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FORMATION OF BENZO[C]PHENANTHRIDINES BY OXIDATIVE C-N BOND FISSION OF PROTOBERBERINES FOLLOWED BY INTRAMOLECULAR RECYCLIZATION IN CELL CULTURES OF CORYDALIS INCISA

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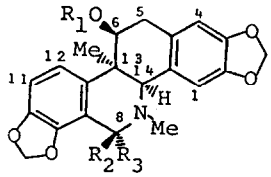
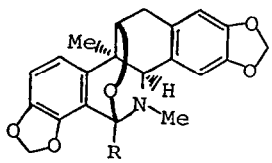
Abstract. Only 9 of the 6-hydroxy-13-methylprotoberberine N-metho salts 9 and 10 was transformed by cell cultures of C. incisa into the benzo[c]phenanthridines, corynoline (1) and corynoloxine (2). Corycavine (7) was incorporated more effectively into 1 and 2 than 9.

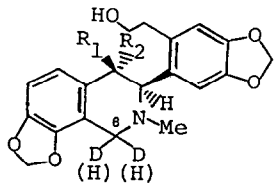
The hexahydrobenzo[c]phenanthridines, such as (±)- and (+)-corynoline (1) and (±)- and (+)-corynoloxine (2), have been obtained from C. incisa Pers.[1,2] They possess a methyl group at C-13 and an alcoholic oxygen function at C-6. We have demonstrated that only the cis-B/C fused N-metho salts (3, 4, and 5) of tetrahydroprotoberberines such as stylopine, tetrahydrocorysamine, and mesotetrahydrocorysamine are converted into the corresponding protopines regardless of the configuration of the hydrogens at C-13 and C-14, while the trans fused salts are ineffective precursors.[2] The protopine-type alkaloids, 6 and 7, have been proven to be bioconverted into hexahydrobenzo[c]phenanthridine-type alkaloids, chelidonine (8) and 1,[2] respectively.

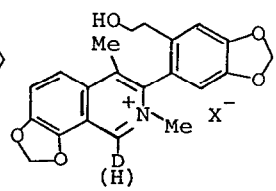
The purpose of the present investigation is to clarify nitrogen-C(6) bond cleavage process in the bioconversion of protoberberines into the hexahydrobenzo[c]phenanthridines. The other purpose is to examine the stereospecificity of the conversion of the 6-hydroxylated derivatives of 4 and 5 with the cis and trans relationship of H-13 and H-14, respectively, into benzo[c]phenanthridines. For these purposes, incorporation experiments with 7 and [8,8-D_2]-derivatives (9′ and 10′) of the carbinolammonium compounds (9 and 10)[3] were carried out using cell cultures of C. incisa.

Cultured cells of C. incisa were grown on agar medium containing 9′ or 10′ at 25°C for 5 weeks. After incubation, the medium and cells were extracted for alkaloids. Four bases were isolated by preparative thin layer chromatography of the extracts from the fraction to which 9′ was fed. The structures of these products were confirmed to be the deuterated corynoline (1′), corynoloxine (2′), cis aminoalcohol (11), and its dehydro derivative (12), by comparison of the mass and ^{1}H NMR spectra with those of the authentic samples. Two compounds









1 R_1= R_2= R_3= H
1' R_1= H R_2= R_3= D or H
14 R_1= Ac R_2= R_3= H

2 R = H
2' R = D

11 R_1= Me R_2= H
13 R_1= H R_2= Me

12

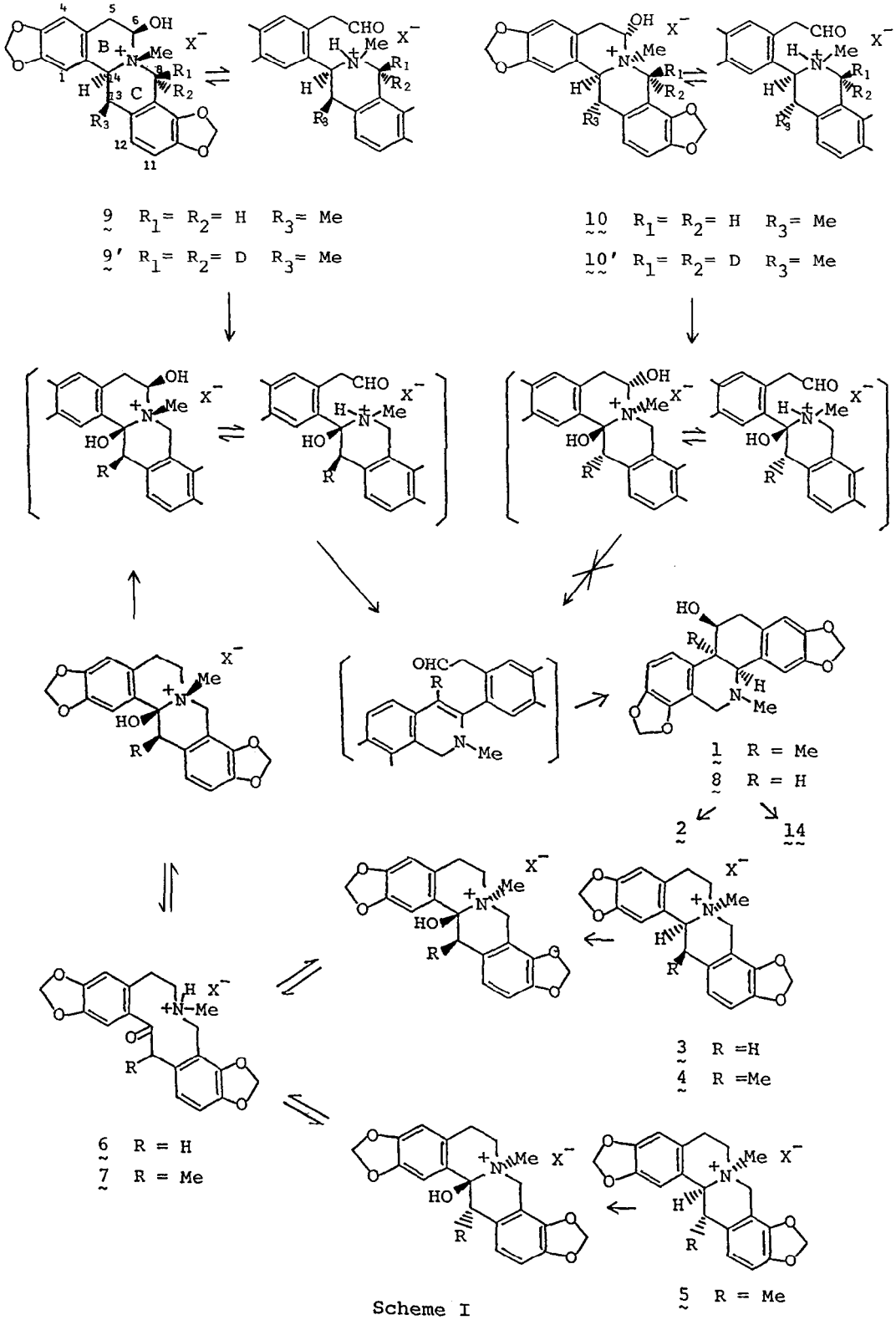
obtained from the fraction to which 10' was administered were found to be the trans aminoalcohol (13) and its dehydro derivative (12). Deuterium retention of the products was determined by ^{1}H NMR and mass spectra (Table I).

Table I Deuterium retention determined by ^{1}H NMR and mass spectra

Substrate	Product	Position of D	D-Retention (%)[a] from ^{1}H NMR	from mass	
9'	1'	C(8A)-D	29	D_1 32	D_2 24
		C(8B)-D	67		
	2'	C(8A)-D	100[b]	D_0 79	D_1 21
	11	C(8A)-D	61	D_1 100	D_2 66
		C(8B)-D	92		
	12	C(8)-D	94[c]		
10'	12	C(8)-D	100[d]		
	13	C(8A+8B)-D	49	D_1 100	D_2 75

a content of D in product/that in substrate x 100
b retention of C(8A)-D in 1'
c retention of C(8A)-D in 11
d retention of C(8A)-D or C(8B)-D in 13

Compound (9'), with the cis configuration of the protons at C-13 and C-14, was biotransformed into 1' and 2', but 10', possessing the trans configuration, was not (Scheme I). This suggests that a stereospecific enzymatic process operates at C-13 and C-14 during biosynthesis of 1 and 2. Formation of 1 from 9 gives the evidence supporting the intermediacy of a 6-hydroxyprotoberberine,[3] that is, an aldehyde,[3] during the conversion from the 13-methylprotoberberines into 1. An enamino aldehyde could be generated either by stereospecific oxygenation at C-14 of 9 followed by the elimination of water or by dehydrogenation at C-13 and C-14. If direct removal of the two adjacent hydrogens at C-13 and C-14 occurs from 9 to set up an enamino aldehyde, it is expected that 9 would be a more effective precursor than 7. In order to clarify this point, experiments with 7 and 9'were carried out under the same conditions. Corycavine (7) was incorporated 4 times



9 R_1= R_2= H R_3= Me

9′ R_1= R_2= D R_3= Me

10 R_1= R_2= H R_3= Me

10′ R_1= R_2= D R_3= Me

1 R = Me

8 R = H

2 14

3 R =H

4 R =Me

6 R = H

7 R = Me

5 R = Me

Scheme I

and 6 times better into 1 and 2, respectively, than 9! Acetylcorynoline (14), which is formed from 1, was isolated from the fraction to which 7 was fed. This indicates that 14-hydroxyprotoberberine N-metho salt (7) is a more effective precursor than 6-hydroxyprotoberberine N-metho salt (9). An enamino aldehyde could be generated by oxygenation at C-14 in 9 or at C-6 in 7 followed by the elimination of water from C-13 and C-14, and it could not be produced by a cis-dehydrogenation from 9 (Scheme I).

The further following statements can be made from the experiment with 9': (1) The retention of deuterium in 2' corresponds to that of the axial C-8α deuterium in 1'. This is in keeping with a stereospecific hydrogen removal from C-8 of 1' to be bioconverted into 2. (2) Biotransformation from 9' into 1' or 11 involves inequivalent loss of deuterium from C-8 of 9'. This deuterium loss suggests that C-8 is affected during the bioconversion into 1 or 11. This is in disagreement with the result in Chelidonium majus plants that C-8 is uneffected during the transformation from stylopine into 8.[4] (3) The amino-alcohols, 11 and 13, are not effective as a precursor of 1 and they might be metabolized to 12.

We conclude that the hexahydrobenzo[c]phenanthridine alkaloids are biosynthesized from the protoberberine alkaloids via oxygenations at C-14 and then at C-6, followed by C(6)-N bond fission and elimination of water leading to an enamino aldehyde, and then intramolecular condensation between the positions corresponding to C-6 and C-13 of the protoberberine skeleton. A route to the benzo[c]phenanthridine alkaloids in accord with all our present data and earlier work is shown in Scheme I.

REFERENCES AND NOTES

1. N. Takao, Chem. Pharm. Bull., 11, 1306 and 1312 (1963).
2. N. Takao, K. Iwasa, M. Kamigauchi, and M. Sugiura, Chem. Pharm. Bull., 24, 2859 (1976); N. Takao, M. Kamigauchi, and K. Iwasa, Annual Meeting of the Pharmaceutical Society of Japan, Okayama, April, 1978, Abstracts, p343.
3. The synthesis of the labeled materials, 9' and 10', will be described elsewhere. The stereochemistry of 9 and 10 was determined by ^{1}H NMR spectroscopy and X-ray analysis. Compound 9 and 10 exist as equilibrium mixtures of the trans-B/C and cis-B/C fused salts. The ratios of the cis-B/C to trans-B/C in methanol were 0.37 and 3.0 in 9 and 10, respectively. Interconversion between the two isomers occurs through the aminoaldehyde form (Scheme I). Details will be reported in a full paper.
4. A. R. Battersby, J. Staunton, H. R. Wiltshire, J. F. Francis, and R. Southgate, J. Chem. Soc. Perkin I, 1147 (1975).

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