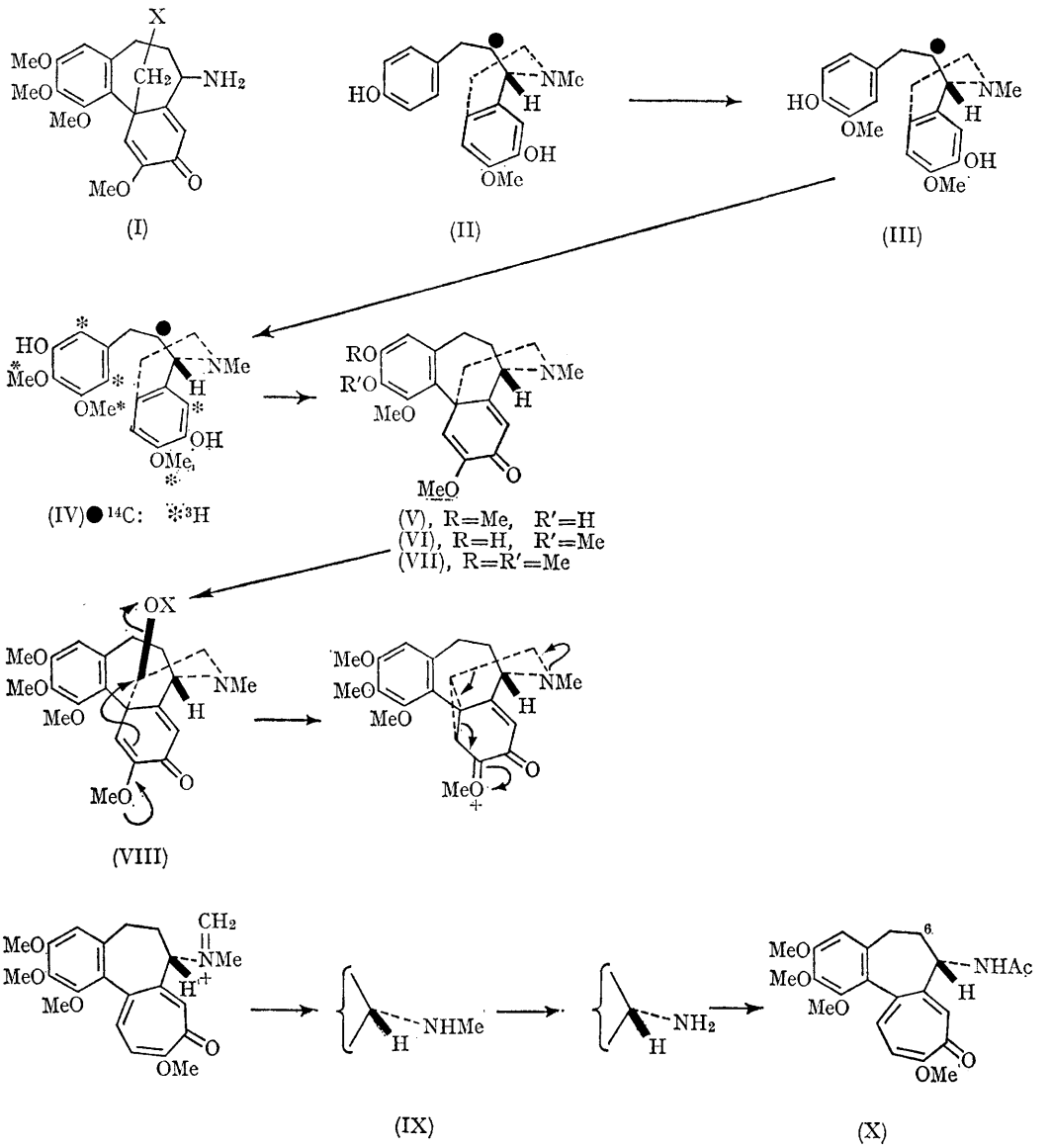
Biosynthesis of Colchicine from a 1-Phenethylisoquinoline

By A. R. BATTERSBY, R. B. HERBERT, E. MCDONALD, R. RAMAGE, and (the late) J. H. CLEMENTS

(*The Robert Robinson Laboratories, University of Liverpool*)

Androcymbium melanthioides is a close relative of *Colchicum autumnale* and both species contain colchicine. The constitution (V) was recently established[1] for androcymbine, the major alkaloid of the former plant. Earlier tracer experiments[2–4] on *C. autumnale* had led to the suggestion[3,4] that the dienone (I) is a biosynthetic precursor of colchicine and this was subsequently supported by a different labelling experiment.[5] Reasoning from the structure of androcymbine, it was suggested[1] that colchicine is a modified 1-phenethylisoquinoline alkaloid and that the partial structure (I) for the intermediate should be extended to give dienone (VI). The biosynthetic pathway to colchicine



then becomes (II) → (X). We now outline the results of tracer experiments which provide strong support for much of this sequence.[6]

Androcymbine (V) was methylated with diazomethane in the presence of tritiated water[7] to afford [^{3}H]-*O*-methylandrocymbine (VII) labelled at that *O*-methyl group shown as R′O. When this dienone was fed to *C. autumnale* plants, it was converted well (15%) into colchicine. Oxidative degradation of the radioactive colchicine gave 3,4,5-trimethoxyphthalic anhydride (99·2% of original activity). This establishes the specific incorporation of the dienone (VII) and therefore the relationship of colchicine to the 1-phenethylisoquinoline series. [^{3}H]-*O*-Methylandrocymbine was also incorporated by *C. byzantinum* plants into demecolcine (IX; 4·9% incorporation) and into colchicine (0·65%).

Direct evidence that 1-phenethylisoquinolines are the precursors of colchicine and demecolcine was obtained by synthesis of the racemic base (as IV) singly labelled with carbon-14 at the position

TABLE

	Ar–T[a]	2 × OMe (ring-A)[a]	OMe (ring-C)[a]
(±)-Precursor (as IV)	4·40	10·1	9·5
Colchicine (X)	3·08 (70% retention)	10·3	9·6

[a] The figures record the ratios of activity relative to the ^{14}C internal standard.

marked ●. Many feeding experiments have been carried out with this substance and the incorporations into colchicine and demecolcine in *C. autumnale* have been as high as 10·1% and 0·81%, respectively. Oxidation of the derived colchicine as earlier[3] afforded radio-inactive trimethoxyphthalic anhydride and radioactive succinic acid (98% of total activity); Schmidt degradation of the succinic acid gave inactive carbon dioxide thus proving the colchicine to be labelled specifically at position 6. Further, this result made it possible to use a skeletal ^{14}C-label as the internal standard for experiments with multiply labelled forms of the racemic base (as IV). These were synthesised by routes to be described in our full publication.

The biosynthetic scheme requires that (a) one third of the aryl tritium should be lost from the precursor when it is converted into colchicine; (b) the two *O*-methyl groups on ring-A should be retained; (c) the *O*-methyl group on ring-C should also be retained. The Table shows that the experimental results agree closely with these requirements; the degradative methods used were those reported earlier[3] together with appropriate Zeisel *O*-methyl determinations. Further, the racemic precursor (as IV) was synthesised carrying a nitrogen-15 label together with carbon-14 at the usual skeletal position and this product was fed to *C. autumnale* plants. The dilution of carbon-14 during the biological conversion into colchicine was 157 which matched perfectly the dilution of nitrogen-15 found to be 156 by mass spectrometry (the ^{15}N-assay was kindly carried out by Drs. E. W. Underhill and L. R. Wetter). Thus, the nitrogen atom of colchicine is proved to be that of the isoquinoline precursor.

The biosynthetic steps preceding the isoquinoline (IV) were examined by feeding *C. autumnale* plants with the phenols (II) and (III). These were incorporated into colchicine to the extent of 1·4% and 3·8% respectively in agreement with the illustrated stepwise oxygenation process.

Our results define in detail a considerable part of the biosynthetic pathway to colchicine; the mechanism of the ring-expansion step and the relationship of demecolcine (IX) to colchicine (X) will be the subject of a future communication.

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