CHAPTER 1

Introduction to Pharmaceutical Chemistry of Natural Products

INTRODUCTION

For thousands of years, natural products (drugs and pharmaceutical substances obtained from natural origin) have played vital role in treating and preventing human diseases. Humans always have been interested in naturally occurring compounds from prebiotic, microbial, plant and animal sources. In earlier period of treatment, naturally obtained ingredients were believed to be combined with witchcraft or mysticism. Later these treatments were found effective and the results were documented in the development of early herbal medicine. The science of pharmacognosy (a branch of pharmaceutical science), which deals with the naturally obtained drugs, developed from these records to provide an authentic, scientific description of the natural products used in treating and preventing human ailments.

The term *Pharmacognosy* was coined by a German scientist from two Greek words *pharmakon* (means a drug) and *gignosco* (means to acquire the knowledge of), in the year 1815. Pharmacognosy is defined as the 'study of the physical, chemical, biochemical and biological properties of medicinal products obtained from our living environment as well as the search for new drugs from natural sources'.

Pharmacognosy is an established basic pharmaceutical science, which has changed considerably from being largely descriptive botanical and mycological field to having more of chemical and biological focus. It serves as an important linkage between basic biology and medicinal chemistry. The advent of phytochemistry and pharmacological screening programme (heart of the drug discovery process from crude extracts) helps the natural products to find their way into medicine as purified phytochemicals, rather than in the form of conventional preparations.

Chemistry of Natural Products is a branch of chemistry that deals with the isolation, identification, structure elucidation and study of the chemical characteristics of chemical substances produced by living matters. The study of pharmaceutical applications of these natural products led to the establishment of a new avenue called *Pharmaceutical Chemistry of Natural Products*.

DRUG DISCOVERY FROM NATURAL PRODUCTS

Investigation of biological components of plant products and other organisms are carried out by adopting any of the five recognized approaches as given:

1. Random screening.
2. Selection of specific taxonomic groups, such as families or genera.
3. Chemotaxonomic approach, in which some classes of secondary metabolites, such as terpenoids, alkaloids, proteins, etc., are selected.
4. Information-managed approach, in which some of the species are selected based on database surveillance.
5. Selection by an ethnomedical approach (based on its use in traditional medicine).

The value of natural products in the field of medicine can be assessed using the following three criteria:

1. The rate of introduction of New Chemical Entities (NCEs) of wide structural diversity, including serving as templates for semi synthetic and total synthetic modification using these natural products.
2. The number of diseases treated or prevented by these substances.
3. The frequency of use of these natural products in the treatment of diseases.

Table 1.1 Drugs derived from natural sources

Natural products	*Medicinal uses*
Acarbose	Antidiabetic
Artemisinin	Antimalarial
Azithromycin	Macrolide antibiotic
Cefetamet pivoxil	Antibacterial
Cefozopran	Antibacterial
Cefpimizole	Antibacterial
Clarithromycin	Macrolide antibiotic
Irinotecan	Anticancer
Ivermectin	Antiparasitic
Miglitol	Antidiabetic
Mizoribine	Immunosuppressive
Mycophenolate mofetil	Immunosuppressive
Paclitaxel	Anticancer
Pentostatin	Anticancer
Policosanol	Nutritional supplement
Sirolimus	Immunosuppressive
Tacrolimus	Immunosuppressive
Teicoplanin	Antibacterial
Vinorelbine	Antimitotic
Topotecan	Topoisomerase I inhibitor
Voglibose	Alpha-glucosidase inhibitor

An analysis of the origin of the drugs developed between 1981 and 2010 showed that natural products or natural product-derived drugs comprised 36% of all NCEs launched into the market. In addition, 28% of the NCEs were synthetic or natural mimic compounds, based on the study of pharmacophores (the section of the molecule containing the essential organic functional groups which directly interact with the receptor-active site and hence, confers the desired biological activity) related to natural products. Between 1990 and 2000, a total of 41 drugs derived from natural products were launched in the market by major pharmaceutical companies (Table 1.1); some of these drugs are azithromycin, paclitaxel, sirolimus (rapamycin), synercid, tacrolimus and topotecan.

The above analysis suggests that the natural products are an important source for new drugs and are also good lead compounds suitable for further modification during drug development. The large proportion of natural products in drug discovery has stemmed from the diverse structures and then intricate carbon skeletons of natural products. Since secondary metabolites from natural sources have been elaborated within living systems, they are often perceived as showing more 'drug-likeness and biological friendliness than totally synthetic molecules', making them good candidates for further drug development.

Nomenclature

The chemical structures of natural products are generally complex in nature, some are remarkably complex and hence naming the compound by systematic nomenclature will not be possible. Therefore, the names are given on the basis of trivial nomenclature, in which the discoverer has the right to name the compound. In general, the source of the compound is selected to supply the root name, e.g. digoxin and digitoxin from *Digitalis purpurea*. The suffix *-in* indicates 'a constituent of', *-oside* to show the compound is a sugar derivative (e.g. glycoside, sennoside), *-genin* for the aglycone obtained upon hydrolysis of the sugar derivative (e.g. digoxigenin), *-toxin* for a poisonous constituent (e.g. podophyllotoxin), or may reflect chemical functionality, such as *-one* (e.g. menthone) or *-ol* (e.g. menthol). Conventionally *-ine* is always used for alkaloids or amines (e.g. quinine and reserpine). The analogues are then named as derivatives of the original natural compound by using the standard prefixes like *hydroxy-*, *methoxy-*, *methyl-*, *dihydro-*, *homo-*, etc., for added substituents to the main compounds and *deoxy-*, *demethyl-*, *demethoxy-*, *dehydro-*, *nor-*, etc., for removed substituents from the main compounds. Some groups of compounds like steroids, fatty acids and prostaglandins are named by adopting the systematic and conventional approach from an accepted root name. For example, the name *cholesterol* (cholest-5-en-3α-ol) is given on the basis of stem name *cholestane*.

Classification of Natural Products

The pharmacologically useful bioactive compounds of natural origin can be classified into various categories in a different manner. The classification of natural products is described as follows:

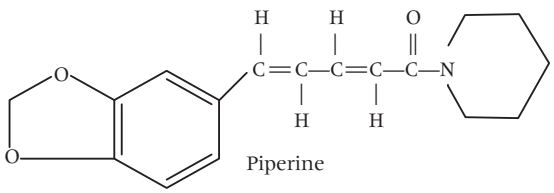
Chemical classification

Based on the chemical nature of the secondary metabolites, they may be classified as alkaloids, terpenoids, flavonoids, etc. Further, each of these can be classified on the basis of its chemical nature, like open-chain aliphatic, alicyclic and cycloparaffinic, aromatic, benzenoid and heterocyclic.

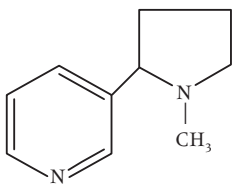
For example, the classification of alkaloids is depicted as given:

Heterocyclic alkaloids

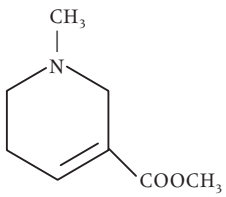
1. Pyridines and piperidines



Piperine

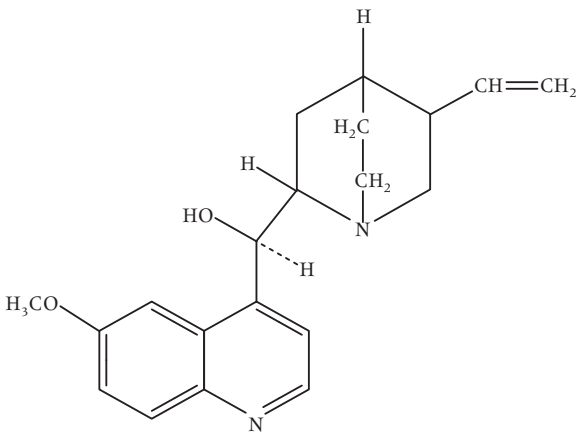


Nicotine

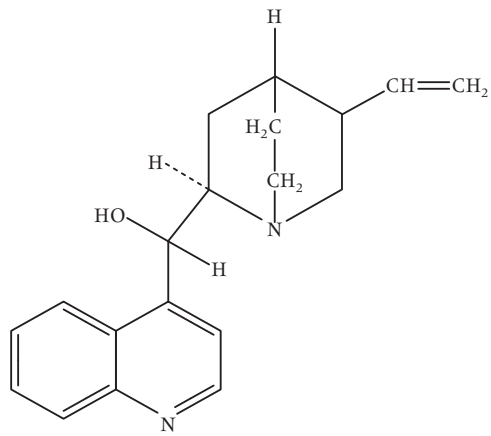


Arecoline

2. Quinolines

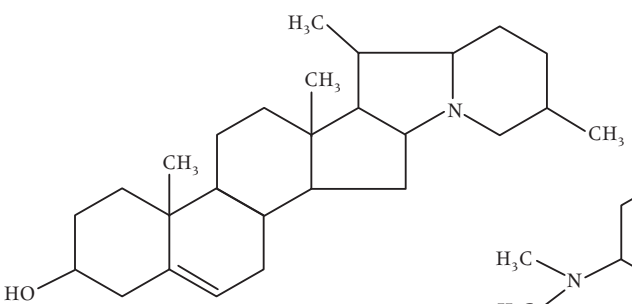


Quinine

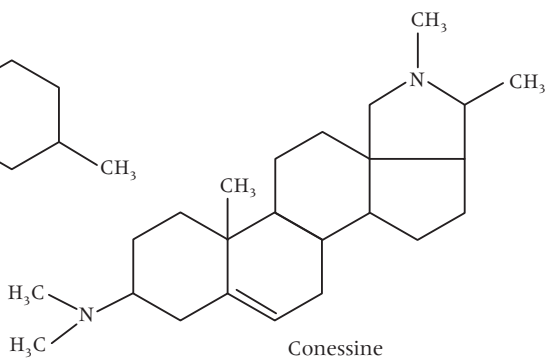


Cinchonine

3. Steroidal alkaloids

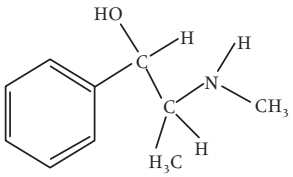


Solanidine

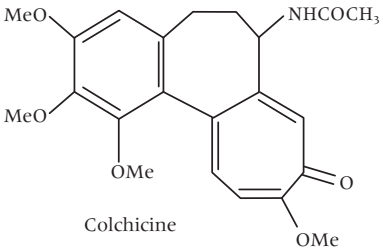


Conessine

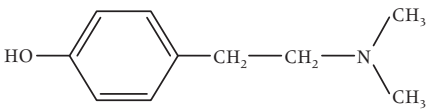
Nonheterocyclic alkaloids



Ephedrine



Colchicine



Hordenine

Pharmacological classification

Natural products are frequently initiated by attempts to isolate and clarify a pharmacologically active principle of plant or animal origin. They may be classified on the basis of its therapeutic activity as given:

Analeptics	:	Strychnine, Brucine
Analgesics	:	Morphine
Anticancer	:	Vincristine, Taxol
Antimalarial	:	Quinine
Local anaesthetic	:	Cocaine

Chemotaxonomic classification

This is performed on the basis of the plants and the chemical nature of the natural products. Chemotaxonomy serves as markers for the evolution as well as the classification of plants. The characters generally studied in chemotaxonomy are secondary metabolites of pharmaceutically important compounds like alkaloids, glycosides, flavonoids, etc.

Biogenetic classification

The primary synthetic process in nature is photosynthesis by which green plants utilize the energy of the sun for the production of organic compounds from carbon dioxide. The initial products of photosynthesis are carbohydrates. Further metabolic alterations lead to the formation of a pool of organic compounds of low molecular weight and simple structures such as carboxylic (-COOH) and amino acid ($NH_2CHRCOOH$) groups, which are vital for all the living organisms. It is responsible for synthetic starting materials for specific, genetically controlled, enzymatically catalysed reactions that lead to the complex compounds that characterize the secondary metabolism of plants and mammals. The reaction pathway leading to a particular natural product is called the *biosynthetic pathway* and the corresponding event is known as *biogenesis*. Different plant and animal species can employ different biosynthetic pathways to produce the same metabolite. This feature can be employed in the classification of plants in terms of their chemotaxonomy.

Among the four major classes of biochemicals (carbohydrates, proteins, nucleic acids and lipids), experiments have indicated that the first three classes could have arisen through prebiotic chemistry. Although the biosynthesis of many natural products can be traced back to acetate (e.g. fatty acids, terpenes and polyketide biosynthesis) or amino acids (e.g. alkaloid biosynthesis), there are many whose biosynthetic origins are either obscure or result from a complex combination of multiple synthetic pathways.

Identification of Natural Products

Medicinal chemistry has evolved from the chemistry of bioactive compounds in early days due to the works at the interface of chemistry and biology. Medicinal chemistry of bioactive natural products spans a wide range of fields, including isolation and characterization of bioactive compounds from natural sources, structural modification for optimization of the exerted activity and other physical properties and semisynthesis and synthesis for a thorough scrutiny of structure–activity relationship (SAR). In addition, synthesis of natural products also provides a powerful means in solving supply problems in clinical trials and marketing of the drug. Obtaining natural products in bulk amount is often very difficult.

In general, chemistry of natural product work is initiated after a given crude drug formulation (typically prepared by solvent extraction of the natural material) is judged 'active' in a particular in vitro assay. If the end goal of the work at hand is to identify which one(s) of the hundreds of compounds are responsible for the observed in vitro activity, the path to that end is fairly straightforward. Steps involved are as follows:

1. Fractionate the crude extract, e.g. by solvent partitioning or chromatography.
2. Test the fractions thereby generated with in vitro assay.
3. Repeat steps (1) and (2) until pure, active compounds are obtained.
4. Determine structure(s) of active compound(s), typically by using spectroscopic methods.

Sometimes in vitro activity does not necessarily translate to activity in humans or other living systems. In that case, a typical protocol to isolate a pure chemical agent from natural origin is bioassay-guided fractionation, meaning step-by-step separation of extracted components based on differences in their physicochemical properties and assessing the biological activity, followed by the next round of separation and assaying.

The unique properties of natural products are disobedience to Lipinski's 'Rule of Five', which has been widely recognized as the most useful 'drug-like' compounds selection criteria. Drug discovery community began to once more appreciate the value of natural products and revived natural products research by integrating rapid isolation and identification with hyphenated technologies, parallel synthesis, computations and many other new techniques into medicinal chemistry of natural products.

Techniques Involved in Natural Product Lead Discovery

The typical process of discovering natural-product drugs and their progression towards development is depicted in Figure 1.1. In this generic scheme, the natural product is extracted from the source, concentrated, fractionated and purified, yielding essentially a single biologically active compound.

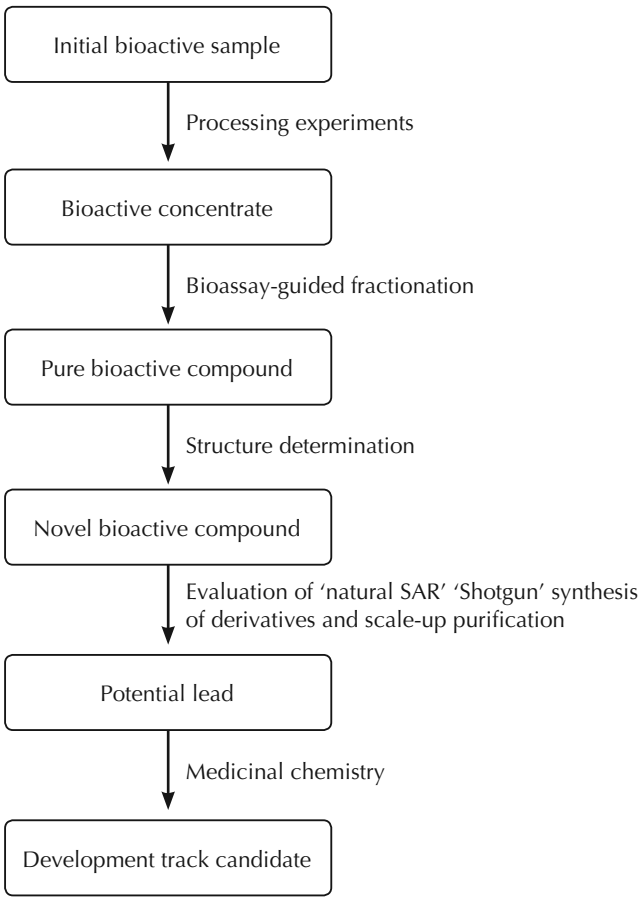


Figure 1.1 Chemical process for natural product drug discovery

Historically, this process has most often suffered from three major hurdles. The first is the rapid identification of known compounds (dereplication) to avoid duplication of effort. This step has been greatly facilitated by the availability of reliable directly coupled HPLC-mass spectrometer (LC-MS) systems, and the general availability of natural product databases. The pivotal development responsible for the success of LC-MS has been the introduction of efficient and general methods for producing ions from the effluent of HPLC separations. The most general of these methods, known as *electrospray ionization* (ESI) and *atmospheric pressure ionization* (API), can generate the ions essential for mass spectrometric analysis for greater than 90% of analytes, ranging from amino acids to proteins and nucleic acids. The correlation of both molecular mass and UV absorption data with known compounds by database searching is ordinarily sufficient to classify sets of compounds.

The second major hurdle in the process—the de novo structure determination of compounds that are (NMEs)—is an area that has been revolutionized by many advances in spectroscopic techniques, particularly in high-resolution NMR technologies. Of the many NMR advances, those

of particular importance to natural product structure determination fall into one of the given two main areas: multidimensional pulse methods and sensitivity improvements. From its inception, and particularly since the advent of two-dimensional NMR methods, high-resolution NMR spectroscopy has seen continuous development and expansion of the array of experimental methods available to elucidate chemical structures. New experiments, particularly multidimensional ones, provide scalar (through bond) ^{1}H-^{1}H and ^{1}H-[^{13}C, ^{15}N, ^{31}P] correlations and ^{1}H-^{1}H dipolar (through space) molecular connectivity data that essentially map out the structure of the compound. In the area of sensitivity, stronger magnetic fields provided by superconducting magnets, cryogenic electronics and micro-probe technologies have dramatically lowered the amount of material needed for structural analysis, to less than a milligram.

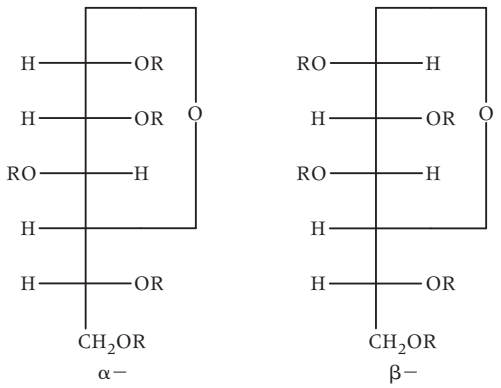
The combination of cryogenic probe electronics with correlation spectroscopy enables the development of further more powerful experiments, such as correlation experiments for low-abundance ^{13}C and ^{15}N nuclei, which are unattainable with conventional hardware. Determination of molecular formula is crucial to the process and is typically done by high-resolution mass spectrometry on microgram quantities of material. One of the most powerful of these techniques is Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS), which is capable of measuring molecular mass with exceptional accuracy. Combining the tools of high-resolution mass spectrometry with two-dimensional NMR spectroscopy allows structure determination to be carried out on sub-milligram or milligram amounts of a compound in a matter of hours or days, rather than weeks or months. Although the determination of complex structures is technically challenging, it is no longer a major impasse in the drug discovery process. In the cases in which the biological activity profile meets criteria for potency and selectivity, preliminary SAR studies are conducted and the process is scaled up. A second avenue for exploring SAR in an expeditious manner is the 'shotgun' approach to chemical derivatization. The knowledge gained through understanding the natural products' SAR and the shotgun approach provided an early foundation on which the overall synthetic strategy was developed. Once the feasibility of modulating biological response through synthetic modification is established, the hit is declared a lead and proceeds for additional optimization by traditional medicinal chemistry.

Natural Products as Pharmacological Tools

There are many historical examples in which the natural product has not just been the medicinal product but has also helped revealing a novel aspect of physiology. For example, digitalis from foxglove showed the role of *sodium-potassium-ATPase*; morphine pointed the way to the receptors affected by endogenous opioids; muscarine, nicotine and tubocurarine helped to explore the different types of acetylcholine receptors, etc. More recently, there has been interest in systematically searching for small-molecule inhibitors of key steps in biochemical processes (chemical genetics). Given that many assays involve identifying phenotypic changes in living cells (as opposed to binding interactions with isolated proteins), it is probable that natural products will provide useful probes for such studies. Moving beyond observations of phenotypic changes to defining the alterations in gene expression or protein function that are responsible will require advances in transcriptomic and proteomic methods.

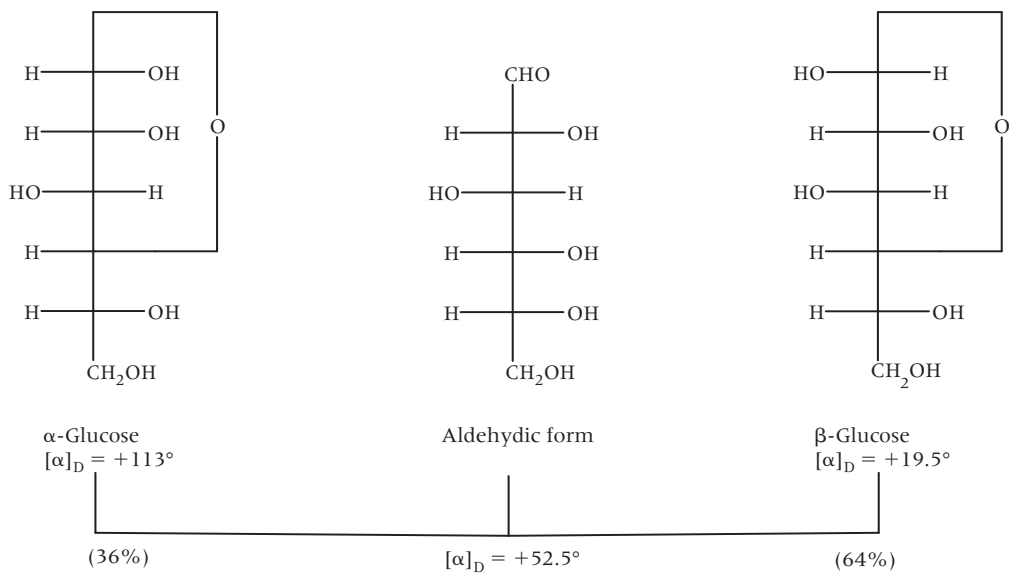
In summary, research on natural products has contributed significantly and has been the most successful strategy for discovering new drugs and for extending human life and improving clinical practice. As the chemical techniques improved dramatically, the active constituents are isolated from natural resources, characterized and synthesized in the pharmaceutical chemistry laboratory. These chemical modifications (semisynthesis) lead to find more active or better tolerated drugs. Gradually, the synthetic compounds superseded many of the natural products, but certain natural products were never surpassed and remain as valued medicines till today. Natural products play an important role in drug discovery programme, as long as nature continues to yield novel, diverse chemical entities possessing selective biological activities.

The two isomers differing only in the configuration of C_1 (in aldoses) or C_2 (in ketoses) are known as *anomers*, while such carbon atom is known as *anomeric carbon* atom.
The existence of two methyl glucosides and penta-acetates can be explained in same way.



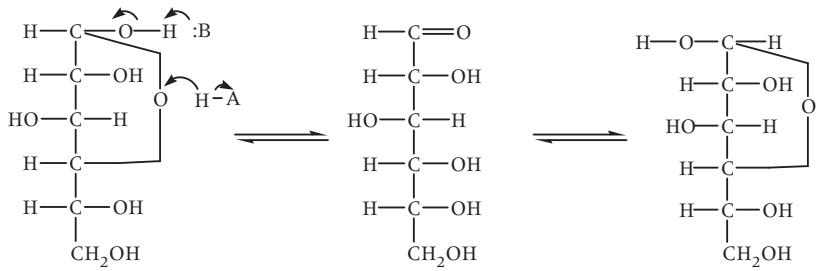
Methyl glucoside when R = CH_3
Glucose penta-acetates when R = $COCH_3$

3. *Mutarotation:* The cyclic structure can open and reclose, which allows the rotation to occur about the carbon bearing the reactive carbonyl to yield two distinct configurations (α and β) of the hemiacetals and hemiketals. The carbon about which this rotation occurs is called *anomeric carbon* and the two forms are called *anomers*. Carbohydrates can change spontaneously between α and β configurations. This process is known as *mutarotation*.
When glucose crystallized from water below 50°C is dissolved in water, its initial specific rotation of +113° falls gradually to a constant value of +52.5°; similarly, when the glucose crystallized from water above 95°C is dissolved in water, its initial specific rotation of +19.5° gradually rises to +52.5°. This phenomenon is known as *mutarotation* in glucose.

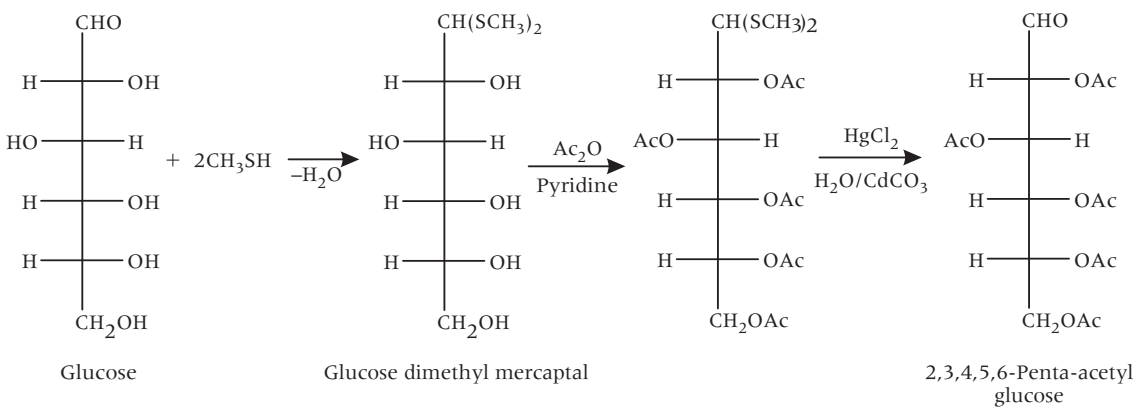


The ring structure for glucose explains that the change in specific rotation is due to interconversion of the α-form [$(\alpha)_D = +113°$] of glucose to β-form [$(\alpha)_D = +19.5°$) and vice versa through the aldehydic structure till an equilibrium is reached between the two structures. The specific rotation value of this equilibrium mixture corresponds to +52.5°.

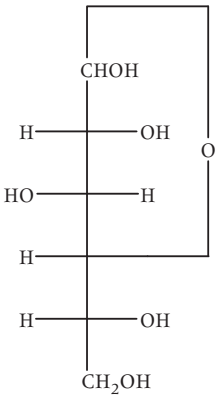
The question at our hand is how the open-chain form of glucose is obtained from the ring form. It can be explained that this happens because protonation by acid HA and deprotonation by base B takes place at the same time.



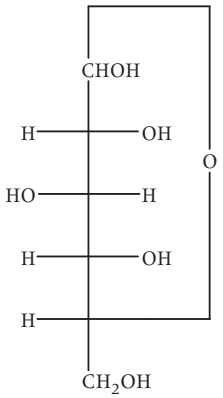
The concentration of aldehydic form can be increased by adding methanethiol and esterification, followed by the removal of methanethiol.



Two types of ring structures: Fischer isolated a new D-glucoside, which differs from that of already known α- and β-glucosides and called it γ-D-glucoside. But later on Haworth explained that this γ-glucoside was found to be a mixture of two different glucosides (α and β) different from that of previously known α and β-glucosides. These observations reveal that the sugars have two different ring structures, namely γ- and δ-oxide ring structures.



γ-Oxide
(5-membered ring structure)



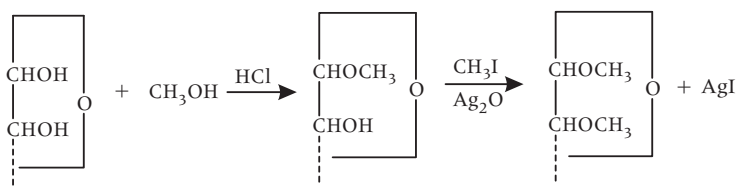
δ-Oxide
(6-membered ring structure)

Determination of the Ring Size

The following methods are generally employed for the determination of the ring size in sugars.

1. Methylation method
2. Periodic acid oxidation method

1. *Methylation method:* This method was first reported by Hirst. The sugar is first completely methylated, which is then hydrolysed with dilute hydrochloric acid. When only the glycosidic methyl group is hydrolysed, the product is finally oxidized. On the basis of the oxidation product obtained, the ring size of the sugar is determined. This method is based on the assumption that during the reactions there is neither a change in the size of the ring nor in the position of any methyl group. The first step of methylation can be effected by different methods.



(i) *Purdie method:* Upon reaction of the sugar with methanolic hydrogen chloride the sugar is converted into its methyl glycoside. The product is then treated with methyl iodide in the presence of dry silver oxide to afford fully methylated product.

Advantages

- As the free sugar is first methylated by methanolic hydrogen chloride the oxidation of the free sugar by silver oxide is prevented.

- As the methylation is performed at mild condition, the structural alterations in the molecule are avoided.
- This method is free from untoward effects such as Walden inversion, racemization or glucosidic interconversion.

Disadvantages

- Silver oxide causes oxidation of a free reducing sugar. Therefore, this method is applicable only to glycosides and other derivatives in which reducing group is either absent or protected by substitution.
- This method can be used only for the sugars of which the suitable solvents are known.
- Reagents used in this method (e.g. methyl iodide and silver oxide) are expensive.

(ii) *Haworth and Hirst method:* The sugar is fully methylated by using dimethyl sulphate in the presence of aqueous sodium hydroxide.

Advantages

- This method is applicable to all kinds of reducing sugars.
- Methylation occurs smoothly in a step-wise and uniform manner, and therefore, the intermediates can be isolated by interrupting the process.
- Unreacted and partly reacted sugars are not formed; therefore, the purification is carried out easily.
- Yield is good and the reagents used are cheap.

Disadvantage

- Dimethyl sulphate is a poisonous compound; therefore, it should be used with great care under controlled conditions.

(iii) *Diazomethane method:* Sugars can also be methylated by the use of diazomethane in the presence of alcoholic ethereal solution.

Advantages

- Nitrogen is the only by-product and it can be eliminated easily.
- The product obtained is very pure.

Disadvantages

- Diazomethane is very poisonous.
- This method is applicable for methylation of acidic compounds.

(iv) *p-Toluene sulphonate method:* Hydroxy compounds are methylated by reacting the compounds with *p*-toluene sulphonate in sodium hydroxide. This method is also called as *tosylation*.

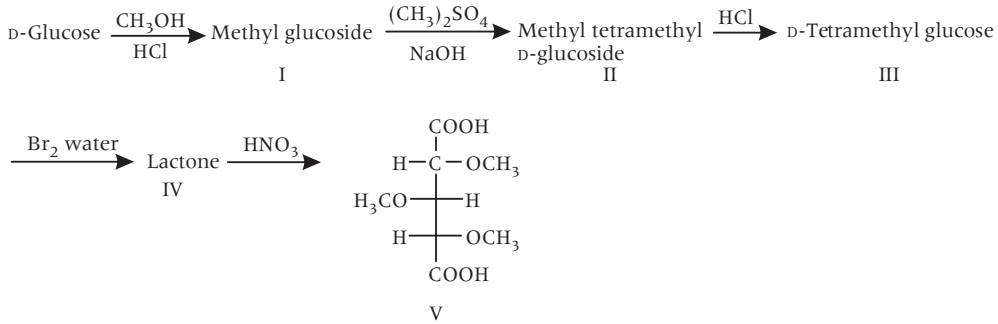
Advantage

It is preferred over the above methods, as *p*-toluene sulphonate is harmless.

(v) *Sodium and methyl iodide method:* By using sodium and methyl iodide in liquor ammonia, the carbohydrates can be methylated.

Let us consider the example of D-glucose and apply the methylation method on both of the possible forms (pyranose and furanose).

Pyranose structure: Pyranose structure is also called *δ-oxide*, *amylene oxide* or *six-membered ring*. Using dimethyl sulphate in the presence of sodium hydroxide (Haworth and Hirst process of methylation), the methyl glucoside (I) (obtained by refluxing the D-glucose with methanolic hydrochloric acid) is methylated to give methyl tetramethyl-D-glucoside (II), which upon hydrolysis gives a tetramethyl-D-glucose (III). Oxidation with nitric acid gives a dicarboxylic acid (V). The dicarboxylic acid (V) was identified as xylotrimethoxy glutaric acid by the fact that it was also obtained by the oxidation of methylated xylose.

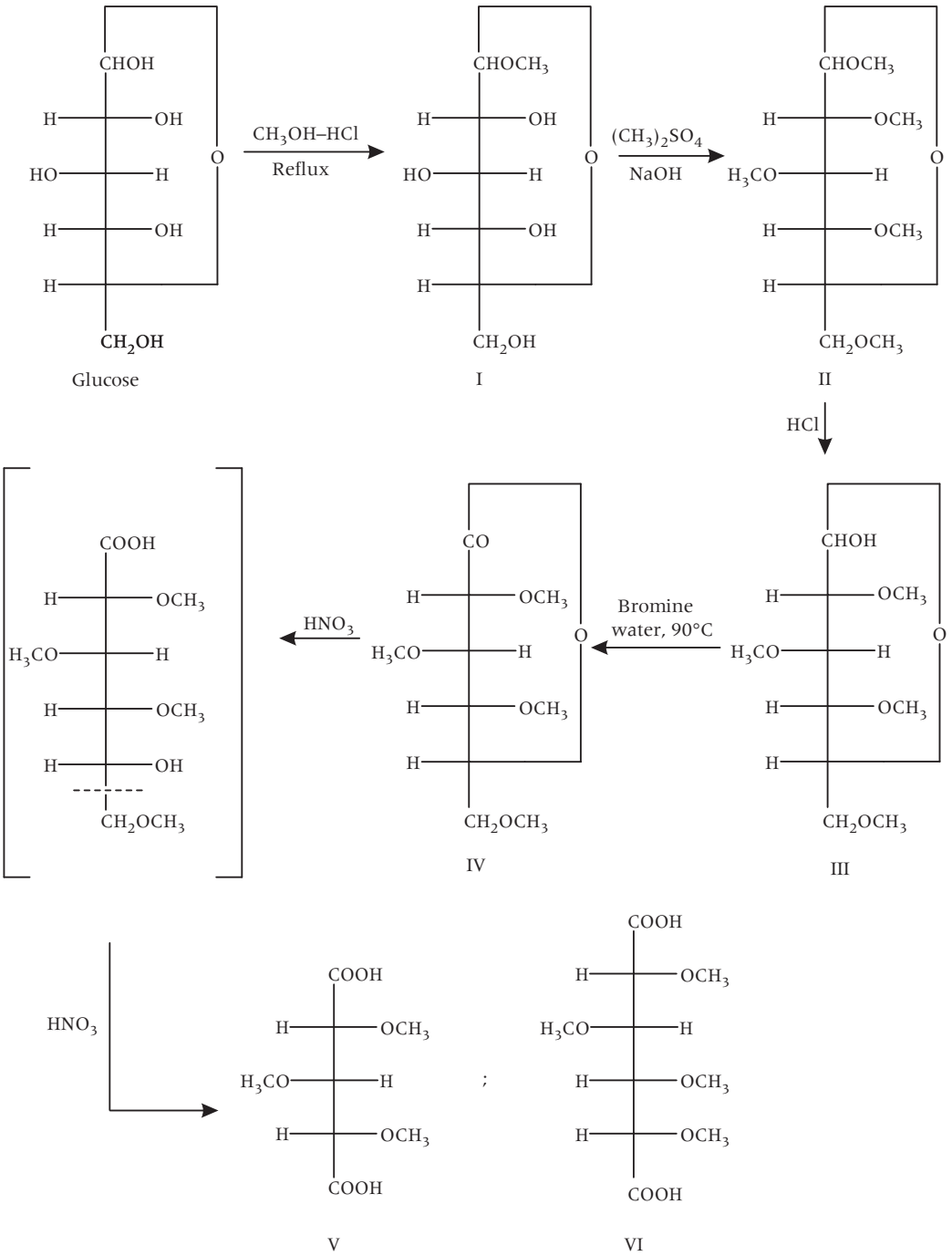


Xylotrim ethoxyglutaric acid

The dicarboxylic acid structure, which helped in determining the structure of the parent compound glucose, is described here.

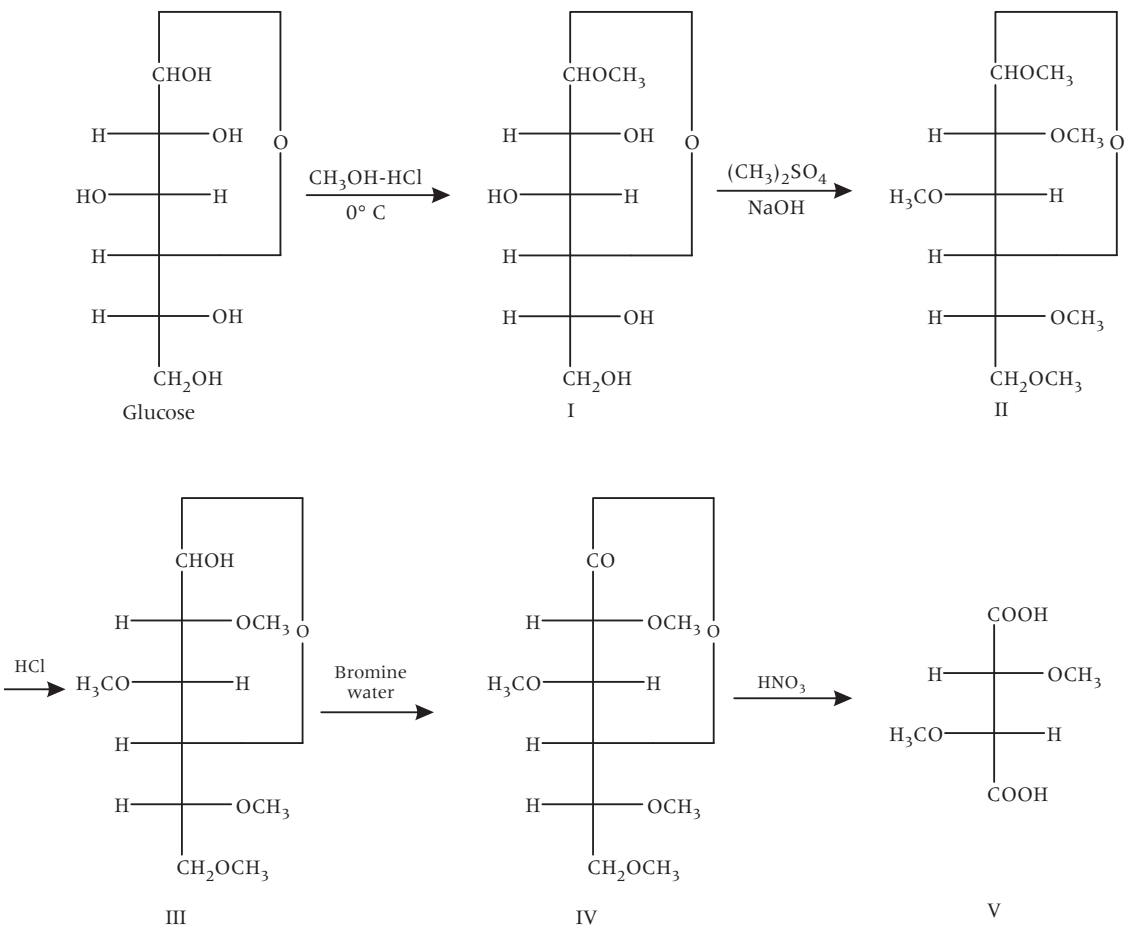
1. From the first carbon atom of the lactone (IV), one of the –COOH groups in acid (V) must be derived and the second –COOH group from the carbon atom is involved in the ring formation in the sugar. As only three hydroxyl groups are present inside the ring of glucose, the ring must be δ-oxide in sugar.
2. Presence of three methoxy groups in the dicarboxylic acid (V) indicates the presence of only three methoxy groups in between the lactone (IV) and hence three hydroxyl groups inside the ring of glucose, i.e. the lactone formation involves C_1 and C_5 and hence the lactone (IV) must be δ-lactone and the sugar must be six membered, which explains all the reactions in the following manner:
3. Finally, the formation of (V) also eliminates the possibility of an ε-ring (when C_1 and C_6 are involved in ring formation) because if at all the case was ε-ring, the product 2,3,4,5-tetramethyl gluconic acid or tetramethylsaccharic acid (VI) would have been formed.

It is important to note the Fischer's first methyl glucosides were actually the pyranosides, which he called furanosides only on the basis of Tollens' assumption that the γ-oxide ring is stable by analogy to γ-lactones.


CHOH
H
OH
HO
H
O
H
OH
H
CH2OH
Glucose
CH3OH–HCl
Reflux
CHOCH3
I
(CH3)2SO4
NaOH
H3CO
OCH3
CH2OCH3
II
HCl
CHOH
III
Bromine
water, 90°C
CO
IV
HNO3
COOH
V
VI
;

Furanose structure: Fischer prepared a new methyl glucoside (actually which was later on found to be a mixture of two glucosides, α and β) by dissolving D-glucose in methanolic hydrochloric acid and then keeping the product at 0°C for some time. The size of the ring in these two new isomeric glucosides was found to be γ-1,4-butylene oxide. The steps used for establishing the size of the ring are same. For example, the methyl glucoside (I, prepared at 0°C as earlier) is completely methylated using methyl sulphate to yield methyl tetra-*O*-methyl-D-glucoside (II), which is hydrolysed with dilute hydrochloric acid to yield tetra-*O*-methyl-D-glucose (III), which is then oxidized first with bromine water at 90°C to yield crystalline lactone (IV) and finally with nitric acid to yield dimethyl-D-tartaric acid (V, dimethoxysuccinic acid) of well-known structure.

The formation of dimethyltartaric acid from D-glucose indicates that within the ring of the sugar and its derivatives, (II), (III) and (IV), there are only two hydroxyl or methoxy groups and hence the ring must involve C_1 and C_4 which are finally oxidized during the set of reaction to the acidic groups. The scheme for conversion of glucose to dimethyltartaric acid may be represented as shown below:

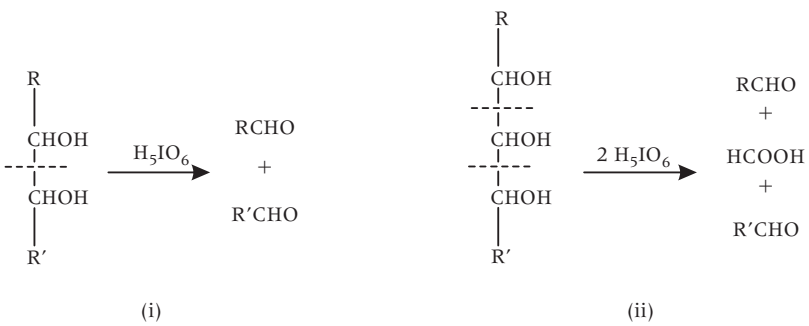


From the above contradictory statements it is concluded that the methyl glucoside prepared at reflux temperature has 1,5-oxide (δ-oxide) ring structure. Hence, with the help of this method it is not possible to say whether glucose itself originally exists in the pyranose (1,5) or furanose (1,4) form or whether the two forms are in equilibrium.

However, X-ray analysis of all the normal monosaccharides indicates the presence of six-membered ring in a manner similar to which α-D-glucose is found to have pyranose structure. Using periodic acid oxidation, the existence of glucose as pyranoside has been confirmed.

2. *Periodate oxidation method:* This is a simple elegant method introduced by Malaprade. It involves the use of periodic acid at about pH 4 in dark to avoid overoxidation and decomposition of the oxidant.

The general principles of periodic acid oxidation have been discussed already. As shown in the example, between every 1,2-glycolic groups one molecule of periodate is consumed.



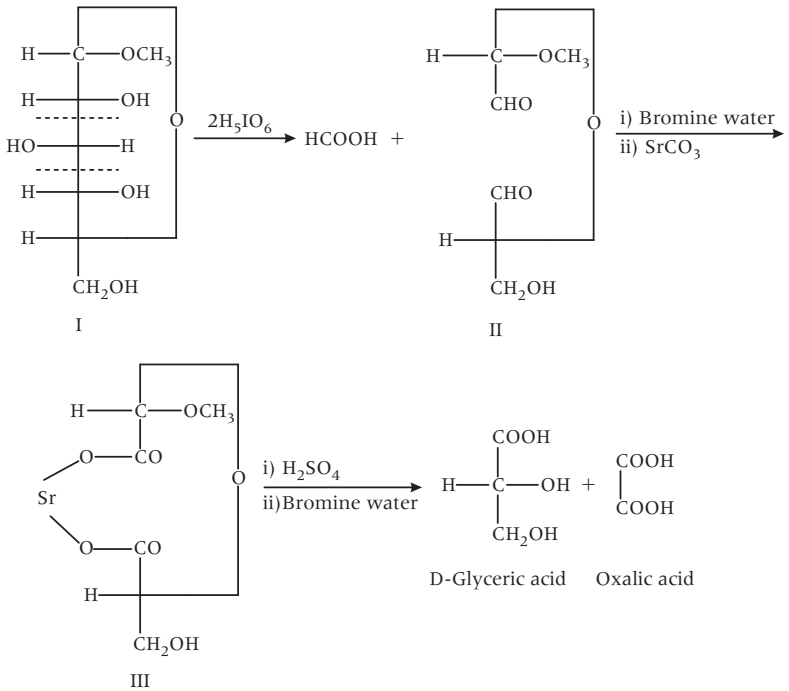
(i) (ii)

Principles useful in structural investigation by periodic acid may be summarized as below:

1. When a –CHOH group is attacked on one side, i.e. only by 1 mole of the reagent, it is converted into –CHO group.
2. One molecule of periodic acid is consumed between every two adjacent hydroxyl groups (1,2-glycolic groups).
3. When a $–CH_2OH$ group is attacked by periodic acid, it is converted into HCHO.
4. When –CHOH group is attacked on both the sides, i.e. one –CHOH group is attacked by 2 moles of periodic acid, it is converted into HCOOH.
5. The reagent does not open the oxide ring of the sugar or its derivatives.

Thus, by estimating the number of moles of the periodic acid consumed and the number of moles of HCHO and HCOOH produced along with the investigation of the oxidation product of the glycoside, this method has been successfully used to determine the size of the ring in glycosides.

Using this method, Hudson proved that the normal glycosides are pyranosides. For example, the compound (I) on oxidation with periodic acid consumes 2 moles of the acid along with the formation of 1 mole of formic acid and a dialdehyde (II) indicating beyond doubt that the compound (I) has three adjacent hydroxyl groups, which is possible only when the glycoside has δ-ring structure.



It has been proved by the above scheme that the structure (II) on oxidation with bromine water in the presence of strontium carbonate gives a crystalline strontium salt (III). The strontium salt (III) on hydrolysis followed by oxidation yields oxalic and D-glyceric acids.