Frontiers in Natural Product Chemistry

Editor:
Atta-ur-Rahman, *FRS*

Bentham Books

Frontiers in Natural Product Chemistry

(Volume 6)

Edited by

Atta-ur-Rahman, *FRS*
Kings College
University of Cambridge
Cambridge
UK

Frontiers in Natural Product Chemistry

Volume # 6.

Editor: Atta-ur-Rahman, *FRS*

ISBN (Online): 978-981-14-4846-1

ISBN (Print): 978-981-14-4844-7

ISBN (Paperback): 978-981-14-4845-4

need for a court order if at any point you breach any terms of this License Agreement. In no event will any delay or failure by Bentham Science Publishers in enforcing your compliance with this License Agreement constitute a waiver of any of its rights.

3. You acknowledge that you have read this License Agreement, and agree to be bound by its terms and conditions. To the extent that any other terms and conditions presented on any website of Bentham Science Publishers conflict with, or are inconsistent with, the terms and conditions set out in this License Agreement, you acknowledge that the terms and conditions set out in this License Agreement shall prevail.

Bentham Science Publishers Pte. Ltd.
80 Robinson Road #02-00
Singapore 068898
Singapore
Email: subscriptions@benthamscience.net

CONTENTS

PREFACE

Frontiers in Natural Product Chemistry presents recent advances in the chemistry and biochemistry of naturally occurring compounds. It covers a range of topics, including important researches on natural substances. The book is a valuable resource for pharmaceutical scientists and postgraduate students seeking updated and critically important information on bioactive natural products.

The five chapters in this volume are written by eminent authorities in the field. Chapter 1 presents an overview of different ways of production to obtain bioactive peptides from different underutilized plant sources, including from food, brewing and bioethanol industries. Chapter 2 deals with the research on the design of new fluoroquinolones with improved features by molecular hybridization technique. Chapter 3 deals with a pathway for waxy rice starch hydrolysis by a compressed hot water process. Chapter 4 deals with the most important marketed plant alkaloidal drugs and their metabolites. Chapter 5 provides an insight into the molecular basis of preventive and therapeutic effects of natural bioactive substances against cancer diseases.

I hope that the readers will find these reviews valuable and thought-provoking so that they may trigger further research in the quest for new and novel therapies against various diseases. I am grateful for the timely efforts made by the editorial personnel, especially Mr. Mahmood Alam (Director Publications), and Mrs. Salma Sarfaraz (Senior Manager Publications) at Bentham Science Publishers.

Atta-ur-Rahman, *FRS*
Kings College
University of Cambridge
Cambridge
UK

List of Contributors

Ahmet Demirbas	Karadeniz Technical University, Department of Chemistry, Trabzon, Turkey
Antonio Lama-Muñoz	Department of Chemical, Environmental and Materials Engineering, University of Jaén, Campus Las Lagunillas, Jaén, Spain
Bikash Debnath	Natural cum Advance Synthetic Lab, Department of Pharmacy, Tripura University (A Central University), Suryamaninagar, Tripura, India
Dev Bukhsh Singh	Department of Biotechnology, Institute of Biosciences and Biotechnology, Chhatrapati Shahu Ji Maharaj University, Kanpur-208024, India
Eulogio Castro	Department of Chemical, Environmental and Materials Engineering, University of Jaén, Campus Las Lagunillas, Jaén, Spain Center for Advanced Studies in Earth Sciences, Energy and Environment, University of Jaén, Jaén, Spain
Francisco Espínola	Department of Chemical, Environmental and Materials Engineering, University of Jaén, Campus Las Lagunillas, Jaén, Spain Center for Advanced Studies in Earth Sciences, Energy and Environment, University of Jaén, Jaén, Spain
Kuntal Manna	Natural cum Advance Synthetic Lab, Department of Pharmacy, Tripura University (A Central University), Suryamaninagar, Tripura, India
Manish Kumar Gupta	Department of Biotechnology, Faculty of Science, Veer Bahadur Singh Purvanchal University, Jaunpur-222003, Uttar Pradesh, India
Manuel Moya	Department of Chemical, Environmental and Materials Engineering, University of Jaén, Campus Las Lagunillas, Jaén, Spain Center for Advanced Studies in Earth Sciences, Energy and Environment, University of Jaén, Jaén, Spain
Minerva Cristina García Vargas	Department of Chemical, Environmental and Materials Engineering, University of Jaén, Campus Las Lagunillas, Jaén, Spain Department of Industrial Engineering of Tecnológico Nacional de México/Instituto Tecnológico de Zitácuaro, Av. Tecnológico, 186 CP 61534, Zitácuaro, Michoacán, Mexico
Neslihan Demirbas	Karadeniz Technical University, Department of Chemistry, Trabzon, Turkey
N. Shimizu	Research Faculty of Agriculture, Hokkaido University, Hokkaido, 060-8589, Japan Field Science Center for Northern Biosphere, Hokkaido University, Hokkaido, 060-8589, Japan
Rajesh Kumar Pathak	School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana-141004, Punjab, India
T. Ushiyama	Graduate School of Agriculture, Hokkaido University, Hokkaido, 060-8589, Japan
Waikhom Somraj Singh	Natural cum Advance Synthetic Lab, Department of Pharmacy, Tripura University (A Central University), Suryamaninagar, Tripura, India

CHAPTER 1

Plant Protein Hydrolyzates from Underutilized Agricultural and Agroindustrial Sources: Production, Characterization and Bioactive Properties

María del Mar Contreras[1,*], Minerva Cristina García Vargas[1,2], Antonio Lama-Muñoz[1], Francisco Espínola[1,3], Manuel Moya[1,3] and **Eulogio Castro[1,3]**

[1] *Department of Chemical, Environmental and Materials Engineering, University of Jaén, Campus Las Lagunillas, 23071Jaén, Spain*

[2] *Department of Industrial Engineering of Tecnológico Nacional de México/Instituto Tecnológico de Zitácuaro, Av. Tecnológico, 186 CP 61534, Zitácuaro, Michoacán, Mexico*

[3] *Center for Advanced Studies in Earth Sciences, Energy and Environment, University of Jaén, Spain*

Abstract: Today, there is a growing interest in the valorization of agricultural and agroindustrial waste/byproducts, including through obtaining bioactive compounds. Besides the use of plant proteins in animal nutrition, obtaining protein hydrolyzates could give an added value, improving digestibility and exerting functional properties by the generation of bioactive peptides. Bioactive peptides encrypted in plant proteins are latent until released and activated by proteolysis. Generally, to obtain bioactive peptides, enzymatic hydrolysis by peptidases is the most common way, with or without previous solubilization and purification steps of the intact protein. This hydrolysis step can be combined with physical and chemical treatments not only to improve the recovery but also to enhance the bioactivity. Therefore, our chapter presents an overview of different ways of production to obtain bioactive peptides from different underutilized plant sources, including from food, brewing and bioethanol industries. In order to characterize bioactive peptides, the application of conventional methods and more sophisticated methods based on mass spectrometry is also described. Moreover, recent literature on the bioactive properties of those plant peptides and current challenges associated with safety issues are discussed.

Keywords: ACE-inhibitor, Antioxidant, Antihypertensive, Antidiabetic, Byproduct, Bioactive peptide, Carbohydrolase, Hydrolysis, Mass spectrometry,

* **Corresponding author María del Mar Contreras:** Department of Chemical, Environmental and Materials Engineering, University of Jaén, Campus Las Lagunillas, 23071 Jaén, Spain;
E-mails: mcgamez@ujaen.es;mmcontreras@ugr.es;mar.contreras.gamez@gmail.com

Atta-ur-Rahman (Ed.)

Microwave assisted extraction, Peptidomics, Peptidase, Protein, Sustainability, Valorization.

INTRODUCTION

Considering the population factor and the state of natural resources, the need to look for more efficient agroindustry processes is recognized due to demographic growth and the current unsustainable practices. The global population is growing, while our standard of living is increasing; thereby, we have to face environmental challenges. In this sense, it is expected that the world's population increases by 2 billion people in the next 30 years and could reach around 11 billion in the next century. Based on this prognosis, it is not difficult to understand why the United Nation's second priority objective for the present century is to "End hunger, achieve food security and improve nutrition and promote sustainable agriculture" [1]. Moreover, the current agricultural and food practices also threaten the health of people and the planet: i) 70% of worldwide water use is required by agriculture; ii) it generates huge levels of pollution and waste; iii) risks associated with poor diets are one of the leading causes of death; iv) a double burden of malnutrition exists since millions of people are either eating not enough or eating the wrong types of food. In 2017, this led to one in eight adults (*i.e*. more than 672 million people) in the world to be obese [2] and forecasts suggest high levels of obesity on the future population [3]. In particular, increased demand for animal-based protein is expected to have a negative environmental impact, generating greenhouse gas emissions, requiring more water and more land [4]. Thereby, plant proteins could be an alternative but sustainable practices are required.

Therefore, against this background, the goal is how to meet the growing global demand for food, including protein and healthy foods, to improve income and employment in rural areas and, at the same time, reduce the environmental impact. This puts pressure on the world's resources to provide not only more but also different types of food, including more sustainable production of existing sources of protein as well as alternative sources for human consumption [4]. This requires us to move from an oil based economy towards a more sustainable circular bioeconomy model, producing more food and bio-based products from renewable resources, including agricultural and agroindustrial byproducts [5]. In this line, the biorefinery concept has emerged as a sustainable processing of biomass into a portfolio of marketable food and feed ingredients, bio-based products (chemicals, materials, proteins, bioactive compounds, *etc*.) and energy (fuels, power, heat) [6 - 8].

When thinking about these resources, plant compounds are usually put forward as their most probable source [9]. This includes macro (cellulose, hemicelluloses,

pectins, starch, lignin, proteins, minerals, *etc*.) and microcomponents (*e.g*. phytochemicals). In particular, plant byproducts are underutilized sources of proteins and, most of the time are addressed to animal nutrition, but the ruminal degradability of proteins is not high. Nonetheless, proteins can be beneficial not only in terms of nutrition but also from a functional point of view through the generation of bioactive peptides. This means that the breakdown of peptide bonds by enzymatic hydrolysis increases the solubility, digestibility, and functional properties of the precursor proteins and byproduct [6]. Bioactive peptides are known for their high tissue affinity, specificity and efficiency in promoting health [10]. Therefore, apart from the use of plant proteins in animal nutrition, obtaining protein hydrolyzates could give an added value in a biorefinery context, with improved digestibility and exerting functional properties through the generation of bioactive peptides. This could also lead to the formulation of functional ingredients that are in line with the increased consumer awareness towards functional foods, nutraceuticals and personalized diets; the driving force of the functional food and nutraceutical market [10]. Moreover, there is a growing interest in the food industry and among consumers in reducing the use of synthetic additives in food preservation and opting instead for natural ones [11]. All this together connects with the concept of bioeconomy since it can promote a new way to diversify plant byproducts.

Generally, hydrolysis by peptidases, with or without a previous protein extraction step, is the most common way to obtain bioactive peptides with a wide range of biological properties, *e.g*. antidiabetic, antihypertensive, antimicrobial, antioxidant, and anticancer properties [12 - 15], but also autolysis and application of microbial suspensions (whole cells) have been applied [13, 16]. Enzymatic hydrolysis can be combined with physical treatments and alkaline extraction not only to improve the recovery but also to enhance the bioactivity [6, 17]. In this context, this book chapter presents an overview of the different ways of production to obtain bioactive protein hydrolyzates from different underutilized plant sources. These sources include byproducts from the cereals industry (wheat germ protein, broken rice by-product), oil industry (olive, and rapeseed/canola byproducts), fruit and vegetable industries (*e.g*. fruits seeds, potato byproducts, cauliflower leaves), and brewing industry (brewer's spent grain). Some techniques applied to characterize the hydrolyzates and the peptides are also covered. Moreover, the biological properties of the hydrolyzates have been revised, and the sequence of some bioactive peptides is shown. Finally, some safety issues are also discussed.

CHAPTER 2

New Developments in the Quinolone Class of Antibacterial Drugs

Neslihan Demirbas[*] and **Ahmet Demirbas**

Karadeniz Technical University, Department of Chemistry, 61080 Trabzon, Turkey

Abstract: The increasing drug resistance and the insufficiency of the newly developing antibiotics constitute a serious and growing health threat in the world. Especially Gram (-) bacteria acquire genetic material encoding antibiotic resistance by multiple mechanisms. Development of novel antibacterial agents with little tendency to bacterial resistance is, therefore, an important and challenging topic in the medicinal chemistry, and synthetic organic chemistry is an indispensable part of the design and synthesis of efficient antibacterial drug candidates. Among the broad-spectrum antibiotics, fluoroquinolones constitute the most attractive drugs in the anti-infective chemotherapy field. These antibiotics target the bacterial type II topoisomerase enzymes (DNA gyrase and topoisomerase IV) which are essential enzymes involved in bacterial cell growth and division. Since their advent, they were widely applied to treat infections. Unfortunately, most of them suffered from the resistance problem by mutations in the bacterial targets due to their wide use. Recently, the synthetic organic and medicinal chemists focused their research on the design of new fluoroquinolones with improved features by molecular hybridization technique. One of the most promising approaches aiming to combat resistant pathogens is the design and synthesis of new hybrid molecules in which different pharmacophore groups with different modes of action are joined together using a flexible linker. This strategy supplies a way to improve traditional drug combination therapies simplifying optimization of the pharmacokinetics/pharmacodynamic (PK/PD) profile, efficacy at both targets is usually synergistic.

Keywords: Aminoglycoside, Drug resistance, Flavonoid, *β*-Lactam, Macrocyclic, Molecular hybridization, Oxazolidinone, Pyrazole, Pyrazine, Pyrimidine, Quinolone, Triazole.

INTRODUCTION

In recent years, the growing incidence of virulent bacterial resistance towards the present antibacterial agents has become the most serious clinical and socio-

[*] **Corresponding author Neslihan Demirbas:** Karadeniz Technical University, Department of Chemistry, 61080 Trabzon, Turkey; Tel/Fax: +90 462 3774252; E-mail: neslihan@ktu.edu.tr

economic problem worldwide [1 - 3]. Although, The World Health Organization, has described the antibiotics as "miracle weapons giving an opportunity to combat with infectious diseases", a large majority of clinically effective drugs actively used to treat bacterial infections have become less effective due to the increasing antimicrobial resistance [4 - 9]. Moreover, the treatment of infectious diseases is more difficult in immunodeficient patients, such as those infected with tuberculosis, HIV *etc* [9]. Multidrug resistant Gram (+) pathogens, such as methicillin-resistant Staphylococcus aureus (MRSA) and *Staphylococcus epidermis* (MRSE), vancomycin-resistant *Enterococci* (VRE), cephalosporin resistant *Streptococcus pneumoniae* are leading significant morbidity and mortality of the infected patients [10 - 12]. Another pathogen, penicillin resistant *S. pneumoniae* has been reported to cause approximately 3 million deaths each year worldwide because of pneumonia, meningitis and sepsis, which are responsible for serious upper airway infections, such as sinusitis and otitis media [13 - 16].

Microorganisms develop resistance to drugs *via* various mechanisms, such as overexpression of drug efflux transporters, like multidrug and toxic compound extrusion (MATE) transporters [17], changes in the target sites of antibiotics [18], optimization of the enzyme (such as β-lactamase) activity resulting in inactivation of antibiotics [19], spontaneous chromosomal mutations [20], and horizontal transfer of genetic elements [21]. Inhibition of the activity of drug efflux transporters appears to be an encouraging strategy for renovating the activity of a drug that is the substrate of these efflux pumps [22].

Keeping all this in mind, it is clearly seen that the development of wholly novel drug discovery methodologies and the optimization of available antibacterial agents have become a crucial and challenging task for the effective treatment of bacterial infections. However, the development of completely new antibacterials suitable for therapeutic applications has not been as successful as expected, and despite a tenfold increase in spending for Research-Development studies in the pharmaceutical industry, the number of leader molecules has remained nearly stable.

To improve the therapeutic profile of the existing drugs by several manipulations in their structures or to design their novel analogs has become one of the most promising strategies for the development of new antibacterial drugs. This strategy has been widely admitted since it does not entail to discover novel scaffolds or validation of new biological targets, which has been accepted as an extremely difficult and time-consuming procedure [27].

In recent years, in order to overcome the "drug resistance nightmare", the concept

of "molecular hybridization" based on the combination of structural features of two or more drug fragments having different modes of action has emerged as an attractive strategy. These new hybrid compounds with improved affinity and efficacy have been proved to be capable of inhibiting two or more conventional targets simultaneously, and this multiple target strategy has led to discover a number of bioactive hybrid molecules [28 - 31].

In recent drug development programs, 4-quinolone-3-carboxylic acid scaffold has been used as one of the most frequently encountered privilege frameworks having potent and broad spectrum activity [32]. Since the introduction of nalidixic acid (the first generation, the prototype 4-quinolone antibiotics) for the treatment of urinary tract infections in humans in 1962, the class of quinolone antibacterials has played an important role saving countless millions of lives in the chemotherapy of bacterial infections [33 - 35]. Their preferable properties including well tolerability with excellent safety profile, favorable pharmacokinetic characteristics, broad antibacterial spectrum and good treatment effectiveness have made quinolones an important class of synthetic antibacterial agents [36, 37]. This class of antibacterials displays direct inhibition activity on the DNA synthesis by binding to the enzyme DNA complex, they stabilize DNA strand breaks created by DNA gyrase and topoisomerase IV [38]. Gyrase is responsible for introducing negative supercoils in DNA and relieving torsional stress expected to accumulate ahead of transcription and replication complexes. Topoisomerase IV provides a potent decatenating activity. Both gyrase and topoisomerase IV are essential enzymes and therefore the compounds that block bacterial growth by inhibiting them are accepted as potential chemotherapeutics [39].

CLASSIFICATION, SYNTHESIS AND STRUCTURAL REQUIREMENTS OF QUINOLONE ANTIBACTERIALS

Until today, four generations of quinolone class antibacterial drugs have been developed.

First Generation

The first generation includes: nalidixic acid, oxolinic acid, pipemidic acid, cinoxacin and rosoxacin which have shown only weak to moderate activity against Gram (-) bacteria, and therefore is rarely used today.

CHAPTER 3

Structure of Fine Starch Prepared *Via* a Compressed Hot Water Process

N. Shimizu[1,2,*] and **T. Ushiyama**[3]

[1] *Research Faculty of Agriculture, Hokkaido University, Hokkaido, 060-8589, Japan*

[2] *Field Science Center for Northern Biosphere, Hokkaido University, 060-0811, Japan*

[3] *Graduate School of Agriculture, Hokkaido University, Hokkaido, 060-8589, Japan*

Abstract: In a "top-down" process, starch nanoparticles can be produced by structural and size refinements through the breakdown of large particles. In this study, the structure of fine starches prepared *via* a compressed hot water process at different temperatures (160 – 180°C) was analysed using dynamic light scattering and size-exclusion chromatography with multi-angle light scattering (MALS) and differential refractive index detection. Changes in the molecular weight, polydispersity, hydrodynamic radius, and radius of gyration were assessed. The intrinsic viscosity of the fine starch solution was derived from the Flory-Fox and Ptitsyn-Eizner equation. The weight-average molecular weight decreased to 7.29×10^{-5} g/mol while the average hydrodynamic radius and weight-average radius of gyration decreased by 34.9 nm and 14.6 nm respectively, in fine starch prepared at 180 °C. In fine starches prepared at 160 °C, 165 °C, and 170 °C, tails in the multi-angle light scattering peaks, upswings in the conformation plots, and upturns in the plots of gyration radii and elution volumes were all the result of branching structures. In fine starches prepared at 175 °C and 180 °C, amylopectin branching was diminished and symmetrical scattering peaks were detected in the MALS analysis. We propose a pathway for waxy rice starch hydrolysis by a compressed hot water process.

Keywords: Fine starch, Intrinsic viscosity, Hydrodynamic radius, Molecular weight, Radius of gyration.

INTRODUCTION

Starch Conformation in Solid State and in Solution

Starch is a renewable and biodegradable biopolymer that is stored in many plants as a source of energy for photosynthesis. It is the second most abundant biomass in nature, and is typically isolated from plants in the form of microscale granules.

* **Corresponding author Shimizu N:** Research Faculty of Agriculture and Field Science Center for Northern Biosphere, Hokkaido University, Japan; Tel/Fax: +81-11-706-3848; E-mail: shimizu@bpe.agr.hokudai.ac.jp

Fine starches with average particle sizes ranging from the micrometre to nanometre scale have been developed as functional materials with applications in foods, cosmetics, medicines, and various composites. The major characteristics of fine starches are rapid dissolution and enhanced bioavailability after consumption.

Recent studies have reported that nano-scale starch particles can be readily prepared from starch granules, which have unique physical properties [1]. Starch granules consist of numerous nano-size semi-crystalline blocklets [2]. Physical treatments may disintegrate the starch granules, thus releasing the nano-blocklets. The preparation of starch nanoparticles may be classified into "top-down" and "bottom-up" processes. In the "top-down" process, nanoparticles are produced from structural and size refinement through the breakdown of large particles [3, 4]. In the "bottom-up" process, starch nanoparticles self-assemble into starch particles. Starch nanoparticles are important vehicles for nano- and micro-encapsulation in the food industry [5 - 7]. The physicochemical properties of polymers such as molecular weight, polydispersity, radius of gyration, hydrodynamic radius, and the molecular structure in solution are derived before and after modification processes [8, 9]. The intrinsic viscosity of the fine starch solution can be derived using the Flory-Fox and Ptitsyn-Einzer equation. It is important to evaluate not only raw starch, but also modified starches, for industrial use [3, 4].

To use starch effectively, techniques to prepare nanoscale starches have been developed and assessed. Nano-scale waxy rice starch particles can be prepared *via* hydrolysis using a compressed hot water process. Starch nanoparticles can also be prepared by acid hydrolysis, enzymatic treatments, and physical treatments such as high-pressure homogenization, ultrasonication, reactive extrusion, and gamma irradiation [1]. The compressed hot water process is one of the most useful reactions. The smallest average hydrodynamic radius of 75.2 nm was obtained by using a 180°C compressed hot water treatment, with a starch concentration of 0.1% (w/w), and an initial pressure of 3.0 MPa. The product of this process was evaluated by zeta potential and by using a submicron particle size analyzer [10]. A fine waxy rice starch solution prepared at 160°C with a compressed hot water treatment was spray-dried as a wall material for micro encapsulation [6]. It is important to understand starch macromolecular structures to optimize the practical uses of various products in industry. However, the changes in the hydrodynamic particle size induced by the compressed hot water process are unknown.

In this chapter, we describe the structure of fine starches prepared *via* a compressed hot water process at different temperatures (160°C – 180°C). The fine starches were analysed using dynamic light scattering (DLS) and size-exclusion chromatography (SEC) with multi-angle light scattering (MALS) and differential

refractive index (DRI) detection.

The intrinsic viscosity was calculated using the Flory-Fox and Ptitsyn-Eizner equation and correlations with particle conformation were derived. In SEC-MALS measurements, the branching amylopectin polymers affected the column separation and the MALS signal. Therefore, changes in the branching structures in waxy rice starch by the compressed hot water process could be deduced from the SEC-MALS data.

Starch Composition and Chemical Structure

Starch is commonly extracted from corn, wheat, and tapioca in native and modified forms for applications in the food, paper, and pharmaceutical industries [11 - 14]. After extraction from plants, starch occurs as flour-like white particles that are insoluble in cold water. For example, rice starch granules are semi-crystalline particles ranging from 3 to 8 μm. The smallest known starch particles are those in cereal grains. There is some variation in starch granule size among different rice genotypes. Rice starch granules have a smooth surface, but angular and polygonal shapes. The granules are loosely packed in clusters, and some particles have holes and cracks (Fig. **1**).

The internal architecture of native starch granules is characterized by "growth rings" that represent concentric semi-crystalline shells (thickness 120 – 400 nm) separated by amorphous regions. There is evidence that the crystalline shells consist of regular alternating amorphous and crystalline lamellae repeating at 9 – 10 nm intervals. In this structural organization, parallel double helices of amylopectin side chains assemble into radially oriented clusters (Fig. **2**). Little is known about the structure, organization, and arrangement of the lamellae.

Starch consists of amylase α(1-4)-linked glucose units, amylopectin α(1-4)-linked glucose units, and branched α(1-6)-linkages. The molecular weights (M_w) of amylose and amylopectin in starch vary among different plants; in normal corn they are 1.4×10^6 and 39×10^6g/mol, respectively [15]; that of amylose from rice is $5.1\text{-}6.9\times10^5$ g/mol [16]; that of amylose from waxy barley starch is 1.06×10^8 g/mol [17]; and that of amylose in Amioca (waxy corn starch) ranges from $107\text{-}10^9$ g/mol [18].

Starch molecules have a semi-crystalline structure that significantly affects their physical and chemical performance. Analyses of the fine waxy starch after the compressed hot water process revealed a peak derived from its crystalline structure. This peak was much reduced after the hydrothermal and spray-drying processes, indicating that the crystalline structure in the starch molecules had broken down into the amorphous form.

CHAPTER 4

Major Metabolites of Certain Marketed Plant Alkaloids

Kuntal Manna[*], **Bikash Debnath** and **Waikhom Somraj Singh**

Natural cum Advance Synthetic Lab, Department of Pharmacy, Tripura University (A Central University), Suryamaninagar, Tripura, India

Abstract: The archeological and historical record shows that people across Asia, Europe, and Africa used alkaloidal drugs as early as 2000 BCE. Alkaloids are heterocyclic rings consisting of at least one nitrogen atom. They are the waste products of plant metabolites and serve a wide variety of biological activities to human beings. Nicotine, cytosine, atropine, scopolamine, cocaine, catuabine, quinine, quinidine, dihydroquinine, papaverine, ephedrine, reserpine, ergotamine, caffeine, *etc*. are the most important marketed plant alkaloidal drugs and their metabolites are described in this chapter. Metabolism plays a central role in regulating the toxicity of a variety of phytochemicals. Hepatic microsomal enzymes such as monooxygenase and putative NADPH-FMN-reductase, carboxyl esterase, CYP2B6, CYP3A4, and CYP2D6 are mostly involved in the metabolism of alkaloids. This chapter will be important for future researchers.

Keywords: Cytochrome P650, Heterocyclic Ring, Hepatic Microsomal Enzymes, Marketed Alkaloids, Major Metabolites , Metabolic Pathway, Pharmacological Activities, Secondary Metabolites.

INTRODUCTION

Natural medicines provide a major source of pharmaceuticals, which we use today directly from nature or in marketed form. For the assistance of plants to survive and reproduce, they synthesize many secondary metabolites [1]. These secondary metabolites reveal biodynamic activity beneficial to both human and animal health. Alkaloids, phenols, steroids, glycosides, tannins, terpenoids, and phytoalexins are the secondary metabolites produced by the plants [2]. Among these secondary metabolites, alkaloids are considered the important ones. They are relatively modest molecules existing in plants at <10 g/kg [3]. Due to being toxic in nature, plants use alkaloids to protect themselves against harmful organi-

[*] **Corresponding author Kuntal Manna:**Department of Pharmacy, Tripura University (A Central University), Suryamaninagar, Tripura, India-799022; Tel: +91381-2379404; Fax: +91381-2374803;
E-mail: k_manna2002@yahoo.com

Atta-ur-Rahman (Ed.)

sms. Alkaloids are constituted in the plant kingdom and mainly found in the higher plants, such as those belonging to papaveraceae, menispermaceae, ranunculaceae, leguminosae, and loganiaceae [4, 5]. Groups of nitrogen-containing compounds that may consist of one or more nitrogen atoms (within the heterocyclic ring) are called alkaloids. However, the term 'alkaloid' (alkali-like) is rather interesting as there is no definite borderline between alkaloids and naturally existing complex amines. Typical alkaloids are basic in nature and primarily acquired from plant sources [6, 7]. Alkaloidal drug metabolism can have significance due to its therapeutic effect or its toxicity. It mainly takes place in the liver and the Cytochrome P450 enzymes are involved in a vital role in metabolism [8]. This chapter mainly focuses on biological sources along with major metabolites in some important marketed alkaloid drugs.

Major Metabolites of Certain Important Marketed Alkaloids

1. Metabolite Study of Pyridine Group of Alkaloids

Nicotine 1 (Fig. (**1**) is a naturally occurring alkaloid found in many plants. Dried leaves of *Nicotiana tabacum* belonging to the family Solanaceae constitute the active source of nicotine [9]. Nicotine and its metabolites may be dangerous to the body. Hepatic enzyme Cytochrome P450 2A6 (CYP2A6), UDP-glucuronosyltransferase (UGT), and flavin-containing monooxygenase (FMO) play an active role in nicotine metabolism. Electrospray ionization and high-performance liquid chromatography/tandem-mass spectrometry (LC-MS/MS) methods are used for the determination of nicotine metabolites Fig. (**1**). Quantitatively, the most important metabolites of nicotine in mammals are the lactam derivative and cotinine. In humans, about 70– 80% of nicotine is converted into cotinine. Nicotine N′-oxide is another primary metabolite of nicotine, although only about 4–7% of nicotine consumed by active smokers is metabolized via this route. The kidney is the vital organ for the excision of nicotine [10, 11]. **Cytisine 26** Fig. (**2**) is a selective nicotinic cholinergic agonist alkaloid obtained from the seed of *Laburnum anagyroides* belonging to the family Fabaceae [12]. Astroug *et al.* (2010) described the pharmacokinetics of cytisine. They gave oral and intravenous administration to examine the cytisine pharmacokinetics profile in male and female New Zealand rabbits. After the administration of cytisine, the rabbit serum of both the sexes were collected in a specific time interval to measure the pharmacokinetic parameters using high-performance liquid chromatography (HPLC) method with ultraviolet (UV) detection. The pharmacokinetic analysis suggested a rapid but incomplete absorption of cytisine after oral administration and did not clarify any metabolites of cytisine [13]. Later Jeong *et al*. (2015) developed a liquid-chromatography mass spectrometry (LCMS) method for the pharmacokinetic study of cytisine in human plasma and

urine. No metabolites were detected in plasma or urine collected in their study [14]. Further research is required for a better understanding.

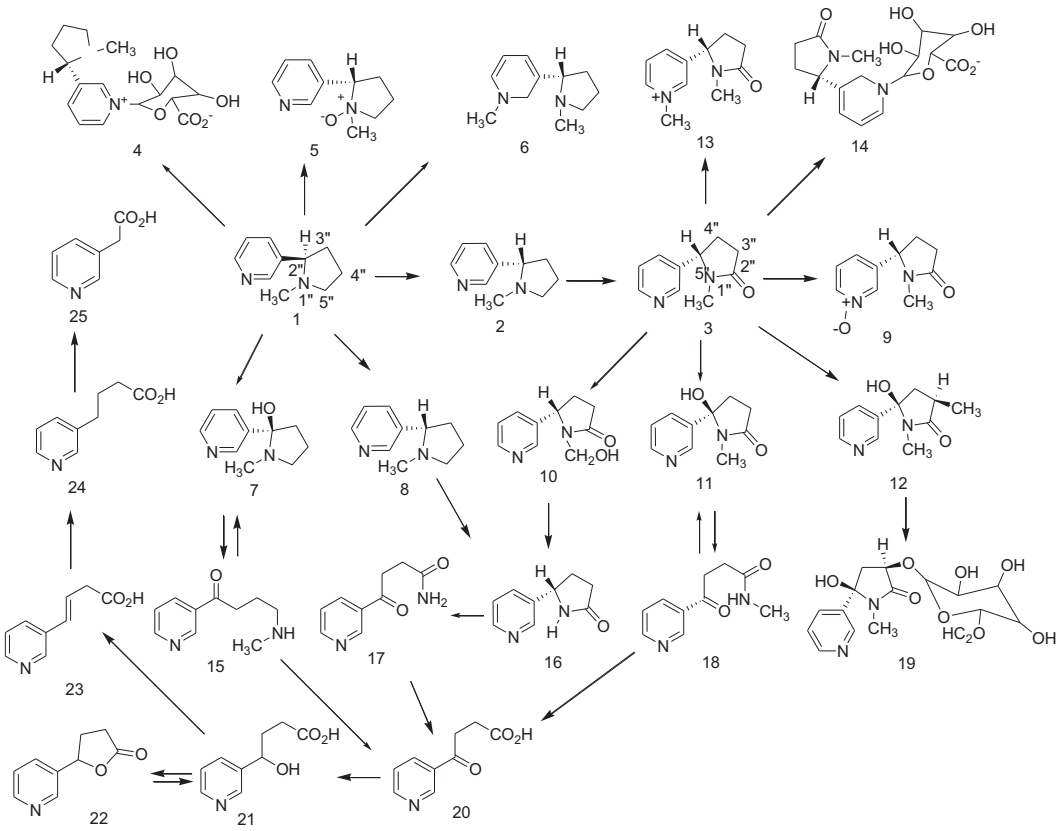


Fig. (1). Metabolism pathway of nicotine (**1**), Nicotine $\Delta^{1'(5')}$ iminium ion (**2**), cotinine (**3**), Nicotine glucoronide (**4**), Nicotine N'- oxide (**5**), Nicotine isomethonium ion (**6**), 2- hydroxy nicotine (**7**), Nornicotine (**8**), Cotinine N- oxide (**9**), N'- hydroxymethyl norcotine (**10**), 5- hydroxycotinine (**11**), *Trans*-3'-hydroxycotinine (**12**), Cotinine methonium ion (13), Cotinine glucorinide (14), 4- (methylamino)-1- (3-pyridiyl)-1-butanone (**15**), Norcotine (**16**), 4-oxo-4- (3-pyridyl)-butanamide (**17**), 4-oxo-4(3-pyridyl) N-methylbutanamide (**18**), *Trans*-3'-hydroxycotinien glucoronide (**19**), 4-oxa-4(3-pyridyl)-butanoic acid (**20**), 4-hydroxy-4-(3-pyridyl butanoic acid (**21**), 5-(3-pyridyl)-tetrahydrofuran-2-one (**22**), 4-(3-pyridyl)-3-butanoic acid (**23**), 4-(3-Pyridyl)-butanoic acid (**24**), 3-Pyridylacetic acid (**25**).

2. Metabolite Study of Tropane Group of Alkaloids

Atropine 27 Fig. (**3**) is obtained from the plant *Atropa belladonna,* a perennial herb belonging to the family Solanaceae. For intoxication in nature, this drug typically provides anticholinergic effects to the body [15]. Atropine is metabolized in the liver, and 30-50% of its unchanged are excreted along with urine [16]. Chen *et al.* (2006) outlined a metabolic pathway of atropine from rat urine, based on the LC-MS/MS technique after the administration of atropine

CHAPTER 5

Natural Products in Cancer Chemoprevention and Chemotherapy

Dev Bukhsh Singh[1,*], **Manish Kumar Gupta**[2] and **Rajesh Kumar Pathak**[3]

[1] *Department of Biotechnology, Institute of Biosciences and Biotechnology, Chhatrapati Shahu Ji Maharaj University, Kanpur-208024, India*

[2] *Department of Biotechnology, Faculty of Science, Veer Bahadur Singh Purvanchal University, Jaunpur-222003, Uttar Pradesh, India*

[3] *School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana-141004, Punjab, India*

Abstract: Cancer is a very fatal, challenging and complex disease. A large number of people across the globe are suffering from various types of cancer. The bioactive substances isolated from different parts of several herbs and spices have shown their valuable preventive and therapeutic role against different forms of cancer. The recent technological innovations have made it possible to explore the molecular targets of these bioactive substances and also enabled us to know the mechanism of action related to disease modulation. Our traditional knowledge related to chemopreventive and chemotherapeutic role of herbs is now being validated and explored by the use of modern biological techniques. However, mechanism of action that governs anticancer effect has not been well elucidated for many herbs and natural products. Herbal extracts are the mixture of different active substances, therefore screening and pharmacological response of each individual compound should be validated separately to gain some insight about mechanism of anticancer property. Polyphenols play significant role in initiation, promotion and progression of cancers by modulating the enzymes and signal of diverse pathways related to cellular proliferations, differentiation, angiogenesis, apoptosis and metastasis. These natural bioactives also serve as lead compounds for drug designing and their biological activity can be further optimize by some desired chemical modification. Optimisation of the therapeutic inhibitors can be enhanced using systems biology modelling of the molecular pathways related to the disease. The aim of this chapter is to provide the insight into the molecular basis of preventive and therapeutic effect of natural bioactive substance against cancer diseases.

Keywords: Apoptosis, Cancer, Chemotherapy, Combination therapy, Metastasis, Natural products, Polyphenols Cell division, Telomerase, Topoisomerase, Transcription factor.

* **Corresponding author Dev Bukhsh Singh:** Department of Biotechnology, Institute of Biosciences and Biotechnology, Chhatrapati Shahu Ji Maharaj University, Kanpur-208024, India; E-mail: answer.dev@gmail.com

Atta-ur-Rahman (Ed.)

INTRODUCTION

Cancer is a very challenging disease for researchers to find an effective approach for the detection and treatment. Cancer is the result of genetic changes that transform the normally dividing cells into malignant cells. It is an uncontrolled division of cells, where cells divides rapidly without reaching to stage of maturity. There are different types of cancer based on the parts of body and mechanism of disease. Cancer is the leading cause of premature death and physical disability world-wide and adversely affects the social and economic status of a country [1]. General causes of cancer are toxic chemicals, radiations, pathogens and genetic factors. Sign and symptoms of cancers are specific to the parts of body where it develops. Many cancers are identified by the name of body parts where abnormal growth of cell or tissues occurs such as breast cancer, colon cancer, lung cancer, liver cancer, prostate cancer, thyroid cancer and pancreatic cancer. Some of the general signs and symptoms are weight loss, pain, bleeding, fever, persistent cough and unusual tissue masses.

Despite of having advancements in screening, detection and therapeutic approaches for the treatment of cancer, the burden of cancer is expected to increase in future. Major cause of cancer death is the lack of detection in early stage. Treatment strategies for cancer depends on the type and stage of cancer. Natural products have proven their effective role in the prevention of different types of cancer. Natural products have also shown their potential role in treatment of cancer [2]. Modern techniques of biology have played very important role in elucidating the mechanism of action behind the preventive and therapeutic role of herbal products. With the discovery of mechanism that are involved in progression of a cancer, designing of a therapeutic molecule targeting the enzyme can be initiated. Surgery, chemotherapy and radiation therapy are the commonly used approaches for the treatment of cancer.

Natural products have shown their potential role against various types of cancers, such as pancreatic, prostate, skin, gastric, lung, oral, blood, colorectal, liver, head and neck, cervical and breast cancers. Natural compounds may have bioavailability, efficacy, specificity, metabolism, and toxicity related issues when used as a drug. These therapeutic issues may be overcome by performing a series of necessary chemical changes in the lead compounds. A number of natural products have shown their therapeutic role against cancer in preclinical and clinical studies.

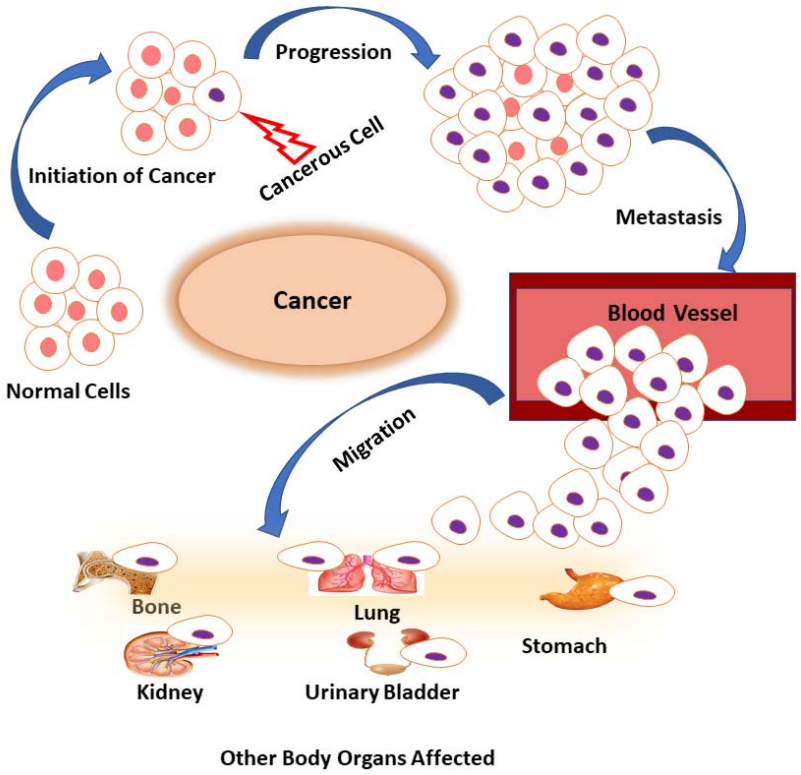


Fig. (1). Initiation, progression and metastasis of cancer.

Cancer is a uncontrolled division of cell due to the accumulation of defects *via* different mechanism, or mutations in DNA and the cells possess the capability to move from one part of body to another Fig. (**1**). Mutations or chromosomal aberrations affecting oncogenes and tumor suppressor genes causes malignant transformation of cells [3]. The most common sites of cancer are breast, cervix lungs, prostate, colorectal, stomach, and liver. Cancer cells can initiate, spread, and grow in various parts of body. The risk of developing cancer increases with age. Altering a diet that includes beneficial phytochemicals can have preventive and therapeutic role against cancer. In cancer chemoprevention, foods containing bioactive chemicals that have anticancer effect can be supplemented in diets. Alkaloids, flavonoids, terpenoids, polysaccharides, saponins, polyphenols and others have been reported as natural products with potential anticancer role [4]. Most of anticancer drugs that are in clinical use for cancer therapy originate from natural products derived from plants, marine sources, and microorganisms. Some natural products have shown their anticancer potential *via* regulating immune function, inducing apoptosis or autophagy, or inhibiting cell proliferation. Other

SUBJECT INDEX

A

B

C

D

E

F

G

H

I

K

L

M

N

O

P

Q

R

S

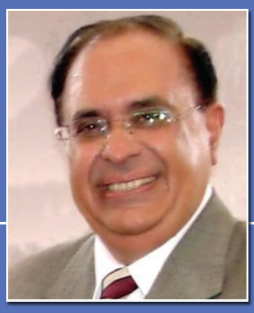
T

U

V

W

Z



PROF. DR. ATTA-UR-RAHMAN, *FRS*

Prof. Atta-ur-Rahman, Ph.D. in Organic Chemistry from Cambridge University (1968) has 1,232 international publications (45 international patents and 341 books). He received the following awards: Fellow Royal Society (FRS) London (2006), UNESCO Science Prize (1999), Honorary Life Fellow Kings College, Cambridge University (2007), Academician (Foreign Member) Chinese Academy of Sciences (2015), Highest Civil Award for Foreigners of China (Friendship Award, 2014), High Civil Award Austria ("Grosse Goldene Ehrenzeischen am Bande") (2007), Foreign Fellow Chinese Chemical Society (2013), Sc.D. Cambridge University (UK) (1987), TWAS (Italy) Prize (2009). He was the President of Network of Academies of Sciences of Islamic Countries (NASIC), Vice President TWAS (Italy), Foreign Fellow Korean Academy of Science & Technology, President Pakistan Academy of Sciences (2003-2006) and (2011 – 2014). He was the Federal Minister for Science and Technology of Pakistan (2000 – 2002), Federal Minister of Education (2002) and Chairman Higher Education Commission/Federal Minister (2002-2008), Coordinator General of COMSTECH (OIC Ministerial Committee) (1996-2012), and the Editor-in-Chief of Current Medicinal Chemistry.