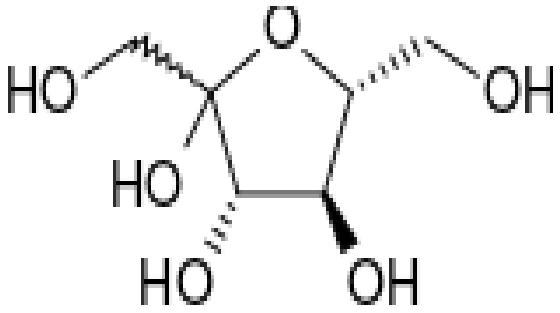


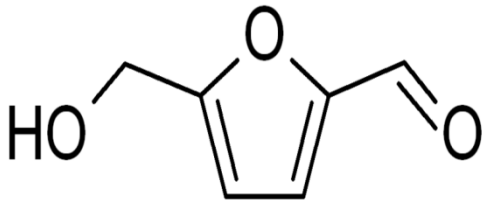
Diacerein (), also known as **diacetylrhein**, is a drug used in the treatment of . It works by inhibiting .

Aloin, also known as Barbaloin, is a bitter, yellow-brown colored compound noted in the exudate of at least 68 *Aloe* species at levels from 0.1 to 6.6% of leaf dry weight (making between 3% and 35% of the toal exudate) (Groom & Reynolds, 1987), and in another 17 species at indeterminate levels [Reynolds, 1995b]. It is used as a stimulant-, treating by inducing movements. The compound is present in what is commonly referred to as the aloe that exudes from cells adjacent to the vascular bundles, found under the rind of the leaf and in between it and the gel. When dried, it has been used as a bittering agent in commerce (alcoholic beverages) [21 CFR 172.510. Scientific names given include *Aloe perryi*, *A. barbadensis* (= *A. vera*), *A. ferox*, and hybrids of *A. ferox* with *A. africana* and *A. spicata*.]. Aloe is listed in federal regulations as a natural substance that may be "safely used in food" when used "in the minimum quantity required to produce their intended physical or technical effect and in accordance with all the principles of good manufacturing practice." This food application is generally limited to use in quite small quantities as a flavoring in alcoholic beverages and may usually be identified only as a "natural flavor."

Biochemize developments, some examples

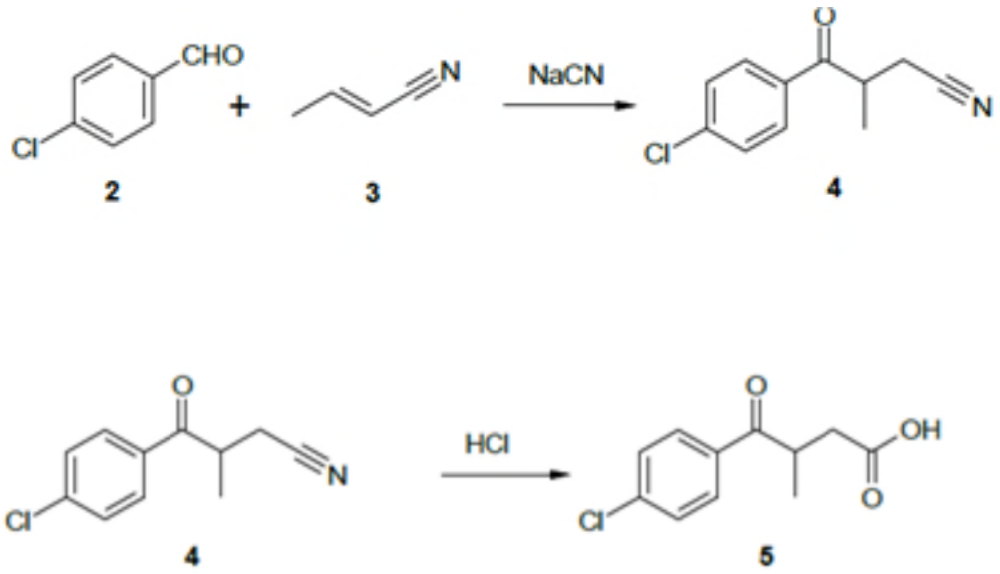
PRODUCTION OF HYDROXYMETHYLFURFURAL FROM FRUCTOSE THROUGH DEHYDRATION





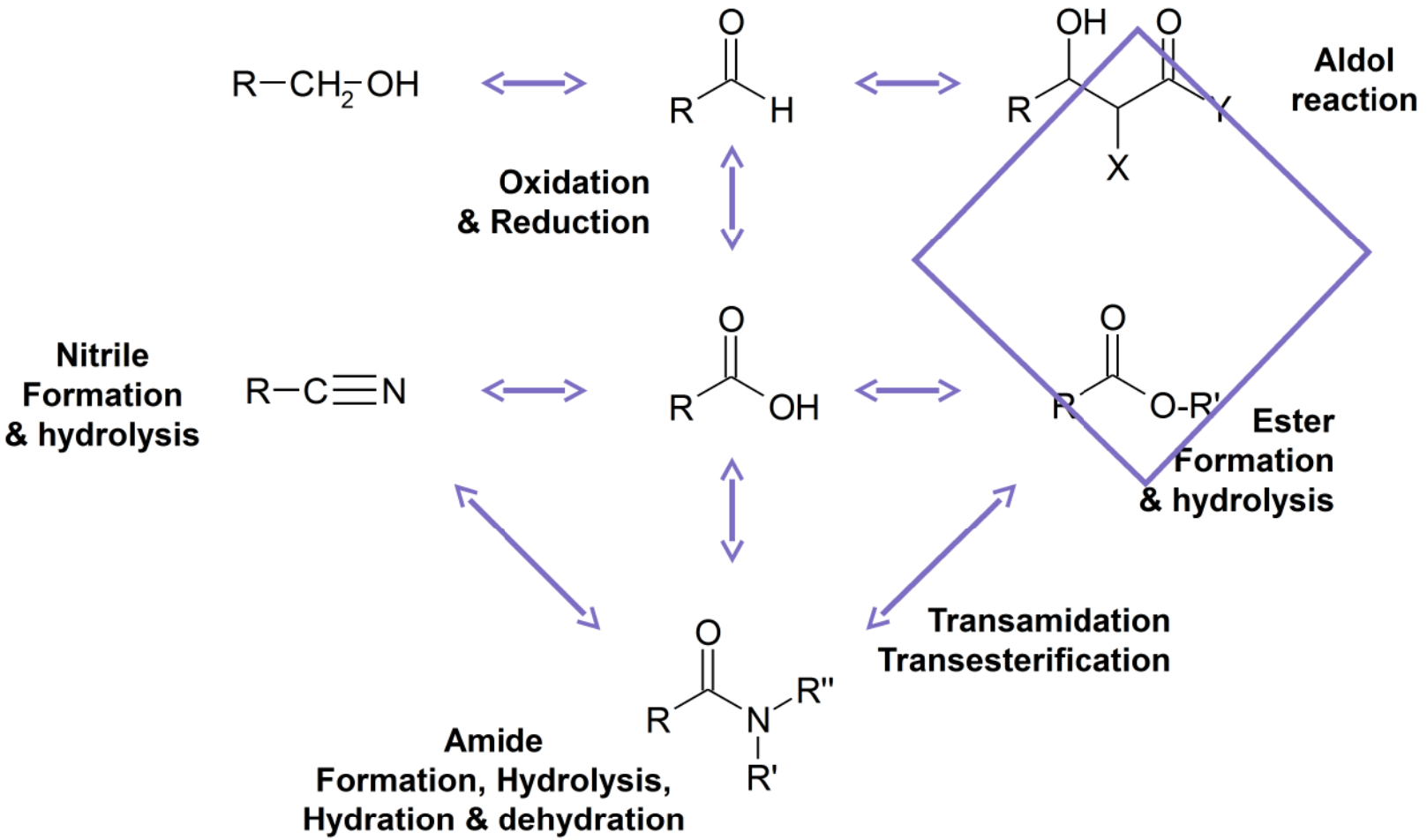
Biochemize developments, some examples

PRODUCTION OF A SYNTHESIS INTERMEDIATE THROUGH MICHAEL ADDITION



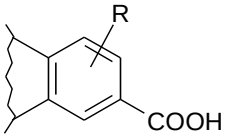
GENERAL OVERVIEW

Today we are able to perform many biocatalyzed transformations. Our most recent experience is based on the following reactions:

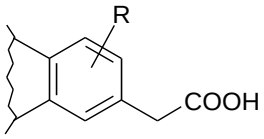


Biochemize developments, some examples

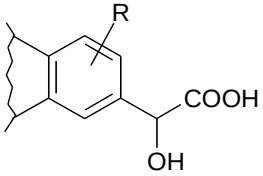
EXAMPLES OF BEST SELLER APIs THAT COULD BE IMPROVED THROUGH ENZYMATIC SYNTHESIS



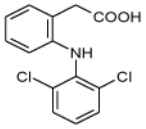
Compuesto 1



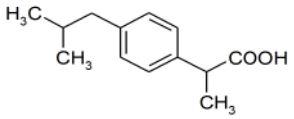
ác. arilacético



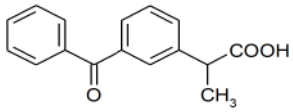
ác. arilpropiónico



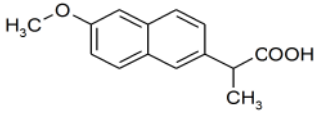
diclofenaco



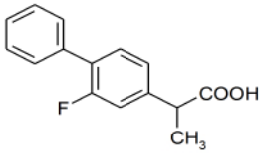
ibuprofen



ketoprofen



naproxen



flurbiprofen

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