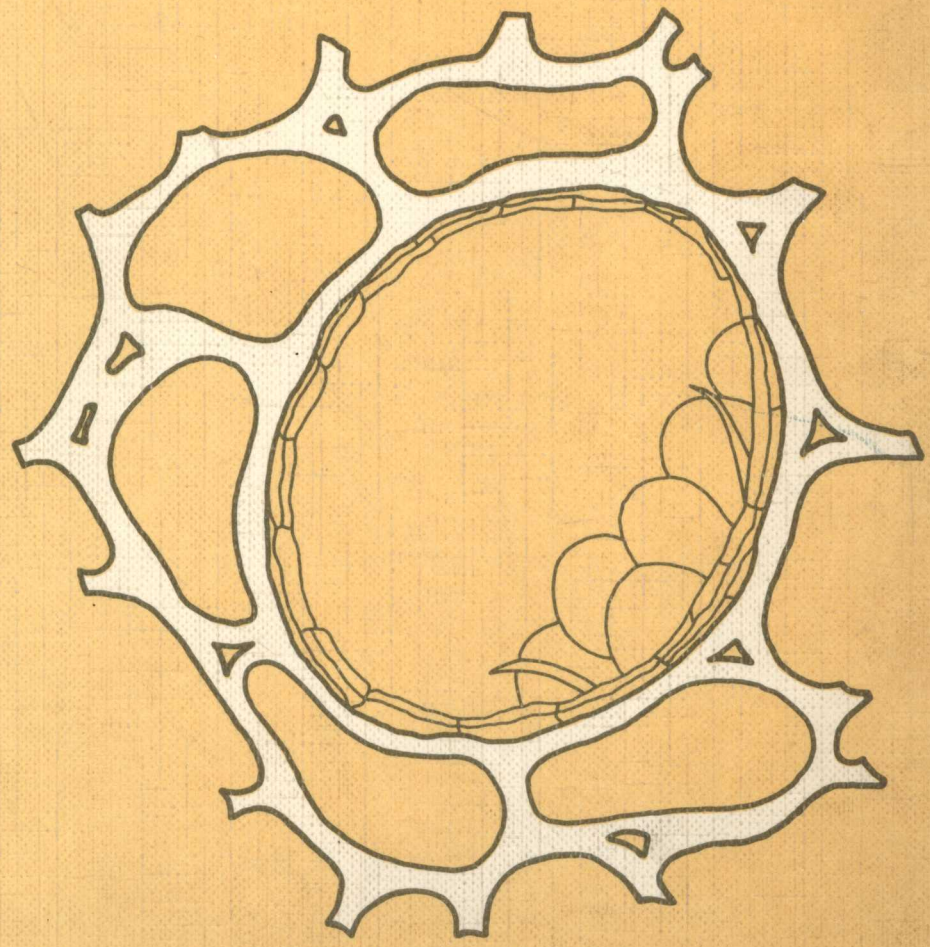
The Practical Evaluation of Phytopharmaceuticals

K. R. BRAIN and T. D. TURNER



WRIGHT-SCIENTECHNICA

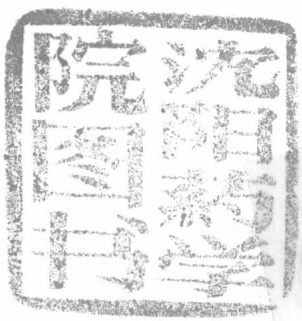
The Practical Evaluation of Phytopharmaceuticals

K. R. Brain, B.Pharm. (Nottingham), Ph.D. (Bath)

Lecturer in Pharmacognosy, Welsh School of Pharmacy, University of Wales Institute of Science and Technology

T. D. Turner, M.Pharm. (Wales), M.P.S., F.R.M.S., F.L.S.

Senior Lecturer in Pharmacognosy, Welsh School of Pharmacy, University of Wales Institute of Science and Technology



WRIGHT-SCIENTECHNICA
BRISTOL
1975

ISBN 0 85608 012 8

PRINTED IN GREAT BRITAIN BY HENRY LING LTD., A SUBSIDIARY OF JOHN WRIGHT AND SONS LTD., AT THE DORSET PRESS, DORCHESTER

PREFACE

The evaluation of plant materials and their derived products has always been an important part of the professional expertise of the pharmacist and analyst. However, over the years the nature and degree of this evaluation have changed. Initially it was considered sufficient to authenticate the plant material by comparison with a standard botanical description or monograph. Later it was realized that for the detection of adulteration this practice must be supplemented with both a microscopical analysis and confirmatory chemical tests for the class of constituent present. This developmental process continued slowly until the middle of this century when the rapid advances in our knowledge of the chemistry of plant drugs, and in new and improved methods for the analysis of the active constituents, resulted in the extension of the evaluative procedures into the areas of the estimation and identification of these constituents and the requirement that drugs should conform to a phytochemical as well as a morphological monograph.

This text is intended to present elements of both the methods and the theoretical background of both the traditional evaluative procedures and those of more recent origin. In certain cases the traditional procedures have become extended into the recent procedure class by virtue of new techniques, and these have therefore been integrated. For example, in the consideration of microscopical analysis the cytomorphology of plant materials is supplemented by micromeretics and the study of microcrystals and optical properties of materials as means of evaluation and identification. Similarly the extraction methods described extend from classical tincture production and non-specific extractive evaluation procedures to the more specific extraction, separation, and estimation methods using chromatographic and other techniques.

There has been no attempt to treat each section exhaustively. The object has rather been to indicate the relationships between the various analytical procedures and the product variables which must be determined. Each descriptive part of the text includes basic principles where necessary and is followed by practical exercises designed to demonstrate the principles described. The experiments are typical of those applied in our own laboratories in the teaching of pharmacognosy to pharmacy undergraduates and of food and drug analysis to public analysts, forensic scientists, food scientists, and cosmetic scientists engaged in postgraduate courses. The bibliography is indicative rather than extensive and it is intended that readers should supplement this text with publications within their own particular fields of interest.

January, 1975

K.R.B.
T.D.T.

CONTENTS

Chapter 1

Introduction

NATURAL product drug materials are a diverse group of products ranging from parts of plants, through simple extracts, to isolated active constituents. The definition encompasses a wide range of natural materials which are important for their therapeutic activity or as pharmaceutical adjuvants. Certain of these drugs have been known and used by man for many centuries, whilst others are still being isolated and evaluated. Many of the older materials are used as *crude drugs*. This term is normally applied to a product, such as a part of a plant or an unrefined extract, which is more or less in the form in which it was collected or prepared. Crude drugs are traditionally classified as ' organized ' or ' unorganized ' according to whether they contain a regular organized cellular structure. It is considered that the terms *cellular* and *acellular products* are more easily understood alternatives to these traditional terms. A crude drug may be subjected to some form of simple extraction procedure, in an endeavour to concentrate its activity and remove unwanted material, to yield a *galenical product*, such as a tincture or liquid extract. If the purification procedure is extended it may allow the isolation of particular *constituents*. In the phytochemical sense a constituent is a distinct chemical entity which can be isolated from a particular source and care must be taken to distinguish between this broad definition and that of an *active constituent* which is an entity which can cause a physiological response.

As the natural drugs are derived from heterogeneous sources they are subject to *variability*. This variability can have both an advantageous and a disadvantageous effect. The main disadvantages are that the activity of the material may vary and that inferior material may be produced. The advantages are that some control of variability can be introduced to produce a superior product. For example, the selection of particular varieties of cinchona raised the average quinine content of the bark drug from 5 per cent to 15 per cent, and the selection of rust-resistant strains of *Mentha* has made menthol production an economic proposition in Brazil. Other methods of increasing the yield of drugs include the production and selection of mutants, polyploids, and hybrids, and the control of the physical and chemical environment of the plant. The stage of development of the plant may be important, and seasonal variations due to differences in light and temperature are not uncommon. For example, the alkaloid level in the basal leaves of belladonna falls from April to June, whilst there is a concurrent increase in the leaf size which keeps the total alkaloid per plant almost constant. Variations in constituent level with age are particularly common in barks and underground organs. For example, the level of quinine in *Cinchona ledgeriana* grown in Java increases markedly for the first five years and then falls slowly. Constituent level may also be linked with morphological development. For example, there is a loss of pyrethrins as pyrethrum flower heads expand, and a loss of volatile oil when clove buds open. In the monographs on a number of drugs defined in official texts such as the *European Pharmacopœia* (E.P.), *British Pharmacopœia* (B.P.), *British Pharmaceutical Codex* (B.P.C.), and the

United States Pharmacopœia (U.S.P.) there are requirements for the collection of only a particular variety of plant, at a certain age or time of the year, or at a certain stage of development.

Collection of the drugs may be from wild or from cultivated plants, and by skilled or unskilled labour, and this can obviously influence the quality and purity of the product. Preservation of the collected material is an important problem, particularly where the constituents are liable to rapid degradation. All living organs contain water, the level varying from about 5 per cent in seeds to about 95 per cent in fleshy fruits. Biochemical reactions can occur only in the presence of water, so that if it is removed the material is protected from both autolysis and attack by micro-organisms. Care must be taken in the removal process, especially if the constituents are heat sensitive, and as enzyme activity increases with temperature up to about 45–50°C the treatment must be rapid and at a sufficiently high (but not excessively high) temperature. Where the product is particularly moisture and heat sensitive (such as digitalis) it is best dried as rapidly as possible at 55–60°C in a preheated oven. Preparation of the drug may occur with the selection of only certain parts of the material. For example, the cork layer is removed from peeled ginger and peeled liquorice. Long storage is not generally desirable as slow changes are inevitable, although there are certain cases where these changes are desirable and indeed necessary (e.g., cascara) and a minimum storage period before use is therefore specified for these materials. As described in detail in Chapter 8 on Extraction, the constituents of any extract will vary according to the particular extraction procedure used.

Of particular importance in the quality control area is the detection of *adulteration* or debasement of an article. This term covers a number of conditions which may be deliberate or accidental. Deliberate adulteration is becoming much less common owing to the use of more sophisticated analytical techniques, although it still occurs quite frequently with expensive drugs and with those sold illegally. Inferiority is a natural substandard condition (e.g., where a crop is taken whose natural constituent content is below the minimum standard for that particular drug) and it can be avoided by more careful selection of the plant material. Spoilage is a substandard condition produced by microbial or other pest infestation which makes a product unfit for consumption, and it can be avoided by careful attention to the drying and storage conditions. Deterioration is an impairment of the quality or value of an article due to destruction or abstraction of valuable constituents by bad treatment or ageing or to the deliberate extraction of the constituents and the sale of the residue as the original drug. For example, spent (extracted) cloves may be supplied for the genuine drug, although in this case detection is simple as the buds float in water if the oil has been removed. Admixture is the addition of one article to another through accident, ignorance, or carelessness, such as the inclusion of soil on an underground organ or the co-collection of two similar species. Sophistication is the deliberate addition of spurious or inferior material with intent to defraud. Such materials are often carefully produced and may appear at first sight to be genuine (for example, powdered ginger may be diluted with starch and a little colouring matter added to give the correct shade of yellow). Substitution is the supply of an entirely different article in place of that required, such as the supply of cheap cottonseed oil as olive oil.

The function of *quality control* and *drug evaluation* is to assess the value of raw materials and to ensure that the final product is of the required standard. The selection of an appropriate approach to any particular analytical problem is essential as, whilst there is frequently more than one pathway to the desired information, one route is almost invariably more rapid, reliable, or accurate than the others. The route selected must depend on the problem under consideration and the information required. The first essential part of a logical approach is to examine the

situation and to define the problem and the aims of the investigation. A little time spent in careful consideration at this point will often save a great deal of time later. The analytical problems encountered in pharmacognosy are varied and include the identification and definition of new drug sources, the assessment of the quality of crude drugs for direct use, or as a source of valuable constituents, and investigations of a trouble-shooting nature in respect of difficulties encountered in the extraction or use of a drug. The following chapters describe the techniques which are utilized in natural drug evaluation and the practical examples are designed in many cases to demonstrate in particular the applications of these methods.

Chapter 2

Morphography

MORPHOLOGY is the study of the form of an object whilst morphography is the description of that form. A number of different bases may be used in the classification of drugs and each has its own advantages and disadvantages. The application of morphology in drug analysis lies in the field of crude drugs where the material is known to occur in a particular form. The morphology may be studied at the whole drug (macromorphology or gross morphology), cell (cytomorphology), or particulate level. If a given sample of a known crude drug is to be evaluated as a potential source of therapeutic activity it is necessary to confirm that: (*a*) it is, in fact, a sample of the drug stated; (*b*) it contains the expected active constituents in reasonable quantity; and (*c*) it contains nothing else.

The usual procedure is first to attempt to authenticate the source of the material, that is to decide if it is actually the material claimed. The essential basis of any authentication procedure is a comparison between the test sample and another, standard, sample the origins of which are known without doubt. No two objects are exactly alike but a given population of objects can be divided into groups according to similarities and differences between individuals. For each group or category of objects there is a character, or group of characters, by which the members of the group are related, whilst at the same time distinguished from the members of other groups. Each group is usually given a name which stands for the sum total of these characters. The actual characters used depend on the objects under consideration, but obviously the larger the number of parameters which are compared the more accurate the comparison becomes. The simplest example of an authentication procedure is a direct comparison of test and standard samples. This has the advantage that there is little need to define the parameters used with great accuracy. It also has the disadvantage that it requires each analyst to keep a complete reference set of standard samples, and also requires completely non-destructive examination methods if the standard samples are to be kept intact. In addition the standard sample cannot demonstrate the accepted range of biological variability if it is to be restricted to a relatively small and manageable size.

The problem of destructive testing can be partially solved by the use of secondary, disposable, standards, but it is much more convenient if a description of the standard, rather than the standard itself, can be used as the yardstick. Consideration must therefore be given to the communication of this description. The description can be made most accurate and unambiguous if it is a combination of verbal and pictorial information. The crude drugs defined in the E.P. and B.P. are defined solely in terms of written descriptions, primarily because of the high cost of reproduction of illustrations. Textbooks of pharmacognosy usually use a combination of verbal and pictorial information, whilst there are atlases of powdered vegetable drugs (such as that by Jackson and Snowdon and by Deryng) which rely almost entirely on the pictorial side. In order to be concise

a verbal description must be made in technical terms. A knowledge of these terms is essential if the reader is to interpret their meaning correctly. The derivation of particular terms is dealt with later in this section.

Sampling

Care must be taken in the selection of samples for examination as these must be truly representative of the material undergoing analysis. In the case of a small consignment all parts should be examined. Where this is impractical portions for analysis should be taken from several different parts of the consignment to ensure that the material which has been supplied is homogeneous. For example, the U.S.P. lays down very definite sampling procedures. If the material is in powdered form or the pieces are less than 1 cm. in any direction samples are taken by extracting cores from top to bottom of the container in each direction. If the sample size is less than 100 kg at least 250 g of material is taken. If the sample size is greater than 100 kg repeated samples are taken, according to the schedule below, and mixed and quartered.

Number of Packages in Shipment	*Number of Packages to be sampled*
1–10	1–3
10–25	2–4
25–50	3–6
50–75	6–8
75–100	8–10

Two of the diagonal quarters are rejected and the remaining two quarters carefully remixed and requartered until two of the quarters weigh as near as possible to, but not less than, 250 g. With samples of vegetable drugs consisting of pieces greater in size than 1 cm the samples are taken by hand from different parts of the container or containers. If the consignment size is less than 100 kg the minimum sample size is 500 g. If the consignment size is greater than 100 kg repeated samples are taken and mixed and quartered as in the first procedure until two of the quarters weigh as near as possible to, but not less than, 500 g.

Organoleptic Evaluation

This includes the examination of the odour, taste, colour, and texture of the drug. To an expert odour and taste are extremely sensitive criteria. However, the description of these features is very difficult so that often the odour and taste can only be described as 'characteristic' and reference made to the analyst's memory. The colour is of use in indicating the general origin of the drug, e.g., material derived from the aerial parts of plants is usually green. The texture is best examined by taking a small quantity of material and rubbing it between thumb and forefinger. The texture is described by terms such as 'smooth', 'rough', and 'gritty'. It is most valuable in indicating the general type of material and the presence of more than one component.

MACROMORPHOGRAPHY

The majority of natural product drugs which occur in more or less entire (intact) form are derived from plants, or parts of plants. It is most usual for only a part of a plant to be used, either because the active constituents are only found in particular parts or because economic considerations dictate the collection of only certain parts of the whole plant. For example, anthraquinones are found in high concentration only in the bark of the cascara tree so that there is little point in collecting material other than the bark.

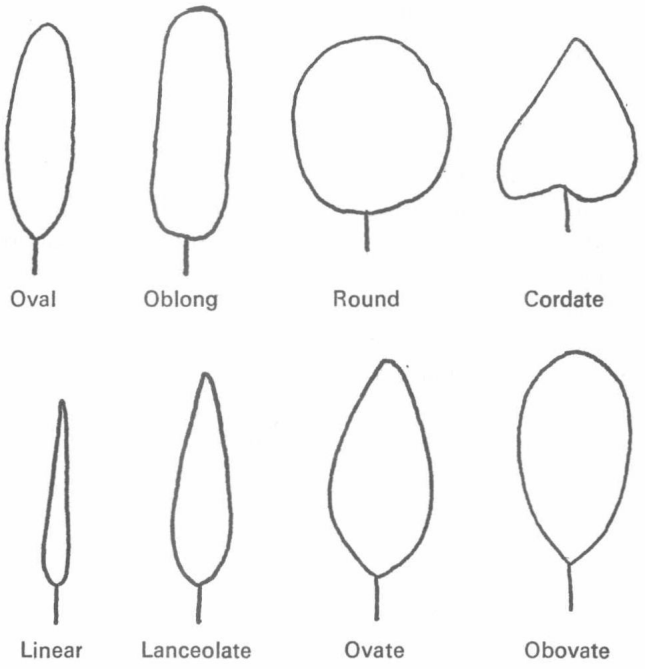


Fig. 2.1: Common descriptive terms for lamina shape.

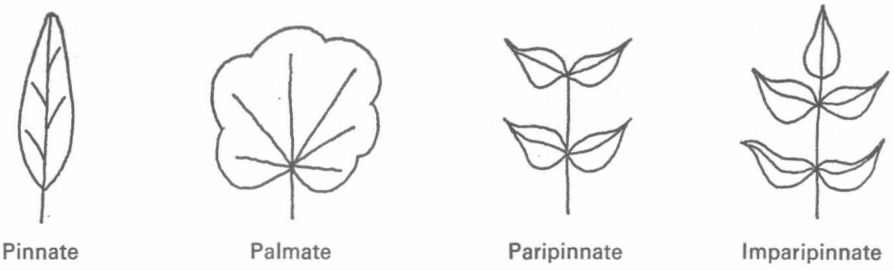


Fig. 2.2: Descriptive terms for leaf composition.

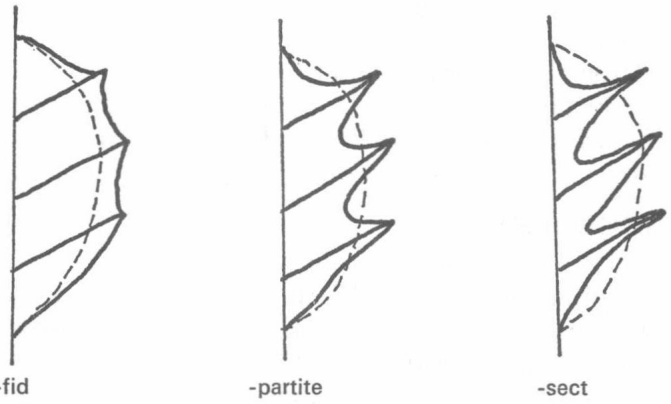


Fig. 2.3: Suffixes applied to denote depth of incision of the lamina.

Most species of plants are readily distinguishable in their natural state, but collection, preparation, and drying produce distortion of the natural form, making recognition more difficult. Natural variations in size and shape are common and are often the result of environmental factors. Macromorphology is of greatest importance to the drug importer and manufacturer, both of whom commonly deal with the drug at this level.

These materials are best considered according to the part of the plant used, and this necessitates a basic knowledge of plant structure. The following groups are commonly used in the description of drugs derived from different parts of the plant:

Herb or aerial parts,	Leaf,	Bark,
Subterranean organ,	Flower,	Wood.
	Fruit and seed,	

The approach to the description of macromorphology will be demonstrated in detail by reference to the particular parameters used in the definition of leaves. As a group, leaves are relatively complex but they have obvious differences in form which even an inexperienced observer can comprehend. The description of other groups is dealt with later in less detail.

Description of Leaf Drugs

Surface Appearance and Texture

The drug may be in whole or broken form and the texture may be described by such terms as ' shrivelled ', ' brittle ', ' leathery ' (coriaceous), ' papery ', or ' fleshy '. The surface colour can be important, together with any obvious differences between the upper and lower surfaces, such as the occurrence of oil glands or hairs. Surface hairs may be present (pubescent) or absent (glabrous). If they are present they are of diagnostic value and may be rough (hispid), long (hirsute), or glandular. They may be distributed over the entire leaf or may be concentrated in particular areas, such as the midrib.

Dimensions

These will obviously vary within a sample but the aspect (length-to-breadth) ratio, mean dimensions, and maximum and minimum values can be useful.

Attachment of Leaf

The attachment of the leaf to the stem is known as 'sessile' or 'petiolate' according to whether the leaf is joined directly to the stem or via a short stalk (the petiole).

Lamina Structure

The lamina is the main flat part of the leaf (leaf blade) and it can show a very wide variation in form.

Shape: Many variations occur, the most important of which are shown in *Fig. 2.1*. These terms may be combined to describe intermediate or variable structures, e.g., 'linear-lanceolate' or 'ovate to obovate'. Where the specimen has been dried the shape can best be determined after soaking.

Composition: The commercial designation ' leaf ' includes both true leaves and the individual leaflets of compound leaves. These can readily be distinguished if the attachment of the leaf to the stem can be examined. Only in the case of a true leaf is a bud found in the axil (the angle between the petiole and the stem). The simple leaves are classified as 'pinnate' or 'palmate' (*Fig. 2.2*). The arrangement of leaflets in compound leaves is known as 'paripinnate' or 'imparipinnate', dependent upon the presence of a terminal leaflet. The blade of the leaflets is almost always asymmetrical, except for any terminal leaflet, which is always symmetrical. The occurrence of

a symmetrical leaflet, together with a larger proportion of asymmetrical leaflets, in a sample indicates that it is derived from an imparipinnate leaf.

Incision: This refers to the occurrence of clefts into the edge of the leaf, and increasing depth of incision is indicated by the addition of the suffixes -fid, -partite, and -sect (*Fig. 2.3*) to the composition term, e.g., palmatifid.

Venation: The arrangement of the veins (vascular tissue) on the lamina. Four types are common: parallel, pinnate, palmate, and reticulate (net).

Margin: The margin or edge of the leaf may be entire or may have some indentation. Note that these marginal indentations are much smaller than those considered as incisions. The most common types are shown in *Fig. 2.4*.

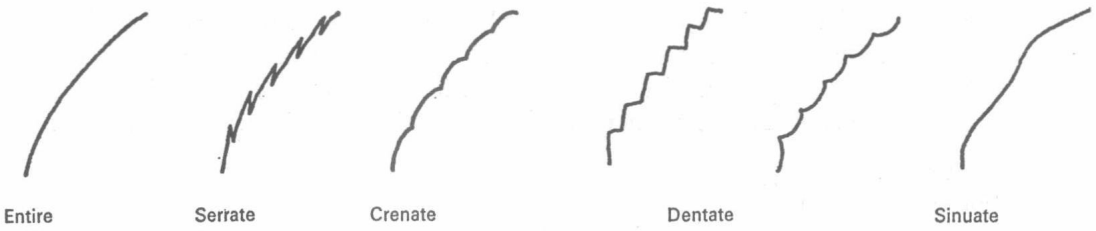


Fig. 2.4: Common descriptive terms applied to the margin of the lamina.

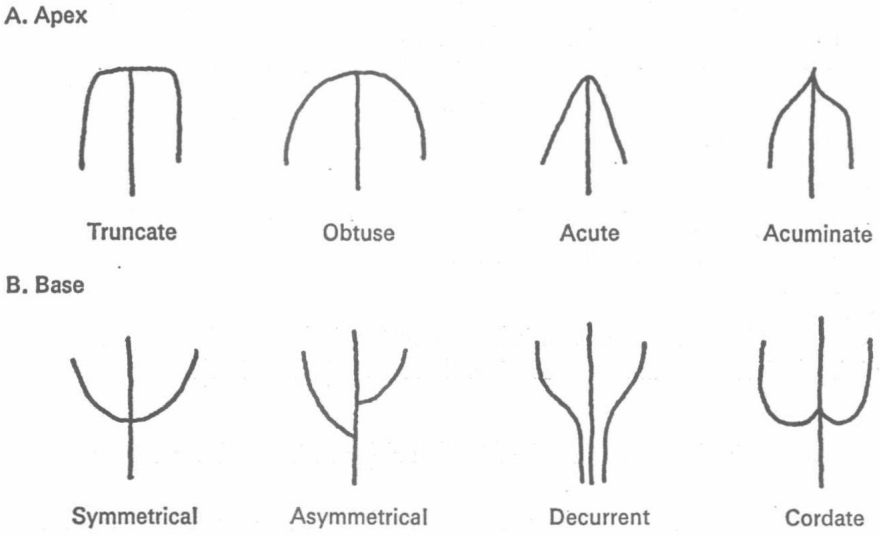


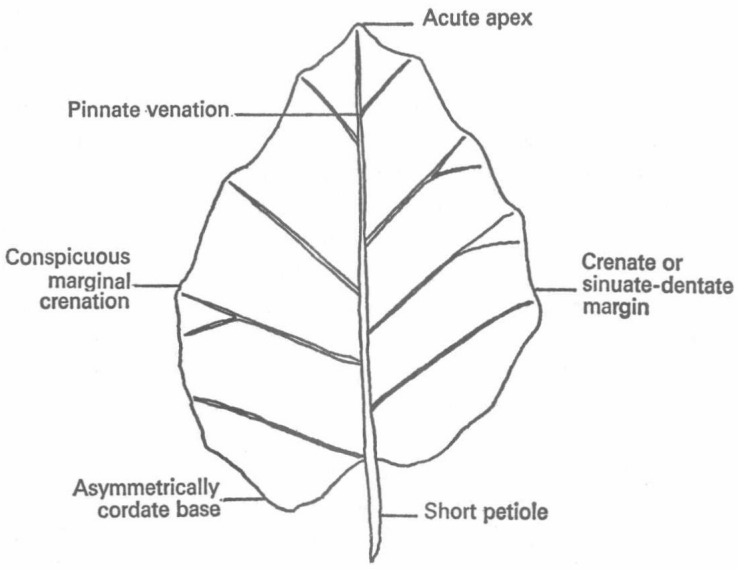
Fig. 2.5: Common descriptive terms for leaf apex and base.

Apex: The tip of the lamina. This may be symmetrical or asymmetrical and of a variety of shapes (*Fig. 2.5*A).

Base: The lower extremity of the lamina. It may be symmetrical or asymmetrical and of a variety of shapes (*Fig. 2.5*B).

The manner of presentation of the description is important and, whilst some variation is permissible, the example given in *Fig. 2.6* shows the type of presentation which is readily understood by a third party. *See also* the monographic presentation used in the E.P., B.P., and B.P.C., and the atlases of powdered drugs.

Fig. 2.6: Example of the description of the macromorphology of a whole leaf. (×3/5.)



Specimen: Commercial leaf of *Hamamelis virginiana*

Organoleptic evaluation

Dark-green to brownish-green, papery, little odour, astringent and bitter taste.

Macromorphology

Somewhat broken and compressed mass of leaves, 4–15 cm long by 3–10 cm wide, and shortly petiolate. Broadly oval or ovate in shape, with pinnate venation. Veins conspicuous on the lower surface. Margin crenate or sinuate-dentate. Lateral veins end at margin in conspicuous crenation. Apex acute or apparently obtuse if broken. Base asymmetrically cordate.

- Describe the surface characters and texture of fresh leaves of *Eucalyptus, Verbascum,* and *Digitalis.* Compare these with the characters of dried samples.
- Examine commercial samples of *Digitalis purpurea* and *D. lanata* leaves. Compare the morphology of these two species of *Digitalis* and prepare both a written description and a line drawing of both samples. Note the points of variation between them.
- Examine a commercial sample of senna leaflets. By reference to standard descriptions determine whether the sample is likely to be of the Alexandrian or Tinnevelly variety.

Description of Herbs

Herbs consist of the entire aerial parts of a plant, usually a young plant. Whilst they are composed primarily of leaves they also contain stem, flowers, and young fruits in smaller quantities. These other materials are usually readily detected in the whole material.

- Examine samples of belladonna, stramonium, and hyoscyamus herbs. Note the differences in leaf form and the marked variation in the flowering and fruiting structures.

Description of Underground Organs

This group includes roots and also underground structures, such as rhizomes and corms, derived from the stem. A rhizome is an organ which appears superficially similar to a root but has the

internal structural arrangement of a stem. Roots and rhizomes can be distinguished by the following criteria. Roots have no central pith, scale leaves, or axillary buds. They have a main tap root, or lateral roots, or their scars. Rhizomes have pith in the centre of the vascular tissue, may have scale leaves with axillary buds, and carry only small adventitious roots, or their scars. The root is developed from the radicle of the germinating seedling whilst the rhizome is formed in the more mature plant by the adaptation of tissues in the region where the root merges into the stem. The root system secures the plant in the soil, absorbs water and nutrients, and is the organ of perennation in herbaceous plants. The rhizome has a diminished absorptive function but it has large food reserves and takes over the rôle of perennation from the root. It also has a greater capacity for the formation of offshoots from the parent plant. This tends to result in a horizontal or oblique direction of growth in contrast to roots, which usually develop vertically. The direction of growth can be determined if the drug sample is held so that the finer roots, or their scars, are in such a position that their growth would have been vertically downwards. Note that in some cases the outer layers of a root or rhizome may be removed to give a ' peeled ' drug. A corm is a solid or swollen stem at, or just below, ground level in which reserve materials are stored. They are not particularly important as drug sources.

The description of an underground organ should include the form in which the drug occurs, e.g., as the root or rhizome (or both), in whole, peeled, or sliced form. The shape may be described by terms such as ' straight ', ' branching ', ' tortuous ', ' cylindrical ', or ' conical '. Surface characters, such as scale leaves, root scars, or lenticels, may be present. The fracture (transverse breakage) may be short, fibrous, splintery, or starchy (*see also* description of fracture of barks). Observation of a transverse surface by means of the naked eye or a hand lens will give information about the tissue arrangement within the structure. The significance of this is dealt with in more detail under Cytomorphology, Chapter 4.

■ **Compare the macromorphology of samples of the rhizomes of ginger, liquorice, and rhubarb, with that of samples of the roots of ipecacuanha. Note the variation within each sample of a given drug and the effects which the preparation and drying processes have had on the form. Prepare a complete description of the macromorphology of liquorice.**

Description of Barks

Barks are a group in which the macromorphology is of particular importance as the form is dependent upon the method of preparation of the drug. The commercial designation ' bark ' includes all those tissues external to the cambium in the mature, secondarily-thickened, stem. The shape of the pieces of bark depends on the type of incision made in removing them from the tree and on the nature and extent of any subsequent shrinkage. Where the bark is removed from large trees it is usually dried under pressure to produce ' flats '. More commonly the bark is removed from relatively small branches and it tends to curl on drying owing to unequal shrinkage of the various layers. Shrinkage takes place in the transverse direction as the main structural elements, such as fibres, run in the vertical direction. As the inner tissue is usually softer it is most frequently found on the concave surface. According to the extent of this curvature different characteristic shapes are assumed, and special terms are used to describe them (*Fig. 2.7*). When only slightly curved on the inside the pieces are described as ' curved ', or if the concavity is on the outside (as occurs in rare instances) ' recurved '. When the curvature on the inside is so great as to form a deep trough the piece is said to be ' channelled '. When still more curvature is present and one edge overlaps the other a ' quill ' is formed. If both edges roll independently