CHAPTER 5

# Sources of the Drug

## 5.1: Scope of Study

In this chapter we shall discuss the pharmacognosy of *Colchicum* and other plants that produce colchicine. Origins, geography, history, commerce, cultivation, preparation, and applications to biology are explained in greater detail for *Colchicum* than is usual in standard works for pharmacists.

The Greek words *pharmakon,* meaning *drug* or *medicine,* and *gnosis,* a knowing, are combined to form the term *pharmacognosy.* Literally, the meaning is a knowledge of drugs. This word is not so old as the study of drugs since it was introduced in 1815 by Seydler through his work, *Analecta Pharmacognostica.* A much older name for this subject is *materia medica,* and while this is still preferred in medicine to *pharmacognosy,* pharmacists prefer the latter word. The two are not entirely synonymous, for the newer term has a more limited meaning. Biologics, such as vaccines, sera, and similar compounds, do not fall within the scope of pharmacognosy but are a part of materia medica. On the other hand, compounds such as waxes, gums, oils, resins, spices, and fibers are included with drugs.

There was much discussion in centuries past as to whether *Colchicum* should be an official drug in the standard formularies of various nations. At certain times *Colchicum* was made official, then dropped, only to be taken up again in a later issue of the formulary. Its extremely poisonous nature and the lack of proper methods to assay the drug caused much of the trouble. It was realized that *Colchicum* was a good cure for gout. Medical men also realized the danger associated with administering the drug. The expressions *official* or *nonofficial, acceptance* or *rejection,* are based on the inclusion of a drug in standard pharmacopeias of a particular government. The drug may be official for one country and not another. Today, the standardization of colchicine is accurate, and the drug is official in every national work on pharmacy.[80] Because of its availability, *Colchicum luteum*

is permitted as a substitute for *C. autumnale* in India.[11] The standards of the *British Pharmacopoeia* do not permit the use of *C. luteum,* because the amount of colchicine in raw material is not high enough.

5.1–1: *Geographical distribution.* Figure 5.1 gives the location of the important species of the genus *Colchicum,* outlining the main areas where species are native. Taxonomists recognize 65 species in this genus,[68] but during the earlier centuries all autumn-flowering species were grouped in the *C. autumnale* type. Actually, the official species is distributed over Europe; line 55 outlines this area on the map (Fig. 5.1). The majority of species described on the map flower in the fall and produce seed in the spring. Another species known to antiquity is *C. variegatum,* number 61. The distribution of *C. luteum,* number 1, is the easternmost representative. All are limited to the Northern Hemisphere and none are reported in the Americas.

## 5.2: Problems in Pharmacognosy

Maintaining quality, protecting the consumer, preventing fraud, and regulating traffic become the responsibility of trained pharmacognosists.[16, 19] During earlier centuries, physicians had to use *Colchicum* according to their judgment. At times this duty was a heavy responsibility (cf. Chapter 1). Even today the problem is not completely solved, for it has been discovered that U.S.P. colchicine may contain another compound, desmethylcolchicine.[24] The substance has biological activity; therefore, purification of so-called pure colchicine is recommended if carefully controlled experiments are to be undertaken.

The preparation of the drug from the fresh state before drying, or through processes of drying, must be correct in order to avoid changes in these complex compounds. Colchicine in solution must not be exposed to sunlight. Slicing, washing, and exposure to insects or bacteria can also introduce changes.

Four principal techniques are used to evaluate drugs. These are (1) organoleptic, (2) microscopic and microchemical, (3) physicochemical, and (4) biological methods. Each particular test is described in the formularies or standard works on assay of drugs. Many of the methods have been applied to colchicine.

## 5.3: Plants Containing Colchicine

One species is famous in every pharmacist's handbook for the production of colchicine. There are many other species that have a capacity for synthesizing the compound in parts of plants. All species of the genus *Colchicum* analyzed to date yield colchicine.[3, 27, 70] An extensive list of them has been collected (Table 5.1). Two genera, *Merendera* and *Colchicum,* have been used interchangeably. Species

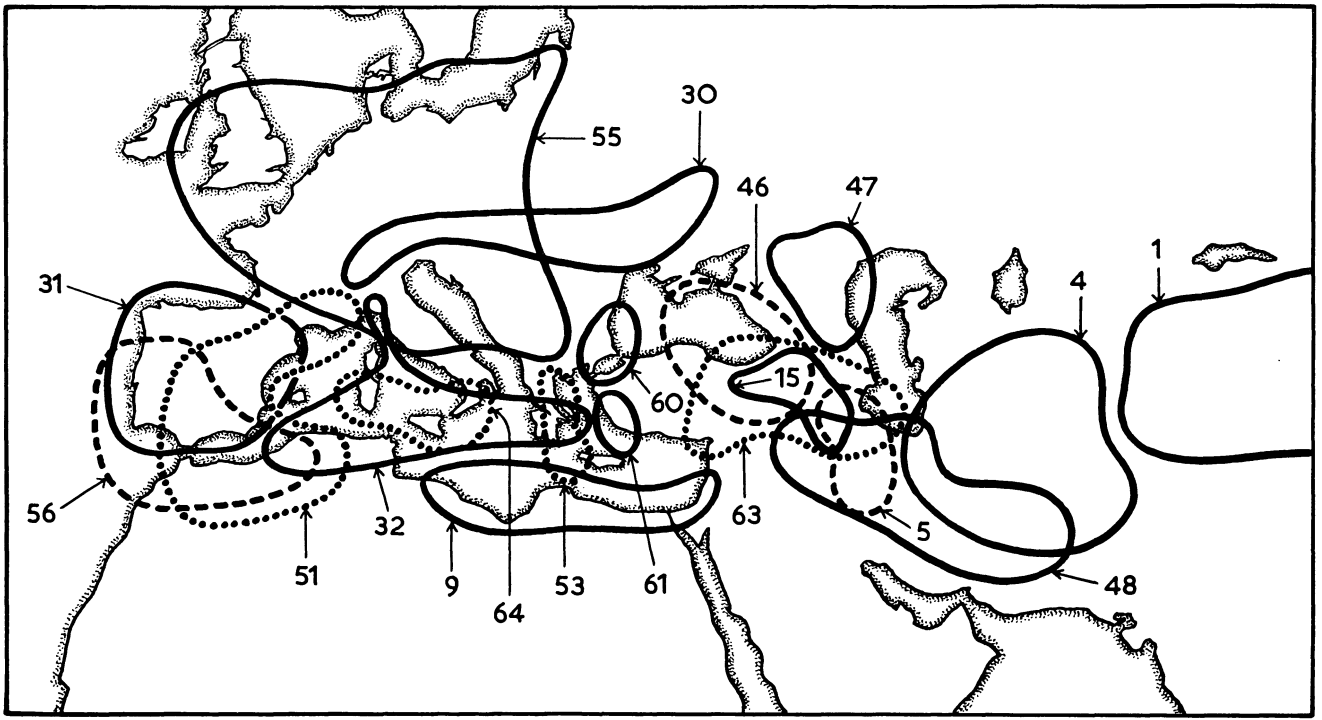


Fig. 5.1—The geographical distribution of important species of **Colchicum.** Those numbered between 1 and 15 are spring flowering and all others are autumnal flowering types. The following are included on the map:

1—**C. luteum**
4—**C. robustum** Stef.
5—**C. szovitsii**
9—**C. ritchii**
15—**C. nivale**
30—**C. vernum**
31—**C. montanum**
32—**C. cupani**
46—**C. umbrosum**
47—**C. laetum**
48—**C. kotschyi**
51—**C. longifolium**
53—**C. lingulatum**
55—**C. autumnale**
56—**C. lusitanum**
60—**C. turcicum**
61—**C. variegatum**
63—**C. speciosum**
64—**C. bivonae**
(After Stefanoff)

of each are found in the northwestern Himalayan area. Both drugs are on sale in the bazaars of the Orient.[17]

Isolated substances from *Colchicum autumnale* and related species have been studied extensively by Professor F. Santavy and his colleagues at the Medical-Chemical Institute of the Polacky University of Olomouc, Czechoslovakia. An up-to-date summary was prepared by Professor Santavy exclusively for this book. Accordingly Tables 5.2 and 5.3 combine the significant details from their numerous published and unpublished works.

The chemical structure of substance F as listed has been determined as desacetyl-N-methyl-colchicine, and differs from colchicine by the loss of the carboxy-group attached to the nitrogen ring as can be seen in the structural diagrams of Chapter 6. Since this compound F has strong c-mitotic properties and is less toxic than the parent alkaloid when used with animals, the further examination of related substances would appear to be worth considerable exploration. A compound "Demecolcin," marketed by Ciba of Basel, Switzerland, has been studied extensively and a preliminary survey shows useful applications to some types of malignant growth. These data are found in references to papers by Bock and Gross (1954), Meier, Schar, and

TABLE 5.1
PRINCIPAL PLANT SOURCES OF COLCHICINE

| | |
|---|---|
| *Colchicum autumnale* L. | *Tofieldia glacialis* Gaud. |
| *C. montanum* L. | *T. calyculata* Whlnd. |
| C. *arenarium* Waldst. and K. | *Veratrum album* L. |
| *C. neapolitanum* Ten. | *V. nigrum* L. |
| *C. alpinum* DC. | *Anthericum ramosum* L. |
| *C. luteum* Baker | *Hemerocallis fulva* L. |
| *C. multiflorum* Brot. | *Ornithogalum umbellatum* L. |
| *Merendera bulbocodium* Ram. | *O. comosum* L. |
| *M. caucasica* Biel. | *Tulipa silvestris* L. |
| *M. persica* Bois and Kotsch. | *Asphodelus albus* Willd. |
| *Gloriosa superba* L. | *Fritillaria montana* Hoppe. |
| *Merendera sobolifera* Fisch. | *Lloydia serotina* Salib. |
| *Bulbocodium ruthenicum* Bung. | *Muscari tenuiflorium* Tausch |

TABLE 5.2

SURVEY OF PLANTS EXAMINED FOR THE PRESENCE OF COLCHICINE AND RELATED SUBSTANCES
SUMMARIZED BY DR. F. SANTAVY

| Plant | Starting Material and References | Substances Isolated or Otherwise Identified | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Substances of Neutral and Phenolic Properties | | | | | | | Substances of Basic Properties | | | | Substances of Glucosidic Properties | | |
| | | Colchicine | B | C | D | $E_1$ | I | Colchiceine | F | S | U | Ta | Colchicoside | To | M |
| *Colchicum autumnale* L. | seeds [61,63,84,85,88,100,101,106] | + | + | + | .. | .. | + | .. | + | + | + | .. | + | .. | .. |
| | bulbs[61,83,89,93,96] | + | + | + | + | + | + | + | + | + | + | + | .. | + | .. |
| | flowers[51,61,99] | + | + | .. | + | + | + | + | + | + | + | .. | .. | .. | + |
| | leaves, pericarp[24,33,90,98] | + | + | .. | .. | + | .. | .. | + | .. | .. | .. | .. | .. | .. |
| *C. speciosum* Stev. | bulbs[3,64,70,82,86,87] | + | + | + | .. | .. | .. | .. | + | + | .. | .. | .. | .. | .. |
| | flowers[87,90] | + | + | .. | .. | + | .. | .. | + | + | .. | .. | .. | .. | .. |
| *C. arenarium* W.K. | bulbs[64,92] | + | .. | .. | .. | .. | .. | .. | + | .. | .. | .. | .. | .. | .. |
| *C. cilicum* Hayek | bulbs[64] | + | .. | .. | .. | .. | .. | .. | + | .. | .. | .. | .. | .. | .. |
| *C. variegatum* L. | bulbs[90] | + | .. | + | .. | .. | .. | .. | + | .. | .. | .. | .. | .. | .. |
| *C. vernum* Ker-Gawl | bulbs[33,95] | + | .. | .. | .. | .. | + | .. | .. | .. | .. | .. | .. | .. | .. |
| *C. agrippinum* Baker | bulbs[95] | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| *C. neapolitanum* Tenore | bulbs[92] | +(?) | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| *C. montanum* L. | bulbs[92] | +(?) | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| *C. alpinum* D.C. | bulbs[92] | +(?) | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| *C. multiflorum* | bulbs[92] | +(?) | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| *C. luteum* Baker | bulbs[90] | + | .. | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| *C. kesselringii* | leaves[38] | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| *C. hierosolymitanum* Feinbr. | bulbs[90,105] | + | .. | + | .. | .. | .. | .. | + | .. | .. | .. | .. | .. | .. |
| *C. ruthenicum* | leaves[33] | +(?) | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| *C. bornmuelleri* | bulbs[64] | + | .. | + | .. | .. | .. | .. | + | .. | .. | .. | .. | .. | .. |
| *C. autumnale album* | bulbs[64] | + | .. | .. | .. | .. | .. | .. | + | .. | .. | .. | .. | .. | .. |
| *C. autumnale major* | bulbs[64] | + | .. | + | .. | .. | .. | .. | + | .. | .. | .. | .. | .. | .. |
| *C. autumnale minor* | bulbs[64] | + | .. | .. | .. | .. | .. | .. | + | .. | .. | .. | .. | .. | .. |

TABLE 5.2 (*continued*)

| Plant | Starting Material and References | Substances Isolated or Otherwise Identified: Substances of Neutral and Phenolic Properties: Colchicine | B | C | D | $E_1$ | I | Colchiceine | Substances of Basic Properties: F | S | U | Ta | Substances of Glucosidic Properties: Colchicoside | To | M |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. autumnale* var. Lilac Wonder | bulbs[64] | + | .. | .. | .. | .. | .. | .. | + | .. | .. | .. | .. | .. | .. |
| *C. autumnale* var. The Giant | bulbs[64] | + | .. | .. | .. | .. | .. | .. | + | .. | .. | .. | .. | .. | .. |
| *C. autumnale* var. Violet Queen | bulbs[64] | + | .. | .. | .. | .. | .. | .. | + | .. | .. | .. | .. | .. | .. |
| *C. autumnale flore pleno* | bulbs[90] | + | + | + | .. | .. | .. | .. | + | .. | .. | .. | .. | .. | .. |
| *Merendera sobolifera* C.A.M | bulbs[90,95] | + | + | + | .. | .. | + | .. | + | .. | .. | .. | .. | .. | .. |
| *M. attica* Boiss et Sprun | bulbs[95] | + | .. | .. | .. | .. | + | .. | .. | .. | .. | .. | .. | .. | .. |
| *M. bulbocodium* Ram | bulbs[17] | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| *M. robusta* | leaves[38] | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| *M. caucasica* Spreng | bulbs[90] | + | + | + | .. | .. | .. | .. | + | .. | .. | .. | .. | .. | .. |
| *Androcymbium gramineum* McBridge | bulbs[56,90] | + | .. | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| | flowers, leaves[90] | + | .. | .. | .. | + | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| *Gloriosa superba* L. | bulbs[12,94,103,104] | + | + | + | .. | .. | + | .. | .. | .. | .. | .. | .. | .. | .. |
| *G. rothschildiana* O'Brien | bulbs[6,94] | + | + | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| *G. simplex* | bulbs[94] | + | + | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| *Tulipa silvestris* L. | bulbs[33,95] | − | These plants do not contain other substances with a tropolone ring. | | | | | | | | | | | | |
| *Hemerocallis fulva* | bulbs[33,64] | − | | | | | | | | | | | | | |
| *Ornithogallum caudatum* Ait. | bulbs[33,90] | − | | | | | | | | | | | | | |

NOTE: Due to the lack of starting material, a number of plants could not be analyzed by us for the presence of all the substances identified in meadow saffron.

SUBSTANCES ISOLATED FROM MEADOW SAFFRON (*Colchicum autumnale* L.) AND THEIR MOST IMPORTANT PHYSICAL AND CHEMICAL PROPERTIES SUMMARIZED BY DR. F. SANTAVY

| Isolated Compounds | Formula | M. P. | $[\alpha]_D$ in $CHCl_3$ | Reaction With Concentrated $H_2SO_4$ | Tropolone Ring | Number of Methoxyls | Number of Phenol Groups | Number of Acetyl Groups | Sugar Residue | Acetyl Derivative: Formula | Acetyl Derivative: M.P. | Acetyl Derivative: $[\alpha]_D$ in $CHCl_3$ | Starting Material and References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Neutral and Phenolic Compounds** | | | | | | | | | | | | | |
| Colchicine | $C_{22}H_{25}O_6N$ | 157° | −121° | yellow | + | 4 | ..... | 1 | ... | ..... | ..... | ..... | whole plant[61,63,93,96–101] |
| B | $C_{21}H_{23}O_6N$ | 266° | −171° | yellow | + | 4 | ..... | 1* | ... | ..... | ..... | ..... | whole plant[61,93,96–101] |
| C | $C_{21}H_{23}O_6N$ | 180° | −132° | yellow | + | 3 | 1 | 1 | ... | $C_{23}H_{25}O_7N$ | 231° | −92° | seeds[24,61,100,101] bulbs[61,93,96] |
| D | $C_{21}H_{23}O_6N$ | 236° | +294° | red-violet | ... | 3 | 1 | 1 | ... | $C_{23}H_{25}O_7N$ | 232° | +283° | whole plant[61,93,96,97,99–101] |
| $E_1$ | $C_{21}H_{23}O_6N$ | 180° | −130° | yellow | + | 3 | 1 | 1 | ... | $C_{23}H_{25}O_7N$ | 194° | −125° | flowers[61,99] leaves[98] pericarp[61,98] |
| I | $C_{22}H_{25}O_6N$ | 186° | +307° | orange | ... | 4 | ..... | 1 | ... | ..... | ..... | ..... | whole plant[61,93,96,97,99–101] |
| J | $C_{22}H_{25}O_6N$ | 278° | −445° | orange | ... | 4 | ..... | 1 | ... | ..... | ..... | ..... | whole plant[61,93,96,97,99–101] |
| Ka | ? | 214° | −140° | yellow | + | ..... | ..... | ..... | ... | ..... | ..... | ..... | seeds[101] |
| N | ? | ..... | ..... | yellow | + | ..... | ..... | ..... | ... | ..... | 229° | −110° | flowers[99] |
| O | $C_{22}H_{25}O_6N$(?) | 256° | −143° | colorless | ... | 4 | ..... | 1 | ... | ..... | ..... | ..... | flowers[99] |
| P | ? | 229° | −226° | orange | ... | 4 | ..... | 1 | ... | ..... | ..... | ..... | bulbs[96] |
| R | ? | 198° | ..... | yellow | + | ..... | ..... | ..... | ... | ..... | ..... | ..... | bulbs[96] |
| Colchiceine | $C_{21}H_{23}O_6N$ | 177° | −256° | yellow | + | 3 | 1 | 1 | ... | $C_{23}H_{25}O_7N$ | 124° | −260° | flowers,[99] bulbs[96] |
| **Basic Compounds** | | | | | | | | | | | | | |
| F (Demecolcin) | $C_{21}H_{25}O_5N$ | 186° | −127° | yellow | + | 4 | ..... | ..... | ... | $C_{23}H_{27}O_6N$ | 231° | −240° | whole plant[61,63,93,96,97,99–101] |
| H 3 | ? | 185° | ..... | yellow | + | ..... | ..... | ..... | ... | ..... | ..... | ..... | bulbs[96] |
| S | $C_{20}H_{23}O_5N$ | 138° | −117° | yellow | + | 3 | ..... | ..... | ... | $C_{22}H_{25}O_6N$ | 202° | −218° | whole plant[63,96,98,99,101] |
| U | $C_{19}H_{21}O_5N$ | ..... | ..... | yellow | + | 3 | 1 | ..... | ... | $C_{23}H_{25}O_7N$ | 229° | −93° | whole plant[96,99,101] |
| Ta | $C_{21}H_{25}O_5N$(?) | 135° | −211° | yellow | + | 3 | ..... | ..... | ... | ..... | ..... | ..... | bulbs[96] |

TABLE 5.3 (*continued*)

| Isolated Compounds | Formula | M. P. | $[\alpha]_D$ in $CHCl_3$ | Reaction With Concentrated $H_2SO_4$ | Tropolone Ring | Number of Methoxyls | Number of Phenol Groups | Number of Acetyl Groups | Sugar Residue | Acetyl Derivative: Formula | Acetyl Derivative: M.P. | Acetyl Derivative: $[\alpha]_D$ in $CHCl_3$ | Starting Material and References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Glucosidic Compounds** | | | | | | | | | | | | | |
| Colchicoside | $C_{27}H_{33}O_{11}N$ | 218° | −360° (water) | yellow | + | 3 | .... | 1 | 1 | $C_{35}H_{41}O_{15}N$ | 177° | − 57° | seeds[83] |
| M | $C_{27}H_{33}O_{11}N$ | 314° | ..... | red-violet | ... | 3 | ..... | 1 | 1 | $C_{35}H_{41}O_{15}N$ | 304° | −244° | flowers[99] |
| To | $C_{26-27}H_{31-33}O_9N$(?) | 234° | − 65° (pyridine) | yellow | + | 3 | ..... | 1 | 1 | ..... | ..... | ..... | bulbs[96] |
| **Other Compounds** | | | | | | | | | | | | | |
| Apigenin | $C_{15}H_{10}O_5$ | 350° | ..... | ..... | .. | ... | .... | ..... | ... | $C_{21}H_{16}O_6$ | 185° | ..... | flowers,[99] bulbs [96] |
| Phytosterol-mixture | ..... | 136° | − 36° | ..... | .. | ... | .... | ..... | ... | ..... | ..... | ..... | whole plant[61,93,96,97,99–101] |
| Benzoic acid | $C_6H_6O_2$ | 122° | ..... | ..... | .. | ... | .... | ..... | ... | ..... | ..... | ..... | whole plant[61,93,96,97,99–101] |
| Salicylic acid | $C_6H_6O_3$ | 156° | ..... | ..... | .. | ... | .... | ..... | ... | ..... | ..... | ..... | |
| 2-Oxy-6-methoxy-benzoic acid | $C_8H_8O_4$ | 133° | ..... | ..... | .. | ... | .... | ..... | ... | ..... | ..... | ..... | |
| Fatty acid-mixtures | ..... | .... | ..... | ..... | .. | ... | .... | ..... | ... | ..... | ..... | ..... | seeds[18] |
| Saccharose | $C_{12}H_{22}O_{11}$ | 187° | + 67° (water) | ..... | .. | ... | .... | ..... | ... | ..... | ..... | ..... | bulbs, seeds[61,93,96,97,99–101] |
| Asparagine | ..... | .... | ..... | ..... | ... | ... | ..... | ..... | ... | ..... | ..... | ..... | bulbs[102] |

* Formyl

Neipp (1954), Moeschlin, Meyer, and Lichtman (1954), and Santavy, Winkler, and Reichtstein (1953).*

Probably the best method of detecting colchicine is the polarographic technique used to great advantage by Santavy and his colleagues.[61] By these newer methods, other compounds have been identified in the seed, corm, and flowers. A section is devoted to this problem.

*5.3–1: Colchicum autumnale* L. We mentioned earlier the unusual character of this autumn-flowering crocus. Not many plants bloom in the fall and mature seeds the following spring. Since the flowering and fruiting cycle is directly correlated with development of corm and seed, and since colchicine production is related to these processes, knowledge of development is important. The content of colchicine will vary from season to season, and with different environmental conditions. Seeds are a rich source of colchicine after maturation. The corms reach a peak of colchicine about June or July. A vast amount of information has been reported over a period of 20 centuries, yet it is surprising to learn how few textbooks bring together a complete report on comparative morphology, anatomy, and physiology in relation to drug production. More than passing attention will be given to such details in this chapter.[34]

The corm has two coverings when dug in early summer, the outer brown membranous and an inner reddish-yellow layer. Beneath these coats lies a yellow body that composes the bulk of the corm and most of the tissues that yield colchicine. The corm is conical, somewhat rounded on the surface, and flattened on one side. At the base of the flattened area a smaller corm, or bud, fits into a groove or depression. When this young bud begins development, the larger, parental corm usually carries the maximum colchicine per dry weight of body.

A bud develops in July, and during August or September stalks of flowers appear. Floral activity is the first index that the young corm has been active. Violet and reddish flowers in a cluster ranging from two to six break through the membranes of the corm just described and appear above ground. Corms that are not placed in the

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* H. Bock and R. Gross, "Leukämie und Tumorbehandlung mit einem Nebenalcaloid aus *Colchicum autumnale* (Demecolcin)." *Acta Haematol.* 11:280–300. 1954.

R. Meier, B. Schar, and L. Neipp, "Die Wirkung von Demecolceinamiden an Zellen *in vitro*." Experientia. 10:74–76. 1954.

S. Moeschlin, H. Meyer, and A. Lichtman, "Ein neues Colchicum-Nebenalcaloid (Demecolcin Ciba) als cytostaticum myeloischer Leukämien." Schweiz. Med. Wschr. 83:990. 1953.

F. Santavy, R. Winkler, and T. Reichstein, "Zur Konstitution von Demecolcin (Substance F) aus *Colchicum autumnale* L." Helvetica Chim. Acta. 36:1319–24. 1953.

soil develop flowers when the time is right. They do so without attention as to water or nutrition. For this reason unusual attention is given to the corm for ornamental purposes.

Each flower measures 10 to 20 cm. from base to tip of petal. The six stamens and six floral parts are united in a tube from the top to the carpels below. Three carpels of an ovulary show the relation to the liliaceous group. At the base of the long tube is the superior, syncarpous ovulary. Regularly, the corm is deep enough in the soil so that about one-half of the flower is above the surface; thus, the ovulary is well beneath the soil surface. Following fertilization, the ovules begin a development that proceeds during the entire winter.[7] A progression of development and colchicine content was noted over the long period of time that elapses from fertilization to maturation. Pollination development begins soon after, but the content of colchicine is low. There is not much increase during the early stages. In other words, the increase in the winter is very small compared to the gain that occurs in content of colchicine as seeds mature. The total time studied extended from August of one year to April of the next.[7, 8, 62]

In early spring the fruit capsule rises out of the soil. Expanding leaves accompany the fruit development. In the vicinity of Olomouc, Czechoslovakia, the green capsules contain small, watery ovules until about the middle of May. From May to July the content of colchicine increases from 0.2 to 0.5 per cent. As capsules mature, the walls split and seeds fall out.[7]

5.3–2: *Colchicum luteum* Baker. Because of its availability in India, the Indian pharmacopeia accepts this spring-flowering species as a source for colchicine.[11, 45, 54]

The product called colchicine is Surinjan-i-talkh. Undoubtedly this drug has been used for many years, certainly before the present studies of pharmacognosy were conceived in their present level. Collection of the corm for colchicine must be coordinated with the flowering and fruiting cycles. Each corm is inclosed in membranous layers, under which lies a hard, white bud. The daughter corm that produces the next season's plant is found in a groove at the base of the parent corm.

At altitudes of 7000 ft., the buds develop early in March or late February. Flowers appear when the snow melts; the plant is one of the earliest to flower in the area. The common name for the species is *Kashmir hermodactyl.*

A scape bearing golden flowers, two or three per cluster, emerges from the corm. Fruiting stalks develop soon after pollination. The capsules mature, and leaves form. Finally the seeds mature, and a cycle is thus completed within one season, from March to May.

*5.3–3: Other sources for colchicine.* Numerous sources of colchicine exist in nature (Table 5.1), and undoubtedly more will be discovered. A notable case is *Gloriosa superba* producing 0.3 per cent colchicine compared with 0.5 per cent for *C. autumnale*. The unusual demand for colchicine made by plant breeders should stimulate search for other sources.[54] These are the problems that pharmacognosists are surveying, particularly in areas where plants have not been thoroughly studied.

When colchicine is extracted from *Colchicum*, other compounds appear in the residue, some of which have proved to be valuable. New products of biological interest might well be revealed through examination of the species that yield colchicine. By new methods of analysis a large amount of important work has been done in recent years with compounds of colchicine and its derivatives.[61]

## 5.4: Cultivation, Collection, and Preparation

An important source of raw material has come from the plants growing in natural habitats.[5] A large area in southeastern Europe supplied much raw material that was purified into colchicine and distributed throughout the world. About 1939 the sudden demand for large portions to be used by geneticists in creating polyploids created a shortage in the market. Almost simultaneously, the war interrupted production and trade in *Colchicum*. The prices increased and colchicine was difficult to obtain.

There are standard practices for cultivating most drug plants, and similar work has been done with *Colchicum*.[21] A general procedure is as follows: Seeds are sown in September, in moist, shady locations and are covered with a thin layer of soil. After germination the next spring, seedlings are set out 60 cm. apart. Cultivation practices are continued for three years. Corms are dug and prepared for the market.

If seed supplies are to be made from cultivated plants, four years of propagation are necessary. Actually a five-year cycle is required. A common practice involves the use of seeds produced in natural habitats. Seeds are collected by bagging the ripening capsules.

Another method for producing raw material under cultivation is to set out the corms that come through the regular corm and bulb markets. Or the corms may be dug in the wild state and transferred to a field for intensive cultivation. Production of colchicine is influenced by environment. A survey from 111 localities in Moravia showed that colchicine produced by seed varied from 0.6 to 1.23 per cent. An average of 0.8 per cent colchicine was obtained.[7, 8, 9]

Drug production can be increased by the application of fertilizer. Increases in colchicine per corm were made when $P_2O_5$ was added.[60] The methods for adding the fertilizer to soil and details of these tests have not been repeated or confirmed. These data are correlated with a variability in production of colchicine found for different localities.

Variation in production of colchicine appeared to be a function of size of seed (Fig. 5.2). The number of seeds per gram varied from 183 to 406. As the number of seeds increased, there was an increase in the percentage of colchicine per 100 grams of raw material. The size of seed is a response to environmental condition, and in turn the production of colchicine is changed by the seed form. Standards set for content of colchicine must account for variation in raw samples of *Colchicum*. Not enough attention has been paid to the relation between environmental conditions and production of colchicine.[69]

*Colchicum luteum* is collected from natural sites exclusively. The corms, rather than the seeds, serve as a source of colchicine. There are large areas of the northwestern Himalayas, notably in the grasslands, where the plants are abundant. Their locations are at levels from 4000 to 7000 ft. While the total content of colchicine is not as high for *C. luteum* as the officially recognized species, enough can be gathered to make this a valuable drug plant.

The dried whole corms are collected from March to May. By this time the fruits have matured and leaves have dried down. The corms are dug and prepared for market according to practices established by collectors who have been working at this trade for many years.

Altitude influences the production of colchicine in the seed more than in the corm, according to a study made in the European Alps for *C. autumnale*. Collections were made beginning at 50 m. and continuing in locations up to 2200 m. The content of colchicine in the seed sample was found to diminish with increasing altitude. The differences were not so great for the corm.[74]

## 5.5: The Crude Drug

Dried corms and seeds of *Colchicum* are official in standard pharmacopeias.[41] Since 1946, *C. luteum* has been accepted in the Indian standards. Dried corms are bitter and have a disagreeable odor. There are two drugs in the Himalayan collections known as the bitter and the sweet surinjan; the former is *C. luteum*.

Collections are made and corms sliced 2 to 5 mm. thick after drying. Each piece should be about 3 cm. wide. A black layer along the side becomes prominent. In transverse section the ground tissues

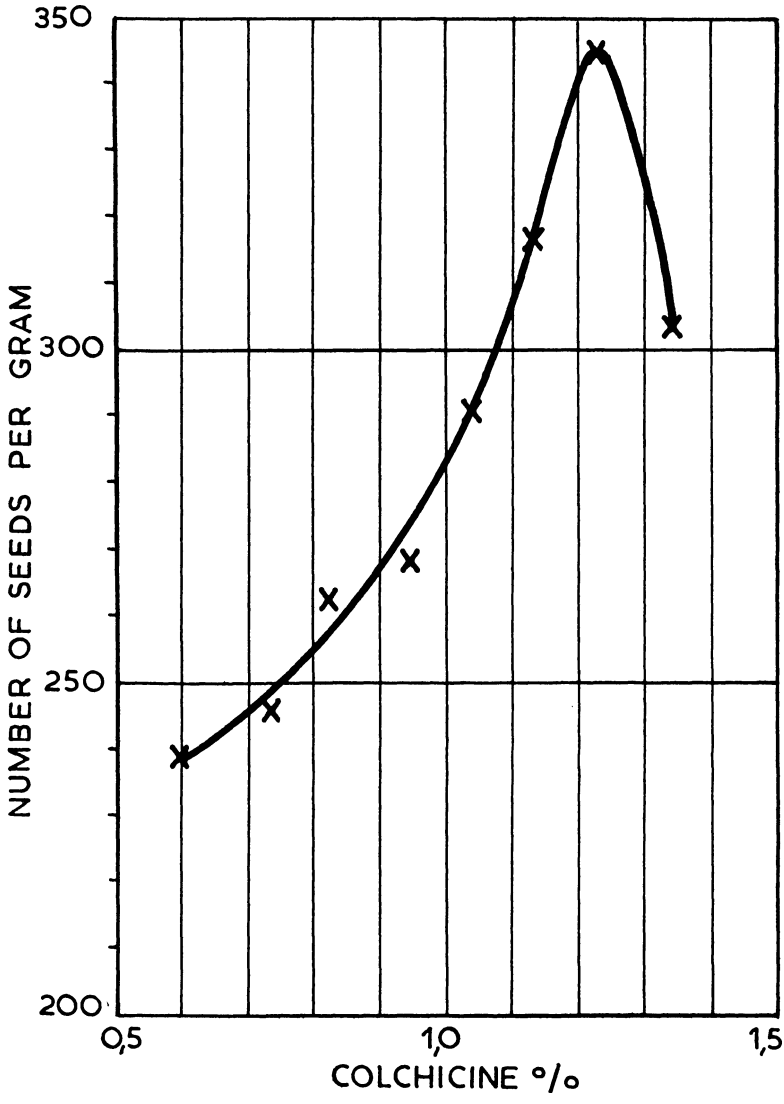


Fig. 5.2—Size of seed can be correlated with percentage of colchicine per gram. The smaller seeds yield more colchicine per gram of raw material. Environmental conditions influence the size of seeds. Larger yields occur when number of seeds per gram exceed 300. (Adapted from Buchnicek)

appear grayish at certain points; these mark the vascular bundles of the corm and are distinct features. In the apical and basal regions the pieces are subconical and plano-convex, respectively. The use of specific marks of identification help to prevent the substitution of material not genuine.

Histologically, the crude drug can be identified by the presence of typical cells. Epidermal cells are rectangular and polygonal, measuring 60 microns on the average. The walls are brown and thickened. Ground tissues are full of starch grains, usually simple; if compound, the components are from two to three parts. Vascular bundles run longitudinally through the corm and are of the collateral type. Xylem vessels are narrow, spiral, or annular, and about 30 mm. in diameter.

Seeds of *Colchicum* are subspherical, 2 to 3 mm. in diameter, having a dark brown and rough seed coat. A large, hard, yellow endosperm surrounding a small embryo is embedded near the surface of the seed. Strong HCl colors the endosperm yellow, indicating the presence of oils.[17, 18] The seeds are bitter, but they do not have the same disagreeable odor found with corms. Large enough amounts of colchicine are contained in seeds that poisonous effects can be produced if warm-blooded animals eat a certain quantity.

### 5.6: Compounds Isolated From *Colchicum*

From 1901 to 1949, many reports have been made to establish the amount of pure substance to be expected from a given amount of dried raw material. The corm, seed, fruit, and flowers have been studied, and variations recorded.[23, 66, 67] Some of the basic reasons for variation have been mentioned. There are sources of variation that occur because different methods of extraction and assay have been used.[4, 80] A survey of some of the literature shows the variety of methods that have been advocated and used.[2, 3, 5, 11, 14, 15, 18, 19, 22, 31, 33, 35, 37, 41, 42, 43, 52, 66, 73] Improvements in methods have come through the use of polarography and chromatography.[32, 61, 65] A large field of chemistry of plant products has been opened by the application of these new technics to drug plants. The idea that *Colchicum* produces only colchicine must be changed in light of the important compounds that appear with pure drug.[81]

The treatment of corms with boiling water during preparation for market causes water-soluble portions to leach out. Different solubilities and physical properties show that even the so-called pure drug is not a single compound. These impurities have been detected in pollen germination studies. Obviously very few biological experiments have been performed with pure colchicine. There are difficulties in making absolutely pure colchicine in large quantity.

In addition to the compounds obtained from the raw material, there are derivatives made in the laboratory by degradation work from the drug. Enough has been done to prove that specific chemical substances related to colchicine are obtainable. The details of such work are extended in the chapter dealing with chemistry of colchicine.

Santavy and his colleagues have isolated compounds from the corm, seed, fruit, and flowers. Their general method involves the extraction from dried powder of particular portions of the plant. Fats are extracted by petrol ether, followed by alcoholic extraction. The use of water, then ether, and finally chloroform brings out an extract demonstrated to have reducible substances when subjected to polarographic analysis. By chromatographic differentiation, specific and identifiable compounds have been reported. Details of the procedures are given in papers written by Santavy and his associates.[61] Isolated substances, the chemical and physical properties of which have been observed, are tabulated in Table 5.3. The work by F. Santavy and his group extends greatly our knowledge of the specific chemical components that may be obtained from the *Colchicum* plant. Classification is made by grouping substances as neutral and phenolics, basic and glucosidic compounds. The particular part of the plant used is listed so that others may repeat the isolation of similar compounds.

Substances A, B, C, D, E, F, G, J, and I have been derived from the corm, seed, fruit, and flowers. In some cases the substances have been found only in certain parts. Pure colchicine is identified as compound A. Desmethylcolchicine appears to be similar to compound C. Another material, colchicerin 3, corresponds to compound G. Biologically, these compounds have different toxicities and produce different effects upon mitosis. Compound F is less toxic than colchicine yet more active in blocking mitosis.

Sunlight induces changes in a solution of colchicine.[65] Irradiation changes the structure of colchicine to a product known as lumicolchicine. At present two kinds of lumicolchicine, I and II, are obtainable. Lumicolchicine I is identified with substances obtained from the seed and flower. Lumicolchicine II is similar to compound J. By irradiation and also through chemical treatment, compounds may be converted from one structure to another. These tests show that the stability of pure colchicine must be regarded as a possible source of variation in biological experimentation.

Only a small portion of this important development in pharmacognosy has been given here. The possibilities of undiscovered identifiable and active compounds open new fields for experimental work. Colchicine has proved to be a very unique substance. The discovery of related compounds synthesized by the plant is even of greater interest.

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