Biosynthesis of Colchicine: Ring Expansion and Later Stages. Structure of Speciosine

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COLCHICINE (X) was recently shown[1] to be biosynthesised from 1-phenethylisoquinolines by way of the dienone (IV) which is formed in turn from the base (I). It was suggested[1] that colchicine is then derived by hydroxylation of the dienone (IV) to yield (V; X = H), homoallylic assistance[2] of ionisation, probably involving the phosphate (V; X = phosphate) followed by the illustrated fragmentation (VI). On this basis, demecolcine (VIII) which is also present in *Colchicum* plants should be a precursor of colchicine. Experimental support for this sequence is now outlined.[3]

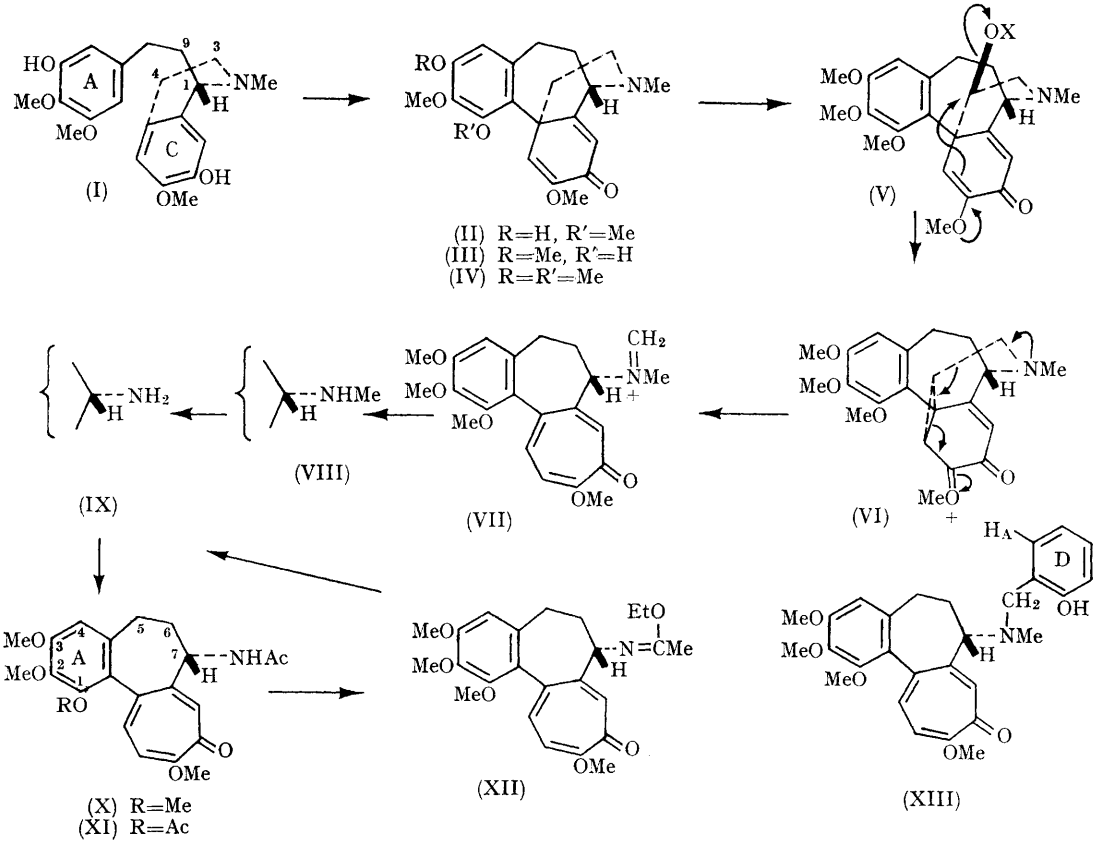
The racemic base (as I), as its *OO*-dibenzyl ether labelled with ^3H at C-1 and at the ring A *O*-methyl groups, was resolved with *OO*-dibenzoyltartaric acid. The (−)-form showed a positive Cotton effect[4] in the region 295—270 mμ proving that it has the illustrated *S*-configuration (as I) which corresponds to that of colchicine;[5] the optical rotatory dispersion curve was kindly determined by Professor W. Klyne and Dr. P. M. Scopes. Debenzylation of the (−)- and (+)-forms afforded the diphenol (I) and its enantiomer. Only the former was an effective precursor of colchicine in *C. byzantinum* plants (Table, Expt. 1, 0·53% incorp.); the incorporation of the enantiomer of (I) was <0·015%. Isolation of ring A from the radioactive colchicine as 3,4,5-trimethoxyphthalic acid allowed the labelling ratio in the alkaloid to be determined (Table). This showed some loss of ^3H-activity from C-1 of (I) but oxidation–reduction is less important in *C. byzantinum* than in the opium poppy.[6]

The biosynthetic scheme requires (a) retention of the *N*-methyl group of (I) in demecolcine (VIII) and its loss when colchicine is formed (b) loss of C-3 from (I) when it is converted into colchicine. Separate experiments (*cf.*, ref. 1) proved that the

TABLE. *Tracer results from precursor* (I)

Expt. No. and chirality	Position and level of labelling	Ratio in precursor[a]	Ratio in demecolcine (VIII)	Ratio in colchicine (X)
1. (−)	1, ^{3}H (8·2) ring A, 2 × $OCH_2{}^3H$ (1·0)	8·2, $^3H/^3H$	*b*	5·3 (65% retention)
2. (±)	N-^{14}C-Me (0·097) 9-^{14}C (0·099) ring A, 2 × $OCH_2{}^3H$ (1·0)	0·196, $^{14}C/^3H$	0·187 (91% retention)	0·107
3. (±)	3-^{14}C (0·137) 9-^{14}C (0·105) ring C, $OCH_2{}^3H$ (1·0)	0·242, $^{14}C/^3H$	*b*	0·126
4. (±)	4-^{3}H (11·0) 9-^{14}C (1·0)	11·0, $^3H/^{14}C$	*b*	4·78 (44% retention)

[a] The figures record the ratio of activity relative to the internal standard of ^{14}C or ^{3}H; the activity of the standard is set arbitrarily as 1·00.
[b] Not examined.



O-methyl groups of (I) are retained quantitatively throughout the biosynthesis. This allows the values quoted in the Table to be calculated (Expt. 2 and 3) and the experimental results are close to those required. Colchicine from Expt. 2 and 3 was hydrolysed and the acetic acid formed carried $<1\%$ of the original activity in each case. Slightly more ^{14}C-activity is retained by the colchicine in Expt. 2 and 3 than required by complete loss of the *N*-methyl and C-3, respectively, and this arises by some "feed-back" of activity from the cleaved fragments through the plant's biosynthetic system. Thus, degradation of colchicine from Expt. 3 showed that the tropolone *O*-methyl group and ring A with its attached atoms together carry ^{14}C activity corresponding to 15·4% of the total.

Hydroxylation of (IV) to generate (V, X = H) could be (a) stereospecific (b) non-stereospecific (c) *via* the corresponding ketone. The corresponding retentions of ^3H for Expt. 4 should be, respectively, (a) 50% (b) *ca.* 85%[7] (c) 0% and the Table shows agreement with (a).

Demethylation[8] of colchicine from Expt. 2 gave *O*-acetyl-1-desmethylcolchicine (XI; $^{14}C:H^3$ ratio 0·22 : 1); the structure of (XI) was confirmed by n.m.r. studies. Half the ^3H activity present in colchicine is lost in the demethylation step which establishes *para*-coupling of (I) to yield (II). No loss of ^3H activity would be observed had *ortho*-coupling occurred to generate (III).

Late stages of the biosynthesis were examined by preparing [^3H-*O-methyl*]demecolcine (VIII) and [^3H-*O-methyl*]colchicine from 3-desmethyldemecolcine[9] and 3-desmethylcolchicine,[9] respectively. Desacetylcolchicine (IX) was obtained from the active colchicine by formation (Meerwein's reagent) and hydrolysis of the imino-ether (XII). Feeding experiments with these precursors to *C. autumnale* plants showed that demecolcine (VIII) and desacetylcolchicine (IX) act as effective precursors of colchicine; the figures after the precursor record the percentage incorporation into demecolcine (or recovery) and into colchicine (or recovery): demecolcine (VIII) 8·8, 13·8; desacetylcolchicine (IX) 1·6, 35·3; colchicine (X) 0, 41·6%. Parallel results were obtained in *C. byzantinum* plants.

The combined results (*cf.*, ref. 1) define the later stages in the biosynthesis of colchicine as (I) → (II) →(IV) → (V) → (VIII) → (IX) → (X). It is probable on chemical grounds that (VI) and (VII) are labile intermediates and the structure of speciosine may be relevant to the imonium salt (VII).

Speciosine is a weak base which occurs[10] in *C. speciosum* and the molecular formula $C_{28}H_{31}NO_6$ was confirmed by mass spectrometry ($M^+ = 477$). The u.v. and i.r. spectra indicated the presence of a non-phenolic aryltropolone system, as in colchicine, together with an additional phenolic chromophore (bathochromic shift in alkali). Strong peaks were present in the mass spectrum at *m/e* 207, 107, and 106; the first suggested[11] that speciosine is related to demecolcine (VIII) and the last two that a readily cleaved entity, C_7H_7O, is present. Structure (XIII) was therefore considered. The n.m.r spectrum of speciosine confirmed every feature of the demecolcine residue and four additional aromatic protons were observed. One at τ 2·96, corresponding to H_A on ring D, appeared as a double doublet ($J = 7$ c. and 1·5 c./sec.) in agreement with the illustrated ring D substitution. Structure (XIII) was established for speciosine by treating demecolcine (VIII) with 2-acetoxybenzyl bromide.[12] Hydrolysis of the product gave the phenol (XIII) which was identical with the natural alkaloid. An interesting possibility is that speciosine arises from the intermediate (VII) in a trapping process but this is not the only plausible route. The necessary tracer experiments are in progress.

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