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METHODS OF ISOLATING ALKALOIDS OF THE COLCHICINE SERIES

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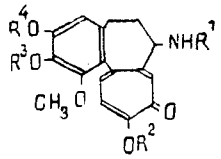
This review considers methods for the isolation of colchicine, colchamine, and colchicoside. The literature for the period from 1884 to 1997 has been used.

Colchicine, a long-known alkaloid of the autumn crocus, has appeared in the pharmacopeias of many countries [1]. Colchamine (synonyms: demecolcine, colcemide), which was discovered later [2, 3], is used in some cases of malignant neoplasms [4]. Colchicine is used for treating gout [1], amyloidosis [5], periodic disease [1, 6], and disseminated sclerosis. Some generalizations concerning the medical use of colchicine have been given in [8]. Colchicine has recently been used in the derivation of new varieties of plants [9].

Some artificial derivatives of colchicine have acquired medicinal value. Abroad, the drug Thiocolceran (deacetylthiocolchicine) is used [10]. Thiocolchicoside (Coltramyl) is employed in rheumatic and nonperiodic diseases [10]. For its pharmacology, see [11]. This drug is synthesized [12] from colchicoside, which is 3-glucosyl-3-demethylcolchicine [13]. Recent patents witness the unabating interest in the practical use of the biological properties of colchicine and its derivatives. In an American patent application antiphlogistic agents based on 2,3-didemethylcolchicine are described [14]. There are patents on medicinal forms of colchicine [15]. A solution of colchamine has been patented for lowering intraocular pressure [16].



	R^1	R^2	R^3	R^4
Colchicine	$COCH_3$	OCH_3	CH_3	CH_3
Colchamine	CH_3	OCH_3	CH_3	CH_3
Deacetylthiocolchicine	H	SCH_3	CH_3	CH_3
Thiocolchicoside	$COCH_3$	SCH_3	CH_3	$C_6H_{11}O_5$
Colchicoside	$COCH_3$	OCH_3	CH_3	$C_6H_{11}O_5$
2-Demethylcolchicine	$COCH_3$	OCH_3	H	CH_3
3-Demethylcolchicine	$COCH_3$	OCH_3	CH_3	H
2,3-Didemethylcolchicine	$COCH_3$	OCH_3	H	H
2-Demethylcolchamine	CH_3	OCH_3	H	CH_3
N-formyldeacetylcolchicine	HCO	OCH_3	CH_3	CH_3
17-Oxocolchicine	$COCH_2OH$	OCH_3	CH_3	CH_3
Colchiceine	$COCH_3$	OH	CH_3	CH_3

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RAW MATERIALS AND ITS DRYING

The colchicine alkaloids are basically obtained from the seeds and bulbs of autumn crocuses – plants of the genus Colchicum. The amounts of colchicine in the common autumn crocus Colchicum autumnale L. are as follows: seeds – 0.22-1.00% [17], dry bulbs – 0.08% [17, 18]. The fresh epigeal parts contain: moisture – about 87%; colchicine in the leaves – 0.004-0.008%; flowers – 0.05%; and the dry matter, respectively, 0.03-0.09 and 0.26% [17]. In the fresh bulbs of showy autumn crocus Colchicum speciosum Stev. there is 63-80% of water [19, 20]; in the flowering period the colchicine content is 0.03-0.14% and the colchamine content 0.01-0.05% [19, 20]; in the dry bulbs the amounts are, respectively, 0.04-0.4% and 0.01-0.08% [19, 20]. In the dry leaves, 0.08% of colchicine and 0.009% of colchamine have been found [19]. We may recall that the epigeal parts of the autumn crocus are the leaves in summer and the flowers in autumn. Colchicoside is obtained from the seeds of autumn crocus growing in Yugoslavia [13, 21].

The epigeal parts of yellow autumn crocus Colchicum luteum Baker have also been proposed for the isolation of colchicine [22]. Colchicine is also present in plants of the genus Merendera [23, 24]. The possibility of the use of the seeds of Gloriosa superba L. [21] and of some other tropical plants of the family Liliaceae [25, 26] has also been discussed.

The storage of the fresh bulbs for 2-3 months is permissible when the following conditions are observed: temperature, humidity, darkness, aeration. The drying of the bulbs of showy autumn crocus at 100°C involves a loss of colchicine of up to 25% and of colchamine of up to 39% [20]. Drying at a temperature not above 19-20°C has been recommended for yellow autumn crocus [27]. The impurities appearing on the drying of the bulbs of common autumn crocus at 100°C have been revealed with the aid of thin-layer chromatography [28]. Impurities are also formed in the slow drying (~25°C) of the leaves and flowers of the same plant. The amount of colchicine does not change but the amounts of 2- and 3-demethylcolchicine and of 2-demethylcolchamine increase. The authors explained this phenomenon by the fact that in the fresh raw material these demethylated derivatives are bound to substances of high molecular mass [17]. It was later found that the drying of the epigeal parts increases the amount of demethylated compounds and alters the amount of the main alkaloids. Demethylation also takes place on the storage of the dry flowers. During the first six months the amount of 2-demethylcolchamine rises, and then it falls [29]. This demethylation on drying must be regarded as irreversible. It has been proposed [17, 21] to methylate the byproducts arising with diazomethane. In this way it is possible to increase the yield of colchicine and colchamine at the expense of the natural demethylated derivatives.

EXTRACTION

In the isolation of colchicine and colchamine, water or aqueous solutions, alcohols, and weakly polar and mixed solvents are used. In one of the first publications the isolation of colchicine from the seeds of common autumn crocus was described. The seeds were then treated with rectified alcohol [18]. For other early publications, see [30, 31]. In the extraction of colchicine by organic solvents, the seeds are first defatted with weakly polar solvents such as petroleum ether [2, 24, 26, 32, 33]. After such treatment, the colchicine can be extracted with 80% ethanol [26]. Other alcohols are also used. For example, the dried epigeal parts of yellow autumn crocus are extracted with methanol [22]. The fresh or dry epigeal parts have also been treated with hot methanol [17, 34]. The use of weakly polar solvents (chloroform [2] and benzene [33]) has been described.

The aqueous extraction of the bulbs of showy autumn crocus has been proposed by Soviet workers [35]. According to thse authors, colchicine is obtained in higher yield and with a small amount of impurities, while the drying of the raw material and the corresponding losses of alkaloids are avoided. It has been proposed to extract bulbs freshly ground in a meat grinder at room temperature, with the addition of an aqueous solution of sodium bisulfate [36, 37]. Somewhat earlier, the aqueous extraction of dry bulbs was used in analysis [38]. Later, a patent was granted for the aqueous extraction of colchicine from the ground seeds of common autumn crocus with preheating to 40°C; and here it was mentioned that aqueous extraction gives a purer colchicine [39]. The same author had previously reported the use of aqueous extraction in the analysis [40]. Aqueous extraction is also recognized as the best in [41]. Here the method of the patent [39] is modified: it has been found more suitable to extract the unground seeds, but at a temperature of 50-60°C and for somewhat longer. The seeds and the water are charged in a ratio of 1:10 and are stirred continuously with a contin-