CHAPTER 6

Chemistry

by James D. Loudon*

6.1: Extraction and General Properties

Colchicine is commonly extracted from the seeds and corms of the autumn crocus, *Colchicum autumnale,* Linn., but it is also present in numerous species of *Colchicum* (Albo[1]) as well as in other Liliaceae (Klein and Pollauf[2]). Extraction is effected by alcohol (Zeisel;[3] Chemnitius[4]) and the concentrates after dilution with water are freed from insoluble fats or resins. The aqueous solution is then repeatedly extracted with chloroform and the colchicine is recovered in the form of a crystalline addition complex with the solvent. From this the chloroform is distilled off in steam or alcohol and evaporation of the residual solution yields amorphous colchicine which may be crystallized from ethyl acetate as pale yellow needles (Clewer, Green, and Tutin[5]). Chromatographic purification of the chloroform solution on alumina greatly facilitates the procedure (Ashley and Harris[6]).

Pure colchicine, $C_{22}H_{25}O_6N$, forms fine, practically colorless needles, m.p. 155°; $[\alpha]_D^{13}$ — 119.9° (c = 0.878 in chloroform), as determined by Mr. T. Y. Johnston at Glasgow. It is readily soluble in alcohol, chloroform, or in cold water, but is less soluble in hot water or in cold benzene and is almost insoluble in ether. From these solvents there is a tendency to crystallize with solvent of crystallization which may markedly affect the melting point. Concentrated aqueous solutions deposit crystals of the sesquihydrate which, despite its relatively sparing solubility in water, does not crystallize from more dilute solution unless induced to do so by seeding (Loudon and Speakman[7]). Dilute mineral acids and alkalis color colchicine an intense yellow, while nitric acid (d,1.4) produces a violet color which slowly changes to yellow and finally to green: other color-reactions are de-

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scribed by Zeisel.[3] Although under suitable conditions colchicine forms precipitates with many of the usual alkaloidal reagents,[3] its classification as an alkaloid is questionable. It is essentially a neutral substance with a homocyclic ring-structure: on the other hand, it is associated in the plant with compounds of allied structure, some seven crystalline and kindred alkaloids being known (Santavy and Reichstein[8]).

6.2: The Functional Groups

Hydrolysis of colchicine by boiling with very dilute hydrochloric acid yields methyl alcohol and *colchiceine*, $C_{21}H_{23}O_6N$, which is acidic, gives a deep olive-green color with aqueous ferric chloride (distinction from colchicine), and on further hydrolysis with more concentrated acid yields equivalent amounts of acetic acid and *trimethylcolchicinic acid*, $C_{19}H_{21}O_5N$ (Zeisel[9]). This last compound is amphoteric and contains a primary amino-group (Johanny and Zeisel[10]); hence the two-stage hydrolysis may be represented as follows:

$$\begin{aligned} &C_{19}H_{18}O_4\,(OMe)\,(NH.COMe) \\ &\quad\rightarrow MeOH + C_{19}H_{18}O_4\,(OH)\,(NH.COMe) \\ &\quad\rightarrow MeCO_2H + C_{19}H_{18}O_4\,(OH)\,(NH_2). \end{aligned}$$

Trimethylcolchicinic acid contains three methoxyl groups which, by prolonged hydrolysis, are demethylated and *colchicinic acid*, $C_{16}H_{15}O_5N$, is produced. Correspondingly in colchicine itself the presence of four methoxyl groups is shown by the usual Zeisel estimation.[9]

The four methoxyl groups and the acetylamido-group together account for five of the six oxygen atoms of colchicine. Since the sixth oxygen is unresponsive to carbonyl reagents, it was at one time thought to be part of a carbomethoxy group (–CO.OMe) or of an oxygen ring system. The former view is in harmony with the ready hydrolysis to colchiceine which has acidic character but which, on the other hand, also shows definite enolic properties and when methylated by diazomethane, yields two readily hydrolyzable *O*-methyl ethers, namely colchicine and *isocolchicine* (Meyer and Reichstein;[11] Sorkin[12]). Similarly trimethylcolchicinic acid reacts with benzenesulphonyl chloride to give two di(benzenesulphonyl) derivatives (Windaus[13]), in each of which one of the acyl groups is attached to nitrogen while the second appears to be attached to oxygen since fairly mild hydrolysis converts both compounds into the same *N*-benzenesulphonyl trimethylcolchicinic acid. This duplication of *O*-derivatives strongly suggests that in colchiceine and in trimethylcolchicinic acid there is a tautomeric enol system capable of giving rise to paired *O*-derivatives which are either steric or structural isomers. Accordingly the sixth oxygen atom is considered to reside in the carbonyl group

of an enolone system in colchiceine and of a corresponding enolone-methyl-ether system in colchicine.

Although neither colchicine nor colchiceine reacts with the usual carbonyl reagents, hydrogenation results provide evidence of the presence of a carbonyl group in each. Bursian[14] found that with a platinum catalyst both compounds absorbed three moles of hydrogen and that thereby colchicine gave a mono-alcohol while colchiceine gave a diol. In each case therefore a new hydroxylic function has been produced and may well arise from reduction of a carbonyl group by one mole of hydrogen. The absorption of two further moles of hydrogen shows the presence of two olefinic groups, while the presence of yet a third olefinic group, which resists hydrogenation, was indicated by the interaction of *hexahydrocolchicine*, $C_{22}H_{31}O_6N$, with perbenzoic acid[14] or with monoperphthalic acid (Tarbell *et al.*[15]) to form an oxide, $C_{22}H_{31}O_7N$.

Summing up: The evidence suggests that colchicine is the methyl ether of an enolone which contains three additional methoxyl groups, an acetylated primary amino-group, and three non-benzenoid double bonds:

$$C_{16}H_9\ (OMe)_4\ (NH.COMe)\ (:O)\ (=)_3.$$

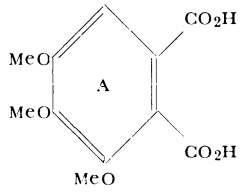
6.3: The Structural Problem

The saturated hydrocarbon, $C_{16}H_{22}$, which corresponds to this assemblage of groups, fall short of the paraffin, $C_{16}H_{34}$, by six hydrogen molecules each of which in default indicates the presence of either a carbon ring or a benzenoid type of double bond. Four of the missing hydrogen molecules are at once accounted for by the demonstrable presence of a benzenoid ring; the remaining two must therefore denote two further ring systems. Colchicine is accordingly tricyclic and the respective rings, both in the alkaloid and in its degradation products, are designated by the letters A, B, and C.

6.3–1: Ring A. The presence of the benzenoid ring (A) is shown by the formation of 3:4:5-trimethoxyphthalic acid (I), or its anhydride, from colchicine and many of its derivatives on oxidation with hot alkaline permanganate (Windaus[16, 17]).

6.3–2: Ring B. The most penetrating insight into the molecular structure of colchicine is obtained through a series of degradation products (Windaus[17, 18]) derived from N-*acetyliodocolchinol*, $C_{20}H_{22}O_5NI$. This compound is formed from colchiceine by the action of iodine in the presence of alkali. It is definitely phenolic and is reduced by zinc and acetic acid to N-*acetylcochinol*, $C_{20}H_{23}O_5N$, which on methylation affords N-*acetylcolchinol methyl ether*. The latter still contains the acetylated primary amino-group and may be deaminated

in several ways: (1) directly, by heating with phosphoric oxide in xylene (Cook and Graham;[19] Barton, Cook, and Loudon[20]) whereby two isomeric compounds, $C_{19}H_{21}O_4$, are formed and are named *deaminocolchinol methyl ether* and iso*deaminocolchinol methyl ether*, respectively; (2) by hydrolysis to the primary amine, *colchinol methyl ether*, followed by reaction with nitrous acid to form a carbinol



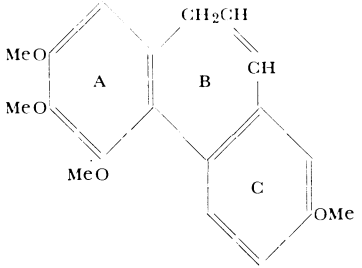
(I)

(Cohen, Cook, and Roe[21]) which on dehydration[20] yields the same pair of isomeric products; (3) by Hofmann degradation of colchinol methyl ether whereby only deaminocolchinol methyl ether has been isolated (Windaus[22]).

Barton, Cook, and Loudon[20] established the structure (II) for deaminocolchinol methyl ether and the structure (III) for the *iso*-compound on the following grounds. Both isomers afforded the same dihydride when hydrogenated in acetic acid with a palladium catalyst; they must therefore differ only in the location of a double bond which must be ethylenic in type. Deaminocolchinol methyl ether was oxidized with sodium dichromate in acetic acid to 2:3:4:7-tetramethoxyphenanthraquinone (VIII), together with a by-product which was recognized as an unsaturated ketone, $C_{19}H_{18}O_5$.

Formation of the quinone, which was identified by synthesis, establishes the presence of a (bridged) diphenyl system and fixes the methoxylation pattern. The nature of the three-carbon bridge in deaminocolchinol methyl ether (II) was next determined by oxidation with osmium tetroxide to a glycol (IV) which, by scission with lead tetra-acetate, yielded not the normally expected di-aldehyde (V) but a mono-aldehyde (VI) formed from (V) by internal condensation. This mono-aldehyde — later synthesized — was identified by oxidation to 2:3:4:7-tetramethoxyphenanthrene-10-carboxylic acid which was also synthesized. Similar stepwise oxidation of *iso*deaminocolchinol methyl ether (III) gave 2:3:4:7-tetramethoxy-9-phenanthraldehyde (VII), identical with a synthetic specimen.

These results leave little room for doubt that deaminocolchinol methyl ether and its *iso*-compound are correctly formulated. Moreover, Cook, Dickson, and Loudon[23] have shown that the synthesized



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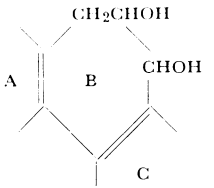

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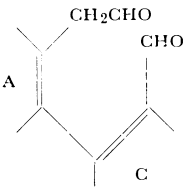

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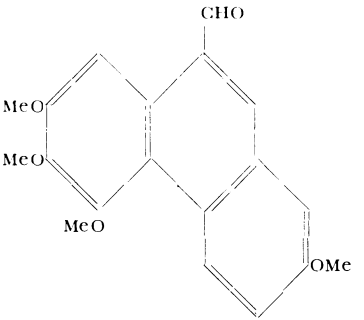

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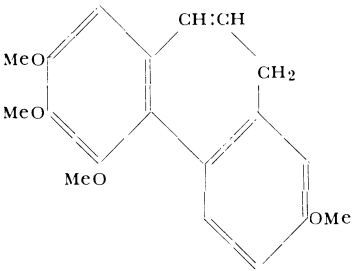

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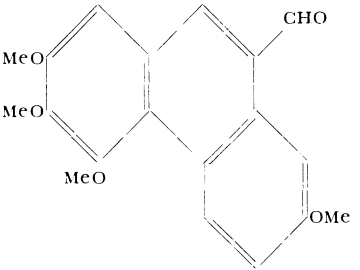

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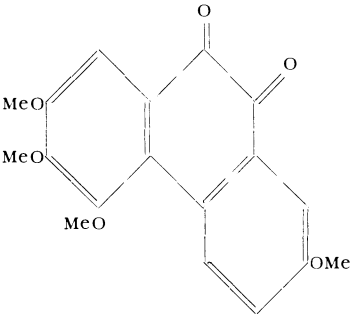
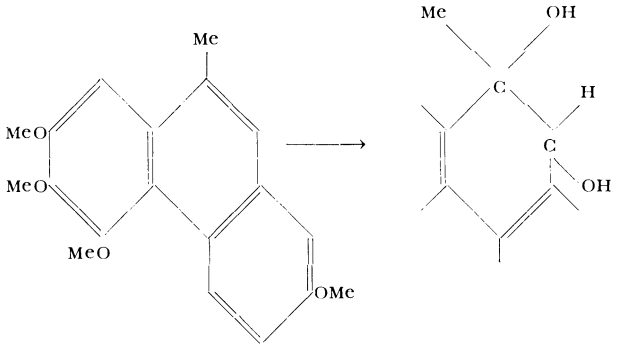


(VIII)

parent hydrocarbon corresponding to (II; H for OMe) reproduces in all essentials the behavior just described and, further, that this hydrocarbon is isomerized to 9-methylphenanthrene by successive heating with hydriodic acid and zinc dust. Such isomerization accounts for the isolation of 9-methylphenanthrene by Windaus[22] during an attempt to demethoxylate deaminocolchinol methyl ether, and it con-



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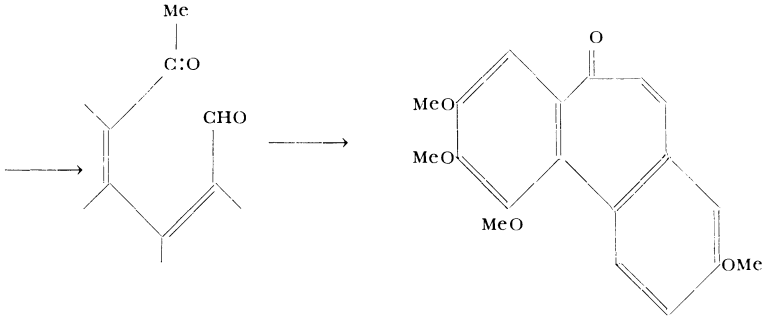
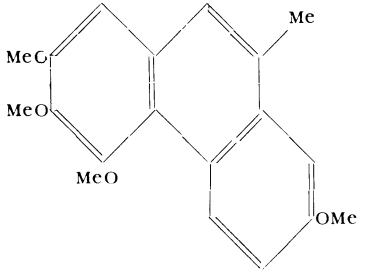


(X)

tributed to his formulating the latter compound as either 2:3:4:6- or 2:3:4:7-tetramethoxy-9-methylphenanthrene, each of which, when synthesized by Buchanan, Cook, and Loudon,[24] proved to be distinct from the degradation product. Tarbell, Frank, and Fanta,[25] who prepared deamino-iodocolchinol methyl ether from *N*-acetyliodocolchinol and oxidized it to a derivative of homodiphenic acid, likewise conclude in favor of a 7-membered ring B as in (II).

The first synthesis of a significant derivative of (II) was effected by Buchanan, Cook, Loudon, and MacMillan.[26] The sequence of reactions used for the ring-contraction (II) → (IV) was applied in the

opposite direction to expand the central ring of 2:3:4:7-tetramethoxy-10-methylphenanthrene (IX). This took advantage of the known reactivity of the 9:10-double bond in phenanthrenes and hydroxylation, scission, and renewed cyclization led to an unsaturated ketone (X) identical with the one produced, as already mentioned, by oxidation of deaminocolchinol methyl ether. Moreover, by applying the same series of reactions to 2:3:4:7-tetra-methoxy-9-methylphenanthrene (XI) Cook, Jack, and Loudon[27] obtained an isomeric unsaturated ketone (XII). This was reduced to the saturated ketone (XIII) and thence by oximation and renewed reduction was converted to the (±)-amine (XVI). Optical resolution of this amine, through its salts with (+)-6:6′-dinitrodiphenic acid, afforded the (—)-base and hence the (—)-acetylé derivative and these respectively were identical with colchinol methyl ether and its *N*-acetyl derivative



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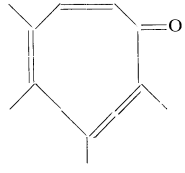

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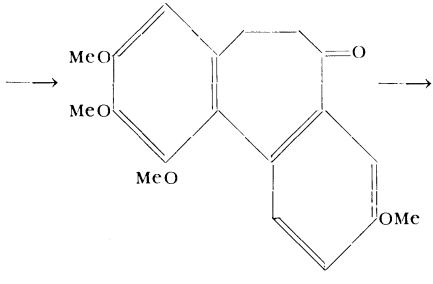

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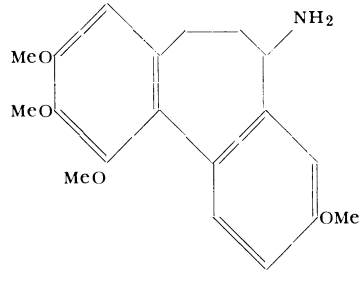
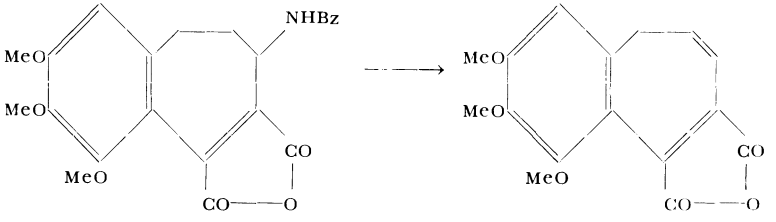


(XIV)

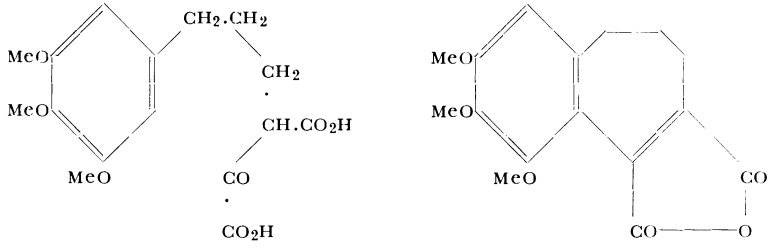
as obtained by degradation of colchicine.[28] By a different route starting from the 9-monoxime of 2:3:4:7-tetramethoxyphenanthraquinone Rapoport, Williams, and Cisney also synthesized the (±)-amine (XIV) and showed it to be identical with racemized colchinol methyl ether.[29]

A second series of degradation products has a bearing on the structure of ring B. Windaus[15] found that *N*-benzoyltrimethylcolchicinic

acid (prepared by di-benzoylation of trimethylcolchicinic acid and preferential hydrolysis of the *O*-benzoyl group) was oxidized by cold alkaline permanganate to two products, namely N-*benzoylcolchinic anhydride,* $C_{23}H_{21}O_7N$, and a corresponding lactone, N-*benzoylcolchide,* $C_{23}H_{23}O_6N$, which he formulated[22] as derivatives of 1:2-dihydro-2-methylnaphthalene. With the recognition of ring B as 7-



(XV) (XVI)



(XVIII) (XVII)

membered in the colchinol series, it was at once evident that *N*-benzoylcolchinic anhydride might be better represented by formula (XV) and *N*-benzoylcolchide by a corresponding lactone structure. To test this view, Cook, Johnston, and Loudon[30] deaminated the anhydride and showed that the resultant deaminocolchinic anhydride was not identical with 6:7:8-trimethoxy-3-methylnaphthalene-1:2-dicarboxylic anhydride – as it would be on the Windaus formulation – nor indeed could it be a naphthalene derivative since it showed ethylenic behavior towards reduction. From the reduction products, Horning, Ullyot, and their colleagues[31] isolated a dihydride and established its structure as (XVII) by synthesis and cyclization of the oxaloacetic acid (XVIII). Thereby the 7-membered rings in *N*-benzoylcolchinic anhydride (XV) and its deamination product (XVI) are unequivocally proved.

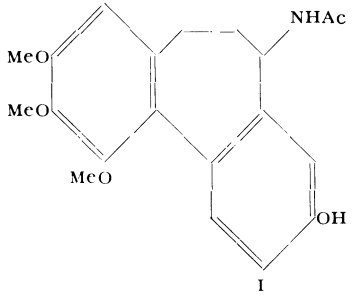
Accordingly both lines of degradation – the first, through *N*-acetylcolchinol, involving a process which makes ring C benzenoid; the second producing *N*-benzoylcolchinic anhydride apparently by

direct oxidation of ring C – consistently lead to the conclusion that ring B of colchicine is 7-membered.

6.3–3: Ring C. It will now be evident that the enolone properties of colchiceine derive from the third ring, namely ring C, and that the structure to be assigned to this ring must also interpret the conversion of colchiceine into *N*-acetyliodocolchinol. This transformation is empirically expressed by

$$C_{21}H_{33}O_6N + I \rightarrow C_2OH_{22}O_5NI + [CHO]$$

and the colchinol derivative so produced may be formulated as (XIX) which is in harmony with the observation that its methyl ether yields 4-iodo-5-methoxyphthalic acid on oxidation.[18, 32] Two further links between the structure of the alkaloid and that of colchinol are known. Cech and Santavy[33] obtained *N*-acetylcolchinol directly by oxidizing colchiceine with alkaline hydrogen peroxide. Again, colchicine (but



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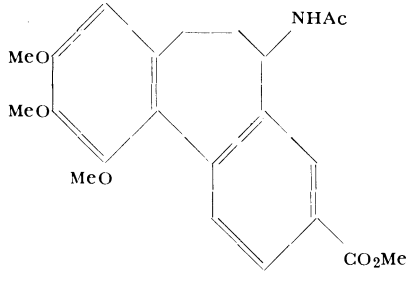


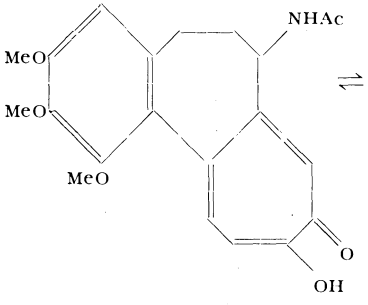
(XX)

not colchiceine) is isomerized when heated with sodium methoxide in methanol (Santavy;[34] Fernholz[35]) forming the methyl ester (*allo*-colchicine) of a carboxylic acid (*allo*colchiceine); and Fernholz[35] converted this acid into *N*-acetylcolchinol by the standard procedure: $RCO_2H \rightarrow RNH_2 \rightarrow ROH$. The structure of *allo*colchicine is therefore securely fixed as (XX).

Even before all of these facts were available, Dewar[36] suggested that ring C of colchiceine was tropolonoid and on this basis the structure of colchiceine is represented by the tautomeric system (XXI) ⇌ (XXII). The validity of this formulation is now generally accepted and an earlier formula, proposed by Windaus,[22] need not be discussed here.

6.4: Comparison With Tropolones

It is necessary, however, to refer briefly at this stage to some of the more general features of tropolone chemistry (for more compre-



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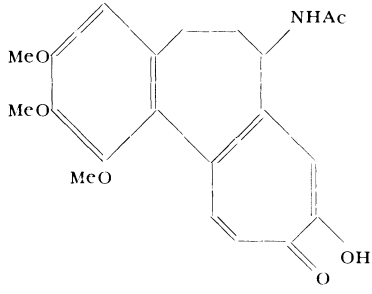


(XXII)

hensive treatment, see Cook and Loudon[37]). Tropolone (2-hydroxy*cyclo*heptatrienone) and its derivatives have aromatic properties, the reactivity of the ethylenic and carbonyl functions being suppressed. Thus the compounds are substituted by electrophilic reagents but do not react with carbonyl reagents. The hydroxyl group is markedly acidic. Salt formation is accompanied by development or intensification of color, and coordination complexes are produced with ferric or cupric ions. Tropolone itself exhibits feebly basic properties and yields a hydrochloride and a picrate. Tropolone ethers resemble esters in their ready hydrolysis. With varying ease individual tropolones (or their ethers) are isomerized by hot alkali, the 7-membered ring undergoing contraction to the benzenoid structure of an appropriately substituted benzoic acid (or ester). Catalytic hydrogenation of tropolones is seldom simple. When complete, it yields octahydrides which are 1:2-diols, but it may involve loss of oxygen, and ketonic intermediates are frequently detectable.

The general analogy with colchiceine, implicit in this account of tropolone behavior, is borne out by more specific comparison. Like unsymmetrically substituted tropolones, colchiceine is known only as a single substance which yields two isomeric methyl ethers, colchicine and *iso*colchicine, corresponding to the tautomerides (XXI) and

(XXII). The ester-like properties of these ethers are revealed in their rapid hydrolysis to colchiceine and in their reactions with ammonia and amines whereby colchicamides are formed,[38] the reactive methoxyl group being replaced by an amine residue. Hydrogenation of colchiceine, or of colchicine, is complex,[14, 15, 39, 40, 41] but there is evidence that hexahydrocolchiceine is a 1:2-diol,[15, 42] and less fully hydrogenated material shows ketonic properties.[39] Polarographic measurements made by Santavy and by Brdicka,[43] and infrared absorption studies by Scott and Tarbell[44] confirm the similarity between colchiceine and tropolones. Moreover, *allo*colchicine (XX) is at once seen to be the benzenoid isomerization product of a methyl ether derived from either (XXI) or (XXII). Its production corresponds to that of methyl benzoate from tropolone methyl ether (Doering and Knox[45]) and explains the origin of the trimellitic acid (benzene-1:2:4-tricarboxylic acid) which Windaus obtained from colchicine by successive alkali fusion and oxidation.[16]

6.5: Structure of Colchicine

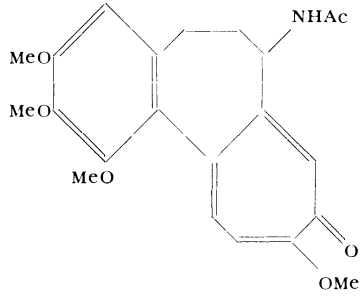
The tautomeric nature of colchiceine allows two possible formulations of colchicine, its methyl ether. It is not easy by chemical means to distinguish between these alternatives but the distinction can be made by X-ray crystallographic analysis. King, De Vries, and Pepinsky[46] in this way examined an addition complex of colchicine and methylene di-iodide and not only confirmed the tricyclic structure with its two fused 7-membered rings but also showed that colchicine is the particular methyl ether (XXIII). It follows that *iso*colchicine has the methyl ether structure corresponding to (XXII).

6.6: Miscellany

So far in this chapter discussion has been directed primarily to the evidence on which the structural formula of colchicine rests. There remain to be noted several reactions and items of chemical interest, which are either at present incompletely evaluated or only indirectly related to the alkaloid's structure. For instance it is known that nitration of colchicine yields a mononitro-colchicine, reducible to an aminocolchicine, but the seat of substitution in these derivatives is not yet definitely ascertained (Nicholls and Tarbell[41]). Bromination of colchicine yields mono-, di-, and tribromo derivatives (Zeisel and Stockert[47]). Bromination of colchiceine yields a tribromo acid which Lettré, Fernholz, and Hartwig[48] formulate as (XXIV) by analogy with the bromination of tropolones[49] and because the compound is readily decarboxylated to a tribromo derivative of *N*-acetylcolchinol. Oxidation of colchicine with chromic acid in aqueous solution yields a ketone, namely oxycolchicine, $C_{22}H_{23}O_7N$, in which a

methylene group of the alkaloid has been oxidized to carbonyl.[22, 50]

Molecular rearrangement is almost commonplace in colchicine's chemistry. It is inherent in the changes, already described, by which the 7-membered rings of the alkaloid or its derivatives become contracted to 6-membered rings. It is also encountered in formation of the carbinol (6.3) by the action of nitrous acid on colchinol methyl



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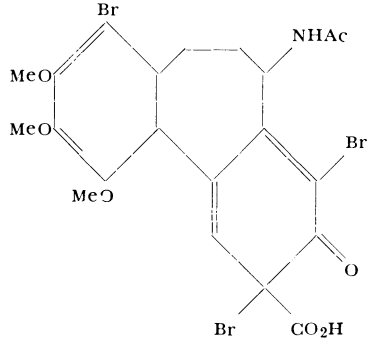
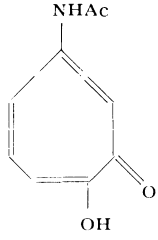


(XXIV)

ether and is again found in dehydration of this carbinol whereby deaminocolchinol methyl ether (and its isomeride) is produced. Both of these reactions are known to involve Demjanow-type rearrangements (Cook, Jack, and Loudon[51]) and through them ring B, initially 7-membered, is contracted and re-expanded in successive steps. Moreover, colchicine itself is sensitive to ultraviolet light and is isomerized in aqueous solution by sunlight. Thereby three isomerides, namely α-, β-, and γ-lumicolchicine are formed (Grewe and Wulf;[52] Santavy[53]) but their molecular structures remain undetermined.

Synthesis – the ultimate challenge of a natural product to the organic chemist – has still to be achieved for colchicine although, at

the time of writing, preliminary work in this direction is engaging much attention.[54-59] The colchicine structure is novel chiefly in respect of the two fused 7-membered rings of its tricyclic system. These rings are retained in a compound, $C_{19}H_{26}O_3$, which Rapoport and Williams[38] prepared from colchicine by a series of hydrogenation reactions. In this product ring A of colchicine is unaltered, but rings B and C are fully reduced and devoid of substituent groups. Synthesis of this compound is potentially more simple, although also less significant, than that of colchicine itself. But even total synthesis of the alkaloid, when achieved, is unlikely to have more than academic importance: synthetic colchicine will not soon provide an economic replacement of the natural product. Here another issue is joined, for it may be possible from a study of the alkaloid and its immediate derivatives to discern some pattern of atoms or groups, which is associated with colchicine's effect on mitosis. By incorporating this molecular pattern in simpler and more accessible compounds it would then be possible to search on a rational basis for synthetic substitutes. Already several attempts have been made to achieve this end and some success has been claimed for compounds modeled on the earlier, partly erroneous formula of Windaus (see work by Lettré discussed in Chapter 17). As would be expected, tropolone derivatives have been investigated for their effect on cell mitosis. For instance, *p*-acetamidotropolone (XXV) — a compound possessing obvious structural similarities to colchiceine — was examined, in *Tradescantia* cells *in vivo*, by Wada[60] who records a strong radiomimetic



(XXV)

action and regards the compound as a possible mutagenic substance. Its effect, however, does not appear to be identical with that of colchicine.

As an aid to biological studies Raffauf, Farren, and Ullyot[61] have prepared C^{14}-labeled derivatives of colchicine by methylation of colchiceine with labeled diazomethane and by acetylation of desacetylcolchicine with labeled acetyl chloride.

Mention was earlier made of congeners of colchicine (6.1). These include a demethylcolchicine — or "substance C" — in which one of the three methoxyl groups of ring A is demethylated. Horowitz and Ullyot[62] find what is probably the same compound present in U.S.P. colchicine to an extent of some 4 per cent. It is also interesting that Bellet[63–65] has isolated a glucoside, namely colchicoside, $C_{27}H_{33}O_{11}N$, from *C. autumnale* and that this glucoside may be hydrolyzed to, and synthesized from, "substance C" and glucose. The glucosidic link probably involves the oxygen atom which in ring A is adjacent to ring B. Santavy and his colleagues have improved the technique of isolating colchicine from *C. autumnale* and have examined its seasonal variation in the plant.[66] They also surveyed various *Colchicum* species for alkaloid content and found *C. arenarium* W.K. to be particularly rich in colchicine. Finally they have made considerable progress towards elucidating the structures of colchicine's co-alkaloids [8, 68] and it is already apparent that at least several of these are simple modifications of the structural pattern of colchicine.

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