

BIOTRANSFORMATION OF A TETRAHYDROPROTOBERBERINE N-METHO SALT TO A
SPIROBENZYLISOQUINOLINE ACCOMPANYING N-METHYL GROUP TRANSFER TO
O-METHYL GROUP BY CALLUS CULTURES OF CORYDALIS SPECIES

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Abstract — The Structure and stereochemistry of base C, a 13-hydroxytetrahydroprotoberberine metabolite in cell cultures, have been determined by X-ray analysis. Thereby the biotransformation of a tetrahydroprotoberberine N-metho salt to a spirobenzylisoquinoline, involving a migration of the N-methyl group to the O-methyl group was first demonstrated.

It has been reported that base C was derived from (-)-epiophiocarpine α -N-methyl chloride (1) bearing a cis-fused quinolizidine ring via 13-oxoalloycryptopine (2) in cell cultures of Corydalis ophiocarpa, Corydalis ochotensis var. raddeana, and Corydalis platycarpa.¹ Base C has been reported to have molecular formula $C_{21}H_{21}NO_6$, a methylenedioxy group, two methoxyl groups, an N-methyl group, one carbonyl group, and a hydroxyl group.¹

Since the structure of base C was not established from the spectral data, a X-ray diffraction study on the hydrochloride was carried out.

Crystal data: $C_{21}H_{21}NO_6 \cdot HCl$, $M = 419.9$, monoclinic, space group $A2/a$, $a = 18.533(4)$, $b = 9.781(1)$, $c = 31.406(8)$ Å, $\beta = 136.20(1)^\circ$, $V = 3940(1)$ Å³, $D_m = 1.413(1)$, $D_c = 1.415$ g cm⁻³, $Z = 8$. A total of 3330 independent reflections were measured by Rigaku AFC-5 diffractometer with graphite-monochromated Cu K α radiation. The structure was solved by heavy-atom methods and successive Fourier syntheses, and refined by full-matrix least-squares to $R = 0.117$. The obtained perspective view is shown in Figure 1. The X-ray study showed that base C was the spirobenzylisoquinoline-type

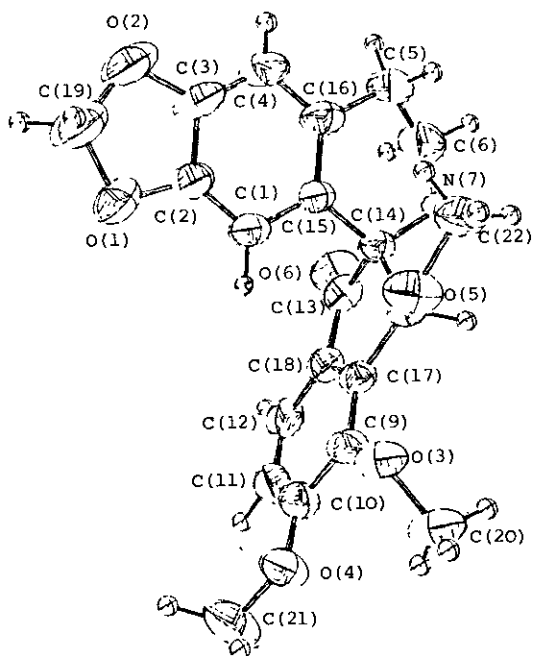
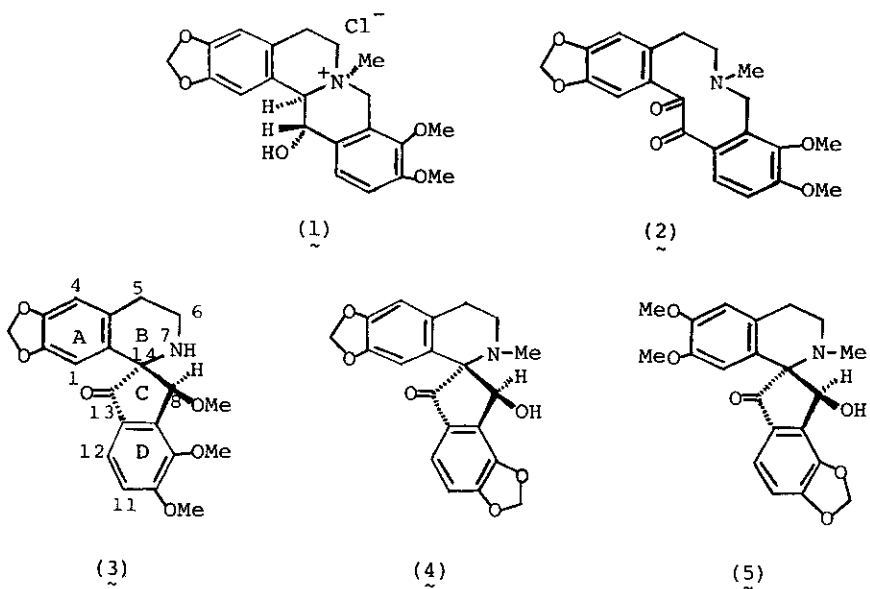


Figure 1. Molecular structure of base C (3)

alkaloid (3) having the aliphatic O-methyl group and the secondary amino group instead of the N-methyl group and the hydroxyl group, respectively, previously suggested from biogenetic view and consideration of the spectral data.¹ The carbonyl group was placed at C-13, and the methoxyl group at C-8 is anti to the nitrogen. The stereochemistry at C-8 is the same as those occur in sibiricine (4)², a minor alkaloid of Corydalis sibirica and raddeanone (5)³ isolated from Corydalis ochotensis var. raddeana. The torsion angle C(6)-N(7)-C(14)-C(13) is 85.6°, indicating a distortion of the B ring half-chair to a more flattened conformation which partially relieves the steric interaction between C-1 atom and O-methyl group at C-8 in the half-chair conformation. This structure maintaining the distortion of the conformation is compatible with the ¹H NMR and ¹³C NMR data for free base (3).¹ Incorporation of ¹³C label of the N-methyl group in the protoberberine precursor (1) into the O-methyl group in base C has been demonstrated by experiments using ¹³C labeled precursor in cell cultures of Corydalis ophiocarpa and Corydalis ochotensis var. raddeana.¹ Consequently, present X-ray determination of the structure of base C established bioconversion of a 13-hydroxytetrahydroprotoberberine N-metho salt into a spirobenzylisoquinoline by cell cultures for the first time. Such a bioconversion in the callus might occur to produce raddeanone (5) in Corydalis ochotensis var. raddeana. It was also established that the O-methyl group at C-8 of a spirobenzylisoquinoline arises from the N-methyl group of a protoberberine skeleton, that is, migration of the N-methyl group to the O-methyl group occurs during the ring rearrangement. This is the most unexpected and interest aspect.

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