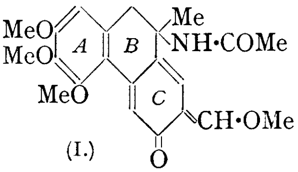
35. *Colchicine and Related Compounds. Part I. Some Observations on the Structure of Colchicine.*

By A. Cohen, J. W. Cook, and (Miss) E. M. F. Roe.

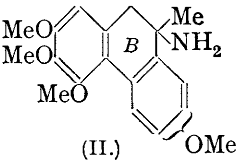
Colchinol methyl ether, a primary amine obtained by degradation of colchicine, has been converted by nitrous acid into a carbinol. The constitution of colchicine is discussed in relation to the stability and properties of this carbinol and of other degradation products of colchicine.

Colchicine, an alkaloid first isolated from the autumn crocus, has been much used in recent years by biological workers who have utilised its effect on cell division. Moreover, Amoroso (*Nature*, 1935, **135**, 266) reported that injections of colchicine resulted in inhibition of tumour growth in mice and a dog, and subsequent workers who have studied the influence of colchicine on tumour-growth have occasionally observed tumour-regression (see, for example, Peyron, Lafay, and Kobozieff, *Bull. Cancer*, 1936, **29**, 874; von Brücke and von Hueber, *Klin. Woch.*, 1939, No. 34, 1160). The high toxicity of colchicine greatly restricts the dosage which may be given, and our primary interest in undertaking this series of investigations has been to seek compounds which would retain the action of colchicine on cell division without having the high toxicity of the alkaloid. Several transformation products of colchicine were found by Brues and Cohen (*Biochem. J.*, 1936, **30**, 1363) to have its characteristic effect on mitosis, and the following paper describes a preliminary study of the synthesis of compounds having some of the structural features of colchicine.

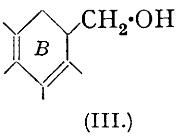
The molecular constitution of colchicine has been largely elucidated by Windaus (*Annalen*, 1924, **439**, 59), who proposed formula (I), which is undoubtedly correct in most details. The positions of substitution in ring *C* have recently been established by Grewe (*Ber.*, 1938, **71**, 907), but it is still possible that the substituents shown in these positions require to be interchanged. The hydrogenation of colchicine and its first hydrolysis product, colchiceine (a hydroxymethylene-ketone), has been studied in detail by Bursian (*Ber.*, 1938, **71**, 245), who has also examined the ultra-violet absorption spectra of colchicine and certain of its transformation products, and finds them consistent with Windaus's formulation.



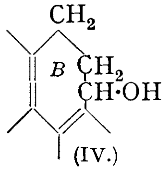
(I.)



(II.)



(III.)

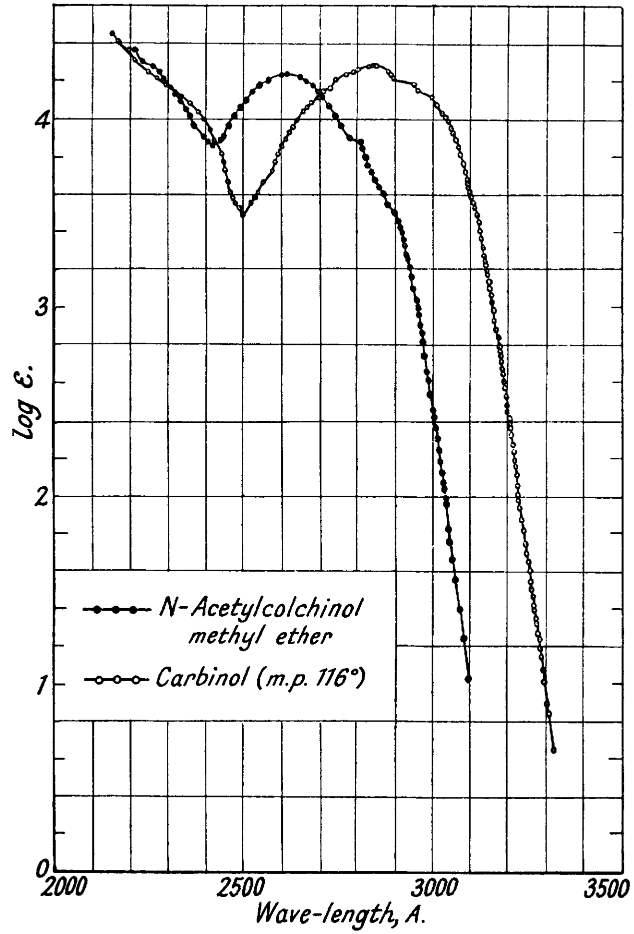


(IV.)

The purpose of the present communication is to draw attention to some respects in which Windaus's structure seems unsatisfactory. A degradation product, colchinol methyl ether, should have formula (II), but this respresents a derivative of 9-amino-9 : 10-dihydrophenanthrene, which should easily pass into the completely aromatic state by loss of ammonia, a behaviour shown by somewhat simpler compounds of analogous structure synthesised by Windaus and his co-workers (*Ber.*, 1924, **57**, 1871, 1875). There is no evidence of such instability. Moreover, by the action of nitrous acid on colchinol methyl ether we have obtained a *carbinol* the stability of which is quite incompatible with the structure of a tertiary carbinol related to 9 : 10-dihydrophenanthrene. This type of difficulty would be removed if colchinol methyl ether were assumed to have its amino-group in the side chain instead of attached to the nucleus. The carbinol would then be represented by (III). This, however, introduces a new difficulty, for on this basis colchinol methyl ether would have unsubstituted hydrogen atoms at positions 9 and 10 and should easily lose a molecule of hydrogen and give the phenanthrene structure. Yet *N*-acetylcolchinol methyl ether was recovered unchanged after an attempt to effect dehydrogenation with platinum-black at 280°.

Several attempts have been made to distinguish between structures of type (II) and type (III) for the carbinol obtained from colchinol methyl ether. A compound of structure (III) should be capable of oxidation to an aldehyde and thence an acid. We have, however, been unable to isolate a crystalline oxidation product. In another approach to the problem use was made of the reaction with phthalic anhydride, which should distinguish between a primary and a tertiary carbinol. However, the carbinol did not react in benzene solution with phthalic anhydride, but gave a *hydrogen phthalate* when fused with phthalic anhydride at 180°. This behaviour is more characteristic of a secondary carbinol than of a primary or a tertiary carbinol, but does not exclude a primary alcohol (compare Houben, " Die Methoden der Organischen Chemie," 1930, III, 20).

The figure shows the ultra-violet absorption curves of the carbinol, m. p. 116°, from colchinol methyl ether, together with that of *N*-acetylcolchinol methyl ether. The measurements were made in alcoholic solution. The curve for *N*-acetylcolchinol methyl ether is very similar to that given by Askew (J., 1935, 513) for 9 : 10-dihydrophenanthrene as regards both wave-length and intensity of absorption. The curve of the carbinol shows much the same intensity, but the band is considerably displaced towards the visible region. It is evident from these comparisons that the carbinol is not a phenanthrene derivative, which might conceivably have been formed from a dihydrophenanthrene structure by the dehydrogenating action of nitrous acid, for the absorption spectrum of phenanthrene is quite different in character. Furthermore, spectroscopic examination failed to reveal the presence of a phenanthrene derivative in a mixture of carbinol and higher-melting material formed in several experiments in which colchinol methyl ether was treated with nitrous acid (see experimental section). The absorption curve of such a mixture was very similar to that of the pure carbinol, with a somewhat lower intensity. Bursian (*loc. cit.*) recorded the absorption spectrum of *N*-acetylcolchinol methyl ether in chloroform solution; his curve has a general resemblance to ours, but the intensity of his maximum is very much higher.



The evidence of the presence of the phenanthrene ring system in colchicine is the conversion by Windaus of colchinol methyl ether by exhaustive methylation into a tetramethoxy-compound which gave 9-methylphenanthrene on demethylation, followed by distillation with zinc dust. The structures of rings *A* and *C* of colchicine seem to rest on a secure foundation, but that of ring *B* is less certain. Possibly the phenanthrene structure arises by intramolecular change in ring *B* in the drastic process of zinc dust distillation. If this were so, ring *B* of colchicine might be seven-membered, leading to a structure (IV) for our carbinol, and this would accommodate all the known facts of the chemistry of colchicine. A decisive answer to this question would be given by the synthesis of Windaus's tetramethoxy-compound formed by exhaustive methylation. If colchinol methyl ether is a dihydrophenanthrene derivative, this tetramethoxy-compound should be 2 : 3 : 4 : 6- or

2 : 3 : 4 : 7-tetramethoxy-9-methylphenanthrene, and the synthesis of both these compounds is being undertaken by one of us. We do not overlook the possibility that the action of nitrous acid on colchinol methyl ether may be attended by molecular rearrangement (compare Wallach, *Annalen*, 1907, **353,** 326).

EXPERIMENTAL.

Degradation of colchicine to colchinol methyl ether hydrochloride was carried out by methods described in the literature, references to which are given by Brues and Cohen (*loc. cit.*).

Action of Nitrous Acid on Colchinol Methyl Ether.—A 10% solution of sodium nitrite (2·8 g.) was added during an hour to a mechanically stirred ice-cooled solution of colchinol methyl ether hydrochloride (13·2 g.) in 10% acetic acid (150 c.c.). After being kept at room temperature for 2 hours, the product was extracted with ether, and the extract washed with dilute sodium carbonate solution, dried, and distilled. The resinous distillate (5·1 g.), b. p. 230°/3 mm., was dissolved in benzene, and light petroleum added until a slight turbidity was produced. The solution slowly crystallised in the refrigerator, and recrystallisation from benzene–light petroleum gave 2·5 g. of large well-formed crystals, m. p. 115°. This *carbinol* was purified by further recrystallisation from benzene–light petroleum, and then formed colourless hexagonal plates, m. p. 115·5—116·5° (Found : C, 69·0; H, 6·4; OMe, 37·3. $C_{19}H_{22}O_5$ requires C, 69·05; H, 6·7; OMe, 37·6%). The p-*phenylbenzoate*, obtained by 1 hour's heating at 100° with *p*-phenylbenzoyl chloride in pyridine, crystallised from alcohol in colourless leaflets, m. p. 146—147° (Found : C, 75·0; H, 5·95; OMe, 23·5. $C_{32}H_{30}O_6$ requires C, 75·3; H, 5·9; OMe, 24·3%).

Attempts were made to oxidise this carbinol with chromic acid and potassium permanganate under various conditions, but crystalline or acidic products were not obtained. In one case 1·5 mg. of a semicarbazone, m. p. 149—150°, were isolated from the products of oxidation of 300 mg. of the carbinol with cold chromic acid in acetic acid.

In other preparative experiments the carbinol was contaminated with an impurity of higher m. p. which was difficult to remove by crystallisation. The pure carbinol was, however, readily isolated by preparation and hydrolysis of the aforesaid *p*-phenylbenzoate. Heating on the water-bath after addition of the sodium nitrite seemed to favour the formation of the by-product and repeated crystallisation of the distilled product then gave a crystalline powder, m. p. 133—134° after sintering. This did not differ in composition from the pure carbinol, so the second substance appears to be an isomeride of the carbinol (Found : C, 69·1; H, 6·9; OMe, 37·4%).

A crude carbinol mixture of m. p. 113—130° was heated with *iso*amyl disulphide at 210° in an attempt to bring about dehydrogenation. The carbinol described above was converted into resinous products, and after vacuum distillation and recrystallisation there was obtained a compound which formed colourless prisms (from benzene), m. p. 157—160° (Found : C, 69·1; H, 6·6%). This may have been the contaminant in the carbinol; it gave a *p*-phenylbenzoate, m. p. 108—109° to a cloudy liquid, not obtained analytically pure.

Action of Phthalic Anhydride on the Carbinol, m. p. 116°, *from Colchinol Methyl Ether.*—When a solution of the carbinol (0·3 g.) and phthalic anhydride (0·3 g.) in benzene was boiled for an hour, the carbinol (0·25 g.) was recovered. A mixture of the carbinol (0·3 g.) and phthalic anhydride (0·3 g.) was therefore heated at 180° for an hour, and the melt afterwards extracted with benzene. It was almost completely removed from the benzene extract by shaking with dilute sodium carbonate solution, and the reprecipitated *hydrogen phthalate* was recrystallised three times from benzene; it then formed small colourless leaflets, m. p. 143—144° (Found : C, 67·7; H, 5·5. $C_{27}H_{26}O_8$ requires C, 67·75; H, 5·5%).

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