Medicinal plant raw materials of antiblastoma action

In the endeavour to discover effective drugs for the treatment of various cancerous diseases, the natural kingdoms, especially the plant kingdom, have been extensively researched. The research involved has been enormous and although the number of successful outcomes appears very modest, the effective drugs produced rank among the most common chemotherapeutic agents employed. Also, the wide diversity and complexity of the compounds isolated have afforded valuable material for the manufacture of semi-synthetic derivatives, often less toxic and clinically superior to the original isolate.

It has been estimated (2005) that over 60% of the anticancer drugs in current use are in some way derived from plants and microorganisms; marine products are in the process of evaluation. A successful anticancer drug should kill or incapacitate cancer cells without causing excessive damage to normal dividing cells. This ideal is difficult, or perhaps impossible, to attain and is why cancer patients frequently suffer unpleasant side-effects when undergoing treatment.

Plants in Cancer Treatment

Plant materials have been used in the treatment of malignant diseases for centuries; a comprehensive survey of the literature describing plants used against cancer listed over 1400 genera***. Recent phytochemical*** examination of plants which have a suitable history of use in folklore for the treatment of cancer has indeed often resulted in the isolation of principles with antitumour activity. Podophyllum was used over 2000 years ago by the ancient Chinese as an antitumour drug, and resins from the root of the plant *Podophyllum hexandrum (syn. P. emodi)* and the related American species, the May-apple (*P. peltatum) have* yielded a number of lignans and their glycosides having antitumour activity. Although the major constituents from these two species, podophyllotoxin and the peltatins, are unsuitable for systemic drug use, two semi-synthetic derivatives of podophyllotoxin, etoposide and teniposide, gave particularly good results in clinical trials.

Etoposide is currently available for the treatment of small-cell lung cancer and testicular cancer, and teniposide is used in paediatric cancers, though both compounds have a similar anticancer spectrum. Other podophyllotoxin-related analogues have been developed and tested. Podophyllotoxin itself may be used topically, and is most effective in the treatment of venereal warts.

From the time of Galen (about ad 180), the juice expressed from woody nightshade (*Solanum dulcamara)* has been used to treat cancers, tumours and warts, and references to its use have appeared in the literature of many countries. The active tumour-inhibitory principle has been identified as the steroidal alkaloid glycoside β-solamarine. Various lichens, e.g. species of *Cladonia, Cetraria and Usnea, also have a history of use in folk medicine* against cancer since about ad 970. These are all rich sources of usnic acid, a compound which has been recognized for many years as an antibacterial and antifungal agent, but only more recently as an antitumour compound.

Very successful higher plant materials used in cancer chemotherapy are the alkaloids of *Catharanthus roseus. Research on this plant, the* Madagascan periwinkle, was stimulated by its mention in folklore, not as a cure for cancer, but in the treatment of diabetes. No hypoglycaemic activity was detected, but treated test animals became susceptible to bacterial infection, and this led the researchers to undertake extensive examination for possible immunosuppressive principles causing these effects. A number of bisindole alkaloids showing antileukaemic activity have subsequently been isolated and two of these, vincaleukoblastine (vinblastine) and leurocristine (vincristine), are now extracted commercially from *Catharanthus roseus and used,* either alone, or in combination with other forms of therapy for cancer treatment.

Another important, more recent, addition to the list of anticancer drugs is paclitaxel (Taxol), a diterpene derivative isolated initially from the bark of the Pacific Yew, *Taxus brevifolia. Although* reportedly used by Native North Americans for various conditions.

The isolation of biologically active constituents, probably minor constituents, from a crude plant extract involves techniques differing from those of conventional phytochemical evaluation. With these, it was customary to study those chemicals which were most easily separated from a plant extract; these were usually those present in the largest quantities and which crystallized readily, or those which represented the researcher's field of interest, e.g. alkaloids, terpenoids, phenols, etc. Only after characterization of their structures were such compounds subjected to biological testing, e.g. for hypotensive, antibacterial, anticancer activities, etc., and this would depend on sufficient material being available. Countless medicinally useful compounds have been missed in this type of approach.

Promising chemicals are subsequently tested against a range of standard experimental neoplasms, and then considered for preclinical toxicological studies if these results are sufficiently encouraging. At this stage, relatively large amounts of material will be required, and larger-scale extractions and fractionation may be necessary.

Very few compounds will reach clinical trials. A low or very narrow therapeutic index (the ratio of maximum tolerated dose to minimum effective dose), undesirable side-effects or high toxicity can outweigh beneficial tumour-inhibitory activity. From some 25 000 screens conducted annually by the NCI (including both synthetic and natural materials), only eight to twelve compounds are likely to be selected for preclinical testing, and perhaps only six to eight go on to clinical trials. Slightly less than half of these may be plant-derived.

Plant tissue cultures might provide a reliable source. If total synthesis of the active chemical is feasible, this will always be the preferred option.

Plants Containing Anticancer Agents in Current Use

pink periwinkle (rose periwinkle) - *Catharanthus roseus*

pink periwinkle *leaves - Catharanthi rosei folia*

The Madagascan periwinkle, *Catharanthus roseus, has been variously* designated *Vinca rosea and Lochnera rosea (Apocynaceae). It is indigenous* to Madagascar but is now widely distributed throughout warm regions and is much cultivated as an ornamental; it grows profusely in southern Florida. Commercial supplies of the drug are obtained from both wild and cultivated plants produced in various locations, including Africa, India, Thailand, Taiwan, eastern Europe, Spain, USA and Australia.

Characters. ***C. roseus is a herbaceous subshrub, 40–80 cm high,*** becoming woody at the base. The leaves are oppositely arranged, oblong with a petiolate acute base, a rounded or mucronate apex and an entire margin. In form the flowers resemble those of the common periwinkle *Vinca major and are coloured violet, rose, white (var. albus)* or white with a red eye (var. *ocellatus). The fruit is a divergent follicle.*

Tetraploid plants are reported to have a more vigorous growth habit and larger flowers than diploid ones.

History. Although the plant has a certain reputation in folk medicine for the treatment of diabetes, modern investigators have been unable to confirm this property. Instead Canadian workers, during 1955–1960, discovered that extracts of the leaves produced leukopenic actions in rats. These observations led researchers at Eli Lilly to undertake an intensive phytochemical investigation of the plant with a view to the isolation of constituents of value in cancer chemotherapy. Six alkaloids proved active in this respect and two are now available commercially.

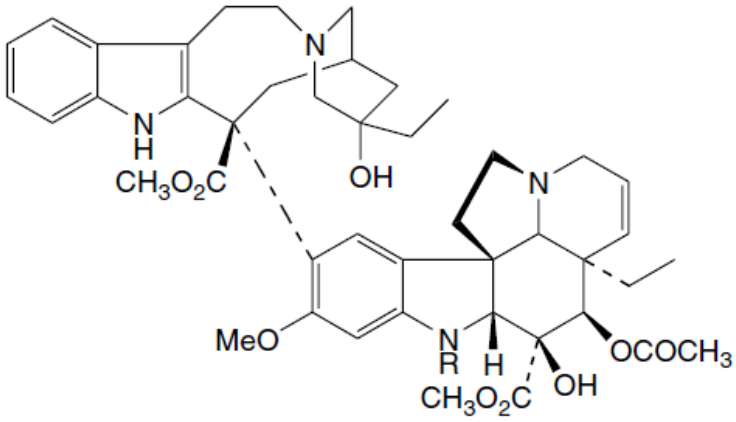
Catharanthus is an example of a drug plant which has been introduced into medicine during recent years, and it is used for the isolation of pure substances rather than for galenical preparation. Indeed, simple galenicals, prepared from the dried plant material and containing a wide spectrum of alkaloids, would be quite useless therapeutically. Hence, in normal circumstances, the raw material is handled by the manufacturer and does not reach the pharmacist as such.

Catharanthus roseus is an evergreen subshrub or herbaceous plant growing 1 m tall. The leaves are oval to oblong, 2.5–9 cm long and 1–3.5 cm wide, glossy green, hairless, with a pale midrib and a short petiole 1–1.8 cm long; they are arranged in opposite pairs. The flowers range from white with a yellow or red center to dark pink with a darker red center, with a basal tube 2.5–3 cm long and a corolla 2–5 cm diameter with five petal-like lobes. The fruit is a pair of follicles 2–4 cm long and 3 mm wide.

It is native and endemic to Madagascar, but is grown elsewhere as an ornamental and medicinal plant, and now has a pantropical distribution.

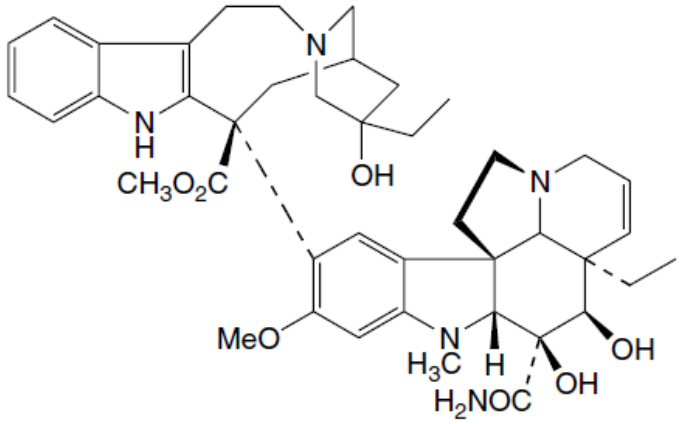
Constituents. About 150 alkaloids have now been isolated from *C. roseus; some, for example, ajmalicine, lochnerine, serpentine and* tetrahydroalstonine, occur in other genera of the family. Of particular interest is a group of about 20 bisindole alkaloids which contains those having antineoplastic activity, including leurocristine (vincristine) and vincaleukoblastine (vinblastine). Vinblastine is produced by coupling of the indole alkaloids catharanthine and vindoline, both of which occur free in the plant. Formation of 3′,4′-anhydrovinblastine from these monomers has been effected with peroxidase isozymes isolated from *C. roseus suspension cultures and with commercial horseradish peroxidase*

Vincristine is structurally similar to vinblastine, but has a formyl group rather than a methyl on the indole nitrogen in the vindoline-derived portion. Because these alkaloids are only minor constituents of the plant (vincristine is obtained in about 0.0002% yield from the crude drug), large quantities of raw material are required and chromatographic fractionations are extensively employed in the isolation procedures. In addition, there is a growing demand for vincristine rather than vinblastine, but the plant produces a much higher proportion of vinblastine. Fortunately, it is now possible to convert vinblastine into vincristine either chemically, or via a microbiological *N-demethylation using Streptomyces albogriseolus.*

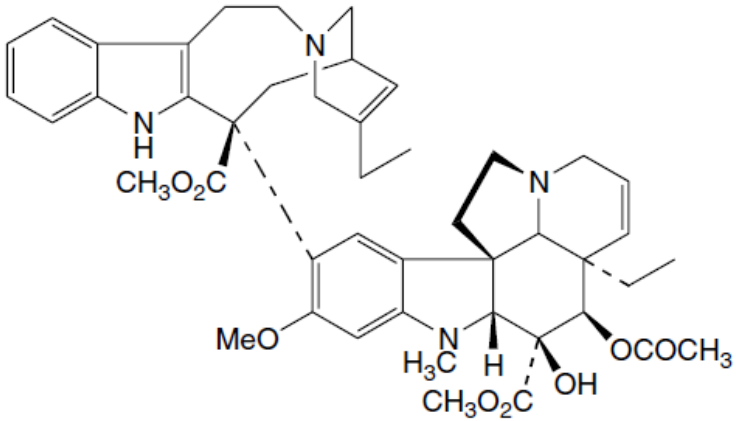


Vinblastine, R = CH_3

Vincristine, R = CHO



Vindesine



Vinorelbine

Uses. Vinblastine is used mainly for the treatment of generalized Hodgkin's disease, and non-Hodgkin's lymphomas. Vincristine is used principally in the treatment of acute lymphocytic leukaemia in children. It has other applications for lymphomas, small-cell lung cancer, cervical and breast cancers. The semi-synthetic vindesine is also used in the treatment of acute lymphoid leukaemia in children.

Vincristine has a superior antitumour activity compared to vinblastine, but is more neurotoxic. Vinorelbine is a newer, orally active, semi-synthetic anhydro derivative of 8'-norvinblastine with a broader anticancer activity and lower neurotoxic side-effects than the other *Catharanthus alkaloids.*

Podophyllum and Podophyllum Resin

May-apple rhizome with roots - *Podophylli rhizomata cum radicibus*
May-apple - *Podophyllum peltatum*

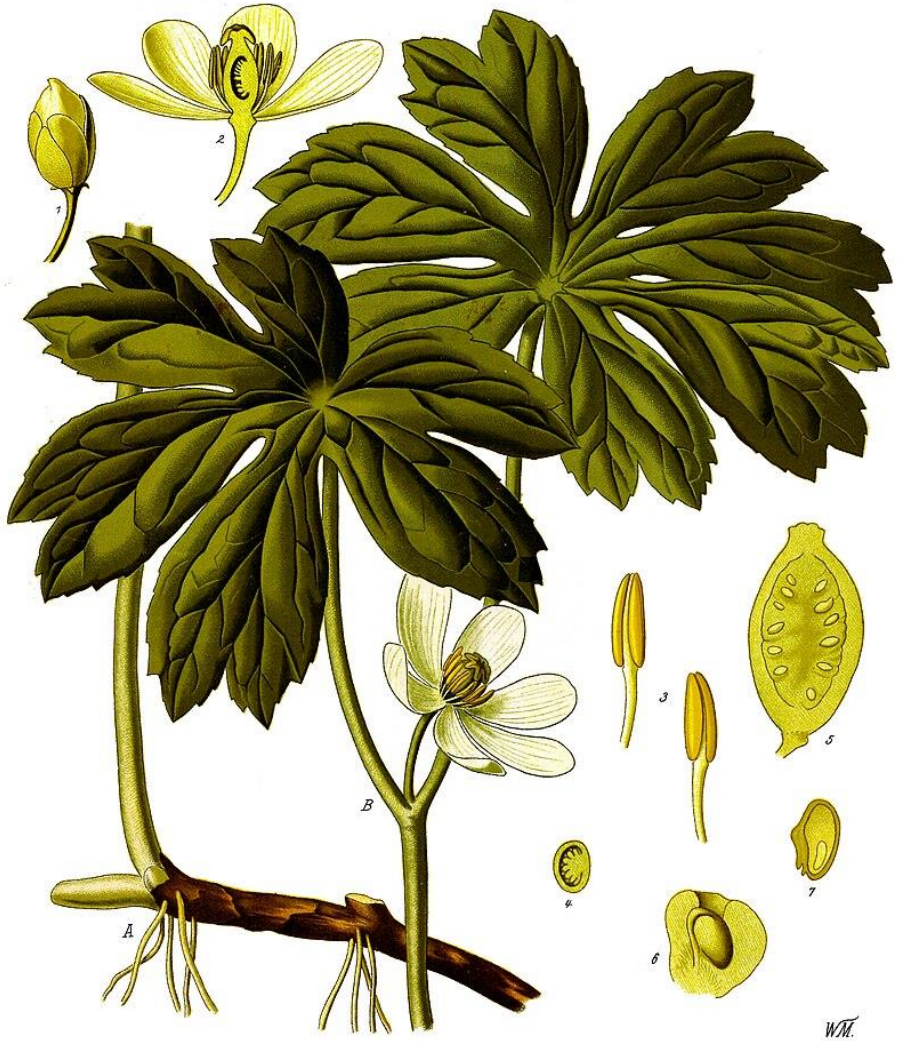
Podophyllum (Podophyllum Rhizome, May-apple Root, Wild Mandrake) consists of the dried rhizome and roots of *Podophyllum peltatum (Berberidaceae, sometimes Podophyllaceae), a perennial* herb common in moist shady situations in the eastern parts of Canada and the USA.

Though the common name is mayapple, in some areas it is the flower that appears in early May, not the "apple". The fruit or "apple" is usually produced early in summer and ripens later in summer.

History. The drug has long been used by the North American Indians as a vermifuge and emetic, and was introduced into the 1864 *Pharmacopoeia. Podophyllin, a crude resin obtained from the rhizomes* and roots, was subsequently employed as a purgative, but usage declined, until in 1942 podophyllin was recommended for the treatment of venereal warts. Since then, extensive research has led to an appreciation of podophyllum's antitumour properties, and the development of successful anticancer agents.

Podophyllum peltatum is an herbaceous perennial plant in the family Berberidaceae. The stems grow to 30–40 cm tall, with palmately lobed umbrella-like leaves up to 20–40 cm diameter with 3–9 shallowly to deeply cut lobes. The plants produce several stems from a creeping underground rhizome; some stems bear a single leaf and do not produce any flower or fruit, while flowering stems produce a pair or more leaves with 1–8 flowers in the axil between the apical leaves. The flowers are white, yellow or red, 2–6 cm diameter with 6–9 petals, and mature into a green, yellow or red fleshy fruit 2–5 cm long.

All the parts of the plant are poisonous, including the green fruit, but once the fruit has turned yellow, it can be safely eaten . The ripe fruit does not produce toxicity.



It is widespread across most of the eastern United States and southeastern Canada. They are also grown as ornamental plants for their attractive foliage and flowers, and they are a larval host for the golden borer moth and the may apple borer.

The unripe green fruit is toxic. The ripened yellow fruit is edible in small amounts, and sometimes made into jelly, though when consumed in large amounts the fruit is poisonous. The rhizome, foliage, and roots are also poisonous. Mayapple contains podophyllotoxin, which is highly toxic if consumed, but can be used as a topical medicine.

Macroscopical characters. Podophyllum occurs in subcylindrical reddish-brown pieces about 5–20 cm long and 5–6 mm thick. The outer surface is smooth (autumn rhizome) or wrinkled (summer rhizome). The nodes are enlarged to from two to three times the diameter of the internodes. On these swellings the remains of the aerial stems are visible

on the upper surface as large cup-shaped scars surrounded by the remains of the cataphyllary leaves, some of which have buds in their axils. On the lower side of each node are about 5–12 root scars or portions of roots. The latter, if entire, are 2–7 cm in length and about 1.5 mm in diameter. The drug breaks with a short fracture and shows a starchy or horny interior. The transverse section of the internode shows a starchy bark and pith and a ring of 20–30 small fibrovascular bundles. The latter are not radially elongated. A section of the node is similar but shows branches from the ring of bundles running upwards to the cup-shaped scar of the aerial stem or downwards to the roots. Odour, slight; taste, disagreeably bitter and acid.

Constituents. The active principles of podophyllum are contained in the resin, podophyllum resin or 'podophyllin', which is prepared by pouring an alcoholic extract of the drug into water and collecting and drying the precipitate. American podophyllum yields about 2–8% and Indian podophyllum (see below) about 6–12% of resin. Podophyllum Resin of the *USP was obtained solely from the American drug but that*

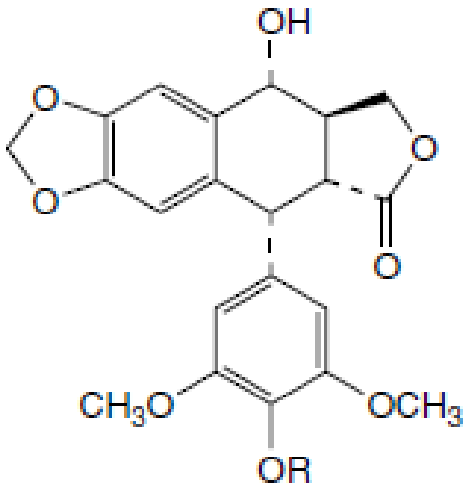
of the *BP may be either American or Indian, although the resins from* these two sources are not identical.

The chief constituents of the root belong to the group of lignans, which are C18 compounds derived biosynthetically by dimerization of two C6–C3 units (e.g. coniferyl alcohol) at the β-carbon of the sidechains. The most important ones present are podophyllotoxin (about 0.25%), β-peltatin (about 0.33%) and α-peltatin (about 0.25%). In the root, all of these occur both free and as glucosides.

Preparation of the resin results in considerable losses of the glucosides. The root also contains smaller amounts of the closely related 4'-demethylpodophyllotoxin and its glucoside, desoxypodophyllotoxin and podophyllotoxone. These compounds all possess cytotoxic or antitumour activity, but activity is lost on mild base treatment.

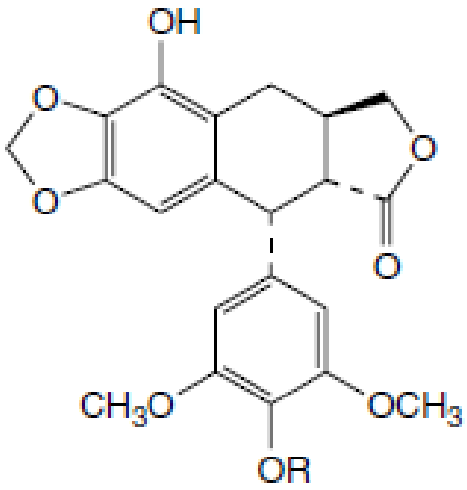
Epimerization, α to the carbonyl, results in the formation of the thermodynamically

more stable *cis-fused lactone ring, rather than* the severely strained *trans arrangement of the natural compounds.*



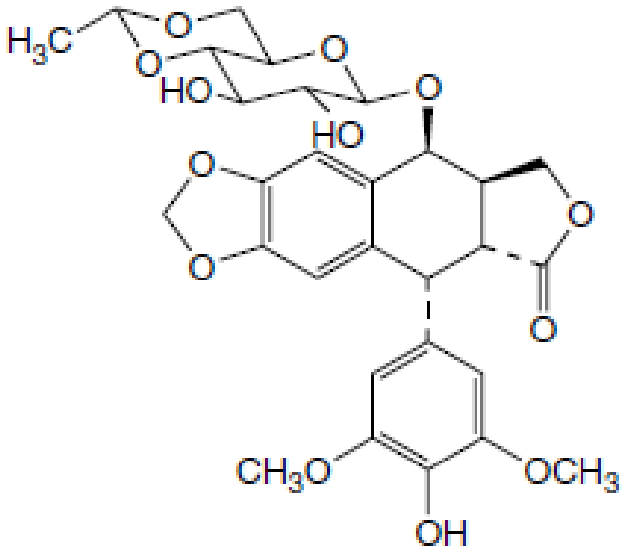
Podophyllotoxin, R = CH_3

Demethylpodophyllotoxin, R = H



β-Peltatin, R = CH_3

α-Peltatin, R = H



Etoposide

C. Canel *et al.* ***have suggested that to*** avoid destruction of the natural population of *P. pelatum by root collection* the harvested leaves of cultivated plants be utilized. The authors found that rehydration of the powdered dried leaves and subsequent organic solvent extraction, gave yields of 5.2% podophyllotoxin exceeding levels previously reported from any source. This increase in yield resulted from hydrolysis of lignan 4-*O*-*β*-*O*-*glucosides in situ* during the rehydration period.

Uses. Podophyllum resin has long been used as a purgative but has largely been replaced by less drastic drugs. It has a cytotoxic action and is used as a paint in the treatment of soft venereal and other warts.

Podophyllotoxin is also used for this purpose. Etoposide (4'-emethylepipodophyllotoxin ethylideneglucoside) is a lignan derivative obtained semi-synthetically from podophyllotoxin and used in the treatment of small-cell lung cancer and testicular cancer as well as lymphomas and leukaemias. The water-soluble pro-drug etopophos (etoposide 4'-phosphate) is also available. The related thenylidene derivative teniposide has similar anticancer properties and though not as widely used as etoposide has value in paediatric neuroblastoma, lymphocytic leukaemia, and brain tumours in children.

Beautiful meadow saffron - *Colchicum speciosum Stev.*
Beautiful meadow saffron bulbotuber fresh - *Colchici bulbotubera recentia*

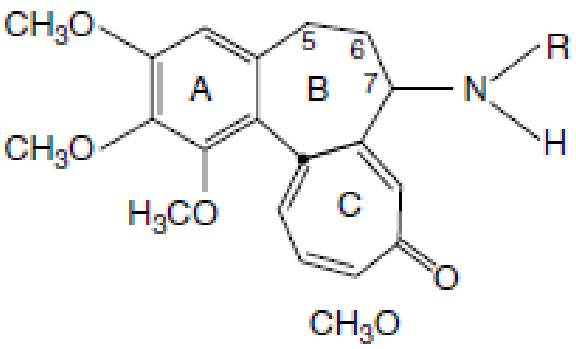
Colchicum speciosum is a species of flowering plant in the family *Colchicaceae*

The corm consists of an enlarged underground stem bearing foliage leaves, sheathing leaves and fibrous roots. If the plants are examined in the latter part of the summer, it will be found that a new corm is developing in the axil of a scale leaf near the base of the old corm, the new plant occupying an infolding in the side of the parent corm. In September the parent corm bears the remains of recently withered leaves and is very much larger than the daughter corm. For medicinal purposes the corm would have been collected shortly after the withering of the leaves (‘early summer’) and before the enlargement of its axial bud. The corms are surrounded by a dark, membranous coat. The young corm develops fibrous roots at its base, and in August or September two to six flowers emerge from it, but its foliage leaves do not appear above ground until the following spring. The flowers are 10–12 cm long. Each has six stamens and a perianth consisting of six lilac or pale-purple segments which fuse into an exceptionally long perianth tube, at the base of which lies the superior ovary. More than half the length of the flower is below ground, and the fruit lies protected throughout the winter by the surrounding corm and earth. of plants may be increased.

The fruit is a three-lobed, three-celled, septicidal capsule, which is carried above ground in the spring by the expanding leaves. The fully grown leaves are radical, linear-lanceolate and about 12 cm long. Duringthese changes the daughter corm grows at the expense of the parent, which now gradually perishes. Before doing so, however, it may produce in its second spring one or more small corms by means of which the number of plants may be increased.

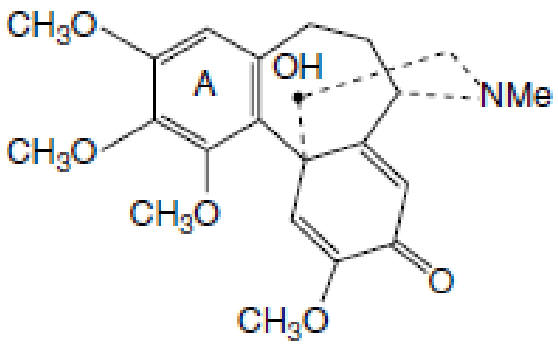
It is native to mountainous areas of northern Turkey, the Caucasus and northern Iran.

On treating the drug with 60–70% sulphuric acid or with concentrated hydrochloric acid, a yellow colour, due to colchicine, is produced.
The corms contain up to about 0.6% colchicine, other related alkaloids and starch.



R = CH_3; Demecolcine
R = $COCH_3$; Colchicine

Ring cleavage
and expansion
(mechanism not indicated)



Dienone related to methyl androcymbine

Colchicine

This is an amorphous, yellowish-white alkaloid, which darkens on exposure to light and gives a yellow coloration with strong mineral acids. Colchicine readily dissolves in water, alcohol or chloroform but is only slightly soluble in ether or petroleum spirit. It is a weak base and may be extracted from either acid or alkaline solution by means of chloroform.

Uses. Colchicum preparations are used to relieve gout, but must be employed with caution. Colchicine is frequently prescribed in tablet form and transdermal preparations containing colchicine are the subject of a Japanese patent (1991). The alkaloid is also used in biological experiments to produce polyploidy or multiplication of the chromosomes in a cell nucleus.
Common side effects of colchicine include gastrointestinal upset, particularly at high doses. Severe side effects may include pancytopenia (low blood cell counts) and rhabdomyolysis, and the medication can be deadly in overdose. Whether colchicine is safe for use during pregnancy is unclear, but its use during breastfeeding appears to be safe.

With overdoses, colchicine becomes toxic as an extension of its cellular mechanism of action via binding to tubulin. Cells so affected undergo impaired protein assembly with reduced endocytosis, exocytosis, cellular motility, and interrupted function of heart cells, culminating in multiple organ failure.