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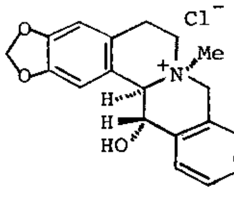
Abstract —— The Structure and stereochemistry of base C, a 13-hydroxytetrahydroprotoberberine metabolite in cell cultures, have been determined by X-ray analysis. Thereby the biotransformation of a tetrahydroprotoberberine N-metho salt to a spirobenzylisoquinoline , involving a migration of the N-methyl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

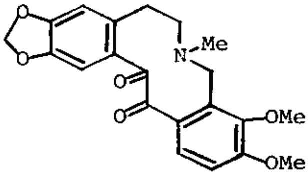
It has been reported that base C was derived from (-)-epiophiocarpine α-N-methyl chloride (1) bearing a *cis*-fused quinolizidine ring *via* 13-oxoallocryptopine (2) in cell cultures of *Corydalis ophiocarpa*, *Corydalis ochotensis var. raddeana*, and *Corydalis platycarpa*.[1] Base C has been reported 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, β = 136.20(1)°, U = 3940(1) Å^3, 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 graohite-monochromated Cu Kα 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



(1)



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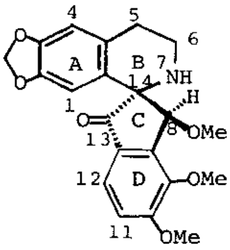

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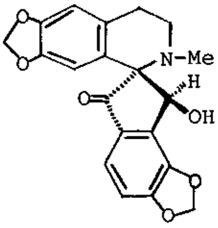

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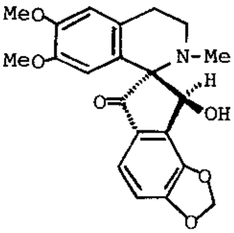

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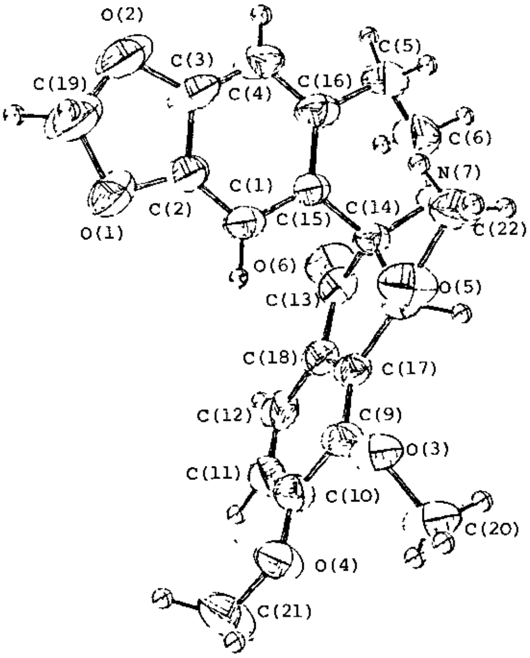

Figure 1. Molecular structure of base C (3)

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 ^{1}H NMR and ^{13}C NMR data for free base (3).[1] Incorporation of ^{13}C 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 ^{13}C 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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